Gerald F. Combs Jr.
James P. McClung

The Vitamins

Fifth Edition

Fundamental Aspects in Nutrition and Health

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Fifth Edition

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Academic Press is an imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1800, San Diego, CA 92101-4495, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-802965-7

Publisher: Nikki Levy
Acquisition Editor: Megan Ball
Editorial Project Manager: Jaclyn Truesdell
Production Project Manager: Caroline Johnson
Designer: Miles Hitchen

Typeset by TNQ Books and Journals

Dedication

To the students and professionals who have used this book and whose comments and suggestions have helped us produce this fifth edition
–The Authors, Fifth Edition

and

to Barbara, ma fleur
–Jerry

and

to Holly, Elliott, and Adeline
–James

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Preface to the Fifth Edition

Understanding the vitamins is key to understanding nutrition. The history of their discovery and the continuing elucidation of their roles in health is the history of the emergence of nutrition as a science from the areas of physiology, biochemistry, medicine, and agriculture.

Capturing the understanding that grew out of that history is both a challenge and a privilege. For us, it involved months of reviewing thousands of publications and looking for clear ways to present complex information without overstating present understanding.

Producing this fifth edition of *The Vitamins* benefitted from the inclusion of a coauthor, which we believe brought a new prospective to the text. James studied the first edition of the *The Vitamins* as a masters student at the University of New Hampshire in 1997. He encountered the second edition of the text as Jerry's student at Cornell University in 2001. We are hopeful that the dynamic relationship we have enjoyed, as student/mentor, colleagues, friends, and now coauthors, has resulted in the most effective edition of this text, as both a reference and a teaching aid.

In writing this fifth edition of *The Vitamins*, we were mindful of comments from users of previous editions, which prompted several changes that we believe enhanced the book. We reorganized several chapters, which reduced their number. We emphasized roles of the gut microbiome in several places of importance. We added sections on biomarkers of vitamin status and modestly expanded the section on biofortification. We added, redrew, and updated several tables and figures. We used extensive footnoting as a means of including explanatory notes as well as for citing primary sources.

We are grateful for the professional assistance from editors, Ms. Jaclyn Truesdell, Ms. Megan Ball, and Ms. Caroline Johnson of Elsevier.

We enjoyed writing this fifth edition of *The Vitamins* together. We hope you will find it useful.

Gerald F. Combs, Jr.
Topsham, Maine
James P. McClung
Westborough, Massachusetts
June 2016

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How to Use This Book

TO THE HEALTH PROFESSIONAL

The Vitamins is designed as a one-stop source of comprehensive, current information on the vitamins. In it you will find information on the history of vitamin discovery, the chemical properties of the vitamins and their isomers and metabolites, the utilization and metabolism of vitamins, the consequences of their deficient and excessive intakes, biomarkers of vitamin status, and the health roles if particular vitamins in beyond the traditional deficiencies. You will find examples of classical and current research findings as well as citations to recent key publications in the footnotes. You may find Appendix particularly useful, as it lists the vitamin contents of a most common foods. Please let us know of any ways you see we might enhance *The Vitamins*.

TO STUDENTS AND INSTRUCTORS

The Vitamins is also intended as a teaching text for an upper-level college course within a nutrition or health-related curriculum; however, it will also be useful as a workbook for self-paced study of the vitamins. It has several features that are designed to enhance its usefulness to students as well as instructors. Here is how we suggest using it.

To the student When you use this text, make sure to have by your side a notebook, pencil (not pen—you may want to make changes in the notes you take). Then, before reading each chapter, take a few moments to go over the "Anchoring Concepts and Learning Objectives" on the chapter title page. *Anchoring Concepts* are the ideas fundamental to the subject matter of the chapter, the concepts to which the new ones presented in the chapter will be related. Those in the first several chapters should already be very familiar to you; if not, then it will be necessary for you to do some background reading or discussion until you feel comfortable in your understanding of these basic ideas. You will find that most chapters are designed to build upon the understanding gained through previous chapters; in most cases, the *Anchoring Concepts* of a chapter relate to the *Learning Objectives* of previous chapters. Pay attention to the *Learning Objectives*; they are the key elements of understanding what the chapter is intended to support. Keeping the *Learning Objectives* in mind as you go through each chapter will help you maintain focus on those elements. Next, read through the Vocabulary list and *mark* any terms that are unfamiliar or about which you feel unsure. Then, make a list of *your own questions* about the topic of the chapter.

As you read through the text, look for items related to your questions and for unfamiliar terms. You will be able to find key terms in bold-faced type, and you should be able to get a good feel for their meanings from the contexts of their uses. If this is not sufficient for any particular term, then look it up in a medical dictionary. Do not wait to do this. Cultivate the habit of being bothered by not understanding something—this will help you enormously in years to come.

As you proceed through the text, note what information the layout is designed to convey. First, note that the major sections of each chapter are indicated with a bold heading. This is done to help you *scan* for particular information. Also note that the footnoted information is largely supplementary and not essential to the understanding of the key concepts presented. Therefore, the text may be read at two levels: at the basic level, one should be able to ignore the footnotes and still get the key concepts; at the more detailed level, one should be able to pick up more background, particularly key citations to the primary literature, from the footnotes. Refer back frequently to your own list of questions and "target" vocabulary words; when you find an answer or can make a deduction, make a note. Do not be reluctant to write in the book, particularly to put a concept into your own words, or to note something you find important or do not fully understand. Studies show that to be an effective learning technique.

When you have completed a chapter, take sometime to list what you see as the key points—those that you would cover in a formal presentation. Then, skim back over the chapter.

You will find that Chapters 6–19 each have one or more Case Studies comprised of more clinical case reports abstracted from the medical literature. For each, use the associated questions to focus your thinking on the features that relate to vitamin functions. As you do so, try to ignore the obvious connection with the subject of the chapter; put yourself in the position of the attending physician who was called upon to diagnose the problem without prior

knowledge that it involved any particular nutrient, much less a certain vitamin. The Case Study in Chapter 21 is different; it is a fictional but highly plausible scenario that calls for a nonobvious decision. Additional case studies are listed in Appendix B.

Take sometime and go through the Study Questions and Exercises at the end of each chapter. These, too, are designed to direct your thinking back to the key concepts of the respective chapter and to facilitate integration of those concepts with those you already have.

We have made a point in Chapter 1 of using the technique of *concept mapping* do demonstrate the integration of complex subject matter. We have found the *concept map* to be a powerful teaching/learning tool. If you have had no previous experience with this device, then it will be worth your while to consult *Learning How to Learn*.[1]

When you have done all of this for a chapter, then deal with your questions. Discuss them with fellow students or look them up. To assist you in the latter, a short reading list is included at the end of each chapter. With the exception of Chapter 2, which lists papers of landmark significance to the discovery of the vitamins, the reading lists consist of key reviews in prominent scientific journals. These reviews and the papers cited in the footnotes will help you find primary research papers on topics of specific interest.

After you have followed all of these steps, *reread the chapter*. You will find this last step to be extraordinarily useful in gaining a command of the material.

Last, but certainly not least, have *fun* with this fascinating aspect of the field of nutrition!

To the instructor The format of this text reflects the way GFC taught a course called "The Vitamins" for some 29 years at Cornell University. To that end, some experiences in using *The Vitamins* as a text for my course may be of interest to you.

I have found that *every* student comes to the study of the vitamins with *some* background knowledge of the subject, although those backgrounds are generally incomplete and frequently include areas of misinformation. This is true for upper-level nutrition majors and for students from other fields, the difference being largely one of magnitude. This is also true for instructors, most of whom come to the field with specific expertise that relates to only a subset of the subject matter.

You can demonstrate this in the following exercise, best done of the first day of class. Raise your index finger (best done with a bit of dramatic flair) and say "vitamin A." Hold that pose for 10 s and then ask "*What came to mind when I said 'vitamin A'?*" Without fail, someone will say "vision" or "carrots," and then an older graduate student may add "toxic." When it looks safe to chime in, others will add what will build to an array of descriptors that, collectively, are more relevant to vitamin A than any is individually. Most of the answers, by far, will relate to the clinical symptoms of vitamin A deficiency and the sources of vitamin A in diets. Catch each answer by dashing it on to a large sticky note and then stick the note haphazardly to a blackboard or wall. If you hear something complex or a cluster of concepts, make sure to question the contributor until you hear one or more individual concepts, which you can record on individual sticky notes. This approach *never fails* to stimulate further answers, and it is common that a group of 15–20 students will generate a list of twice that number of concepts before the momentum fades. Having used sticky notes, it is easy to move them into clusters and, thus, to use the activity to construct a *concept map* of "Vitamin A" based solely on the knowledge that the students, collectively, brought into the room. This exercise can demonstrate an empowering idea that, having at least *some* background on the subject and being motivated (by any of a number of reasons) to learn more, *every* learner brings to the study of the vitamins a unique perspective which may not be readily apparent.

We are convinced that meaningful learning is served when both instructor and students come to understand each others' various perspectives. This has two benefits in teaching the vitamins. First, it is in the instructor's interest to know the students' ideas and levels of understanding concerning issues of vitamin need, vitamin function, etc., such that these can be built upon and modified as may be appropriate. Second, many upper-level students have interesting experiences (through personal or family histories, their own research, information from other courses, etc.) that can be valuable contributions to classroom discussions. These experiences are assets that can reduce the temptation to fall back on the "instructor knows all" notion, which we all know to be false. To identify student perspectives, it is useful to assign on the first class period, for submission at the second class, a written autobiographical sketch. Distribute your own as a model, and ask each student to write "as much or as little" as he or she cares to, recognizing that you will distribute to the class copies of whatever is submitted. The biographical sketches will range from a few sentences that reveal little of a personal nature to longer ones that provide many good insights about their authors; *everyone* will help you to get to know your students personally and to get a better idea of their understandings of the vitamins and of their expectations of the course. The exercise serves the students in a similar manner, thus promoting a group dynamic that facilitates classroom discussions.

The Vitamins can be used as a typical text from which you can make regular reading assignments as preparation for each class. This will free you of the need for lecturing in favor of an open discussion format. In fact, this approach allows more information to be covered, as even a brilliant lecturer simply cannot cover the vitamins in any real depth

1. Novak, J.D., Gowin, D.B., 1984. Learning How to Learn. Cambridge, University Press, New York, NY, pp. 199

within the limits of traditional class periods. This was the original motivation for putting that information into this text, which has allowed shifting responsibility for learning to the student to glean from assigned reading. This allows class time to be used to facilitate learning through discussions of issues of student interest or concern. Often, this means that certain points were not clear upon reading or that the reading itself stimulated questions not specifically addressed in the text. Usually, these questions are nicely handled by eliciting the views and understandings of other students and by your giving supplementary information.

With this approach, the instructor's class preparation involves the collation of research data that will supplement the discussion in the text, and the identification of questions that can initiate discussions. In developing questions, it may be useful to prepare your own concept maps of the subject matter and to ask rather simple questions about the linkages between concepts, e.g., "*How does the mode of enteric absorption of the tocopherols relate to what we know about its physiochemical properties?*" If you are unfamiliar with concept mapping, then consult "*Learning How to Learn*" and experiment with the technique to determine whether it can assist you in your teaching.

The Study Questions and Exercises or Case Studies can be used to give weekly written assignments to keep students focused on the topic and prevent them from letting the course slide until exam time. More importantly, there is learning associated with the thought that necessarily goes into such written assignments. To support that learning, make a point of going over each assignment briefly at the beginning of the class at which it is due and return it by the *next* class with your written comments. You will find that the *Case Studies* are abstracted from actual clinical reports; students enjoy and do well on these assignments.

The model we used in teaching *The Vitamins* at Cornell was to evaluate student's performance on the basis of class participation, weekly written assignments, a review of a recent research paper, and either one or two examinations. To allow each student to pursue a topic of specific individual interest, students were asked to review a research paper published within the last year, using the style of *Nutrition Reviews*. Students were asked to make a short (10 min) presentation of each in class. Their reviews were evaluated on the basis of critical analysis and on the importance of the paper to the field. This assignment was also well received. Because many students are inexperienced in research and will, thus, feel uncomfortable in criticizing it, it is helpful to conduct in advance a discussion of the general principles of experimental design and statistical inference. Exams were also concept-oriented: students were given brief case descriptions and actual experimental data, and were asked to lay out diagnostic strategies, develop hypotheses, design means of hypothesis testing and interpretation of results, etc. Many students may prefer the more familiar short-answer test; such inertia can be overcome by using examples in class discussions and or homework assignments.

The Vitamins was been of great value in enhancing the teaching of the course by that name at Cornell. Thus, it is our sincere wish that it will assist you similarly in your teaching. Please let us know how it meets your needs and how we might enhance it for that purpose.

Gerald F. Combs, Jr.
James P. McClung

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Part I

Perspectives on the Vitamins in Nutrition

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Chapter 1

What Is a Vitamin?

Chapter Outline

Anchoring Concepts

1. Certain factors, called *nutrients*, are necessary for normal physiological function of animals, including humans. Some nutrients cannot be synthesized adequately by the host and must therefore be obtained from the external chemical environment; these are referred to as *dietary essential nutrients*.
2. *Diseases* involving physiological dysfunction, often accompanied by morphological changes, can result from insufficient intakes of dietary essential nutrients.

Imagination is more important than knowledge.

A. Einstein

LEARNING OBJECTIVES

1. To understand the classic meaning of the term *vitamin* as it is used in the field of nutrition.
2. To understand that the term *vitamin* describes both a concept of fundamental importance in nutrition as well as any member of a rather heterogeneous array of nutrients, any one of which may not fully satisfy the classic definition.
3. To understand that some compounds are vitamins for one species and not another, and that some are vitamins only under specific dietary or environmental conditions.
4. To understand the concepts *vitamer* and *provitamin*.

VOCABULARY

Vitamer
Vitamin
Provitamin

1. THINKING ABOUT VITAMINS

Among the nutrients required for the many physiologic functions essential to life are the vitamins. Unlike other nutrients, the vitamins do not serve structural functions, nor does their catabolism provide significant energy. Instead, the physiologic functions of vitamins are highly specific, and, for that reason, they are required in only small amounts in the diet. The common food forms of most vitamins require some metabolic activation to their functional forms.

Although the vitamins share these general characteristics, they show few close chemical or functional similarities; their categorization as vitamins is strictly empirical. Consider also that, whereas several vitamins function as enzyme cofactors (vitamins A, K, and C; thiamin; niacin; riboflavin; vitamin B_6; biotin; pantothenic acid; folate; and vitamin B_{12}), not all enzyme cofactors are vitamins.[1] Some vitamins function as biological antioxidants (vitamins E and C), and several function as cofactors in metabolic oxidation–reduction reactions (vitamins E, K, and C; niacin; riboflavin; and pantothenic acid). Two vitamins (vitamins A and D) function as hormones; one of them (vitamin A) also serves as a photoreceptive cofactor in vision.

2. VITAMIN: A REVOLUTIONARY CONCEPT

Everyday Word or Revolutionary Idea?

The term *vitamin*, today a common word in everyday language, was born of a revolution in thinking about the interrelationships of diet and health that occurred at the

1. Other enzyme cofactors are biosynthesized, e.g., heme, coenzyme Q, and lipoic acid.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00001-0

beginning of the 20th century. That revolution involved the growing realization of two phenomena that are now taken for granted, even by the nonscientist:

1. Diets are sources of many important nutrients.
2. Insufficient intakes of specific nutrients can cause certain diseases.

In today's world each of these concepts may seem self-evident, but in a world still responding to and greatly influenced by the important discoveries in microbiology made in the 19th century, each represented a major departure from contemporaneous thinking in the area of health. Nineteenth-century physiologists perceived foods and diets as sources of only four types of nutrients: protein, fat, carbohydrate, ash,[2] and water. After all, these accounted for very nearly 100% of the mass of most foods. With this view, it is understandable that, at the turn of the century, experimental findings that now can be seen as indicating the presence of hitherto unrecognized nutrients were interpreted instead as substantiating the presence of natural antidotes to unidentified disease-causing microbes.

Important discoveries in science have ways of directing, even entrapping, one's view of the world; resisting this tendency depends on critical and constantly questioning minds. That such minds were involved in early nutrition research is evidenced by the spirited debates and frequent polemics that ensued over discoveries of apparently beneficial new dietary factors. Still, the systematic development of what emerged as nutritional science depended on a new intellectual construct for interpreting such experimental observations.

Vitamin or Vitamine?

The elucidation of the nature of what was later to be called *thiamin* occasioned the proposition of just such a new construct in physiology.[3] Aware of the impact of what was a departure from prevailing thought, its author, the Polish biochemist Casimir Funk, chose to generalize from his findings on the chemical nature of that "vital amine" to suggest the term **vitamine** as a generic descriptor for many such **accessory factors** associated with diets. That the factors soon to be elucidated comprised a somewhat chemically heterogeneous group, not all of which were nitrogenous, does not diminish the importance of the introduction of what was first presented as the *vitamine theory*, later to become a key concept in nutrition: the vitamin.

The term vitamin has been defined in various ways. While the very concept of a vitamin was crucial to progress in understanding human physiology and nutrition, the actual definition of a vitamin has evolved in consequence of that understanding.

3. AN OPERATING DEFINITION OF A VITAMIN

A vitamin is defined as follows (Fig. 1.1). A vitamin

- is an *organic compound* distinct from fats, carbohydrates, and proteins
- is a *natural component of foods* in which it is usually present in minute amounts
- is essential, also usually in minute amounts, for *normal physiological function* (i.e., maintenance, growth, development, and/or production)
- prevents a *specific deficiency syndrome*, which occurs when it is absent or underutilized
- is *not synthesized by the host* in amounts adequate to meet normal physiological needs.

This definition will be useful in the study of vitamins, as it effectively distinguishes this class of nutrients from others (e.g., proteins and amino acids, essential fatty acids, and minerals) and indicates the needs in various normal physiological functions. It also denotes the specificity of deficiency syndromes by which the vitamins were discovered. Further, it places the vitamins in that portion of the external chemical environment on which animals (including humans) must depend for survival, thus distinguishing vitamins from hormones.

Some Caveats

It will quickly become clear, however, that, despite its utility, this operating definition has limitations, notably with respect to the last clause. Many species can, indeed, synthesize at least some of the vitamins, although not always at the levels required to prevent deficiency disorders. Four examples illustrate this point:

Vitamin C: Most animal species have the ability to synthesize ascorbic acid. Only those few that lack the enzyme L-gulonolactone oxidase (e.g., the guinea pig, humans) cannot. For those species, ascorbic acid is properly be called vitamin C.

Vitamin D: Individuals exposed to modest amounts of sunlight can produce cholecalciferol, which functions as a hormone. Only individuals without sufficient exposure to ultraviolet light (e.g., livestock raised in indoor confinement, people spending most of their days indoors) require dietary sources of vitamin D.

Choline: Most animal species have the metabolic capacity to synthesize choline; however, some (e.g., the chick, the rat) may not be able to employ that capacity if they are fed insufficient amounts of methyl donor compounds. In

2. The residue from combustion, i.e., minerals.
3. This is a clear example of what T.H. Kuhn called a "scientific revolution" (Kuhn, T.H., 1968. *The Structure of Scientific Revolutions.* University of Chicago Press, Chicago, IL.), i.e., the discarding of an old paradigm with the invention of a new one.

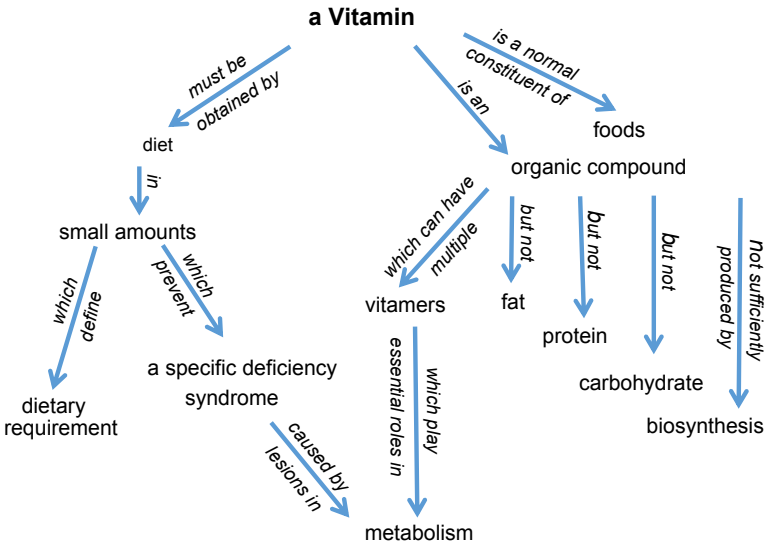

FIGURE 1.1 Concept map of a Vitamin.[4]

addition, some (e.g., the chick) do not develop that capacity completely until they are several weeks of age. Thus, for the young chick and for individuals of other species fed diets providing limited methyl groups, choline is a vitamin.

Niacin: All animal species can synthesize nicotinic acid mononucleotide from the amino acid tryptophan. Only those for which this metabolic conversion is particularly inefficient (e.g., the cat, fishes) and others fed low dietary levels of tryptophan require a dietary source of *niacin*.

With these counterexamples in mind, the definition of a vitamin has specific connotations for animal species, stage of development, diet or nutritional status, and physical environmental conditions.[5]

The "Vitamin Caveat"

- Some compounds are vitamins for one species and not another.
- Some compounds are vitamins only under specific dietary or environmental conditions.

4. THE RECOGNIZED VITAMINS

Thirteen substances or groups of substances are now generally recognized as vitamins (Table 1.1); others have been proposed.[6] In some cases, the familiar name is actually the generic descriptor for a family of chemically related compounds having qualitatively comparable metabolic activities. For example, the term *vitamin E* refers to those analogs of tocol or tocotrienol[7] that are active in preventing such syndromes as fetal resorption in the rat and myopathies in the chick. In these cases, the members of the same vitamin family are called *vitamers*. Some carotenoids can be metabolized to yield the metabolically active form of vitamin A; such a precursor of an actual vitamin is called a **provitamin**.

5. STUDY QUESTIONS AND EXERCISES

1. What are the key features that define a vitamin?
2. What are the fundamental differences between vitamins and other classes of nutrients… between vitamins and hormones?
3. Detail, citing a specific example, a situation in which a vitamin may be nutritionally essential for one species but not another.
4. Using key words and phrases, list briefly what you know about each of the recognized vitamins.

4. The concept map can be a useful device for organizing thought, as its discipline can serve to assist in identifying the nature and extent of concepts related to the one in question. A concept map should be laid out as a hierarchy of related concepts with the superordinate concept at the top and all relationships between concepts identified with a verb phrase. Thus, it can be "read" from top to bottom. One of the authors (GFC) has used concept mapping in graduate-level teaching, both as a group exercise and testing device. For a useful discussion of the educational value of the concept map, the reader is referred to *Learning How to Learn*, 1984, J.D. Novak and D.B. Gowin, Cornell University Press, Ithaca, NY, pp. 199.

5. For this reason, it is correct to refer to vitamin C for the nutrition of humans but ascorbic acid for the nutrition of livestock.

6. These include such factors as inositol, carnitine, bioflavonoids, pangamic acid, and laetrile, for some of which there is evidence of vitamin-like activity (Chapter 19).

7. Tocol is 3,4-dihydro-2-methyl-2-(4,8,12-trimethyltridecyl)-6-chromanol; tocotrienol is the analog with double bonds at the 3, 7, and 11′ positions on the phytol side chain (Chapter 7).

TABLE 1.1 The Vitamins: Their Vitamers, Provitamins, and Functions

Group	Vitamers	Provitamins	Physiological functions
Vitamin A	Retinol Retinal Retinoic acid	β-Carotene Cryptoxanthin	Visual pigments; epithelial cell differentiation
Vitamin D	Cholecalciferol (D_3) Ergocalciferol (D_2)		Calcium homeostasis; bone metabolism; transcription factor
Vitamin E	α-Tocopherol γ-Tocopherol		Membrane antioxidant
Vitamin K	Phylloquinones (K_1) Menaquinones (K_2) Menadione (K_3)		Blood clotting; calcium metabolism
Vitamin C	Ascorbic acid Dehydroascorbic acid		Reductant in hydroxylations in the formation of collagen and carnitine, and in the metabolism of drugs and steroids
Vitamin B_1	Thiamin		Coenzyme for decarboxylations of 2-keto acids (e.g., pyruvate) and transketolations
Vitamin B_2	Riboflavin		Coenzyme in redox reactions of fatty acids and the tricarboxylic acid (TCA) cycle
Niacin	Nicotinic acid Nicotinamide		Coenzyme for several dehydrogenases
Vitamin B_6	Pyridoxol Pyridoxal Pyridoxamine		Coenzyme in amino acid metabolism
Folic acid	Folic acid Polyglutamyl folacins		Coenzyme in single-carbon metabolism
Biotin	Biotin		Coenzyme for carboxylations
Pantothenic acid	Pantothenic acid		Coenzyme in fatty acid metabolism
Vitamin B_{12}	Cobalamin		Coenzyme in the metabolism of propionate, amino acids, and single-carbon units

Chapter 2

Discovery of the Vitamins

Chapter Outline

Anchoring Concepts

1. A scientific theory is a plausible explanation for a set of observed phenomena; because theories cannot be tested directly, their acceptance relies on a preponderance of supporting evidence.
2. A scientific hypothesis is a tentative supposition that is assumed for the purposes of argument or testing and is thus used in the generation of evidence by which theories can be evaluated.
3. An empirical approach to understanding the world involves the generation of theories strictly by observation, whereas an experimental approach involves the undertaking of operations (experiments) to test the truthfulness of hypotheses.
4. Physiology is that branch of biology seeks to elucidate the processes, activities, and phenomena of life and living organisms, while biochemistry seeks to elucidate the molecular bases for such phenomena.
5. The field of nutrition is derived from both of these disciplines; it seeks to elucidate the processes by which animals or plants take in and utilize food substances.

When science is recognized as a framework of evolving concepts and contingent methods for gaining new knowledge, we see the very human character of science, for it is creative individuals operating from the totality of their experiences who enlarge and modify the conceptual framework of science.

J.D. Novak.[1]

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1. Joseph D. Novak (b. 1932) is a prominent American educator known for his research on human learning, knowledge creation, and knowledge representation. Prof. Novak, spent most of his career at Cornell University where he and his colleagues developed the technique of Concept Mapping as a means of representing science knowledge.

LEARNING OBJECTIVES

1. To understand the nature of the process of discovery in the field of nutrition.
2. To recognize the major forces in the emergence of **nutrition science**.
3. To understand the impact of the **vitamine theory**, as an intellectual construct, on that process of discovery.
4. To understand that the discoveries of the vitamins proceeded along indirect lines, most often through the seemingly unrelated efforts of many people.
5. To recognize the key events in the discovery of each of the vitamins.
6. To become familiar with the basic terminology of the vitamins and their associated deficiency disorders.

VOCABULARY

Accessory factor
Anemia
Animal model
Animal protein factor
Ascorbic acid
β-Carotene
Beriberi
Biotin
Black tongue disease
Cholecalciferol
Choline
Dermatitis
Ergocalciferol
Fat-soluble A
Filtrate factor
Flavin

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00002-2

Folic acid
Germ theory
Hemorrhage
Lactoflavin
Niacin
Night blindness
Ovoflavin
Pantothenic acid
Pellagra
Polyneuritis
Prothrombin
Provitamin
Purified diet
Pyridoxine
Retinen
Riboflavin
Rickets
Scurvy
Thiamin
Vitamin A
Vitamin B
Vitamin B complex
Vitamin B_{12}
Vitamin B_2
Vitamin B_6
Vitamin C
Vitamin D
Vitamin E
Vitamin K
Vitamine
Vitamine theory
Water-soluble B
Xerophthalmia

1. THE EMERGENCE OF NUTRITION AS A SCIENCE

In the span of only five decades commencing at the very end of the 19th century, the vitamins were discovered. Their discoveries were the result of the activities of hundreds of people that can be viewed retrospectively as having followed discrete branches of intellectual progress. Those branches radiated from ideas originally derived inductively from observations in the natural world, each starting from the recognition of a relationship between diet and health. Subsequently, branches were pruned through repeated analysis and deduction—a process that both produced and proceeded from the fundamental approaches used in experimental nutrition today. Once pruned, the limb of discovery may appear straight to the naive observer. Scientific discovery, however, does not occur that way; rather, it tends to follow a zigzag course, with many participants contributing many branches. In fact, the contemporaneous view of each participant may be that of a thicket of tangled hypotheses and facts. The seemingly straightforward appearance of the emergent limb of discovery is but an illusion achieved by discarding the dead branches of false starts and unsupported hypotheses, each of which can be instructive about the process of scientific discovery.

With the discovery of the vitamins, therefore, nutrition moved from a largely observational activity to one that relied increasingly on hypothesis testing through experimentation; it moved from empiricism to science. Both the process of scientific discovery and the course of the development of nutrition as a scientific discipline are perhaps best illustrated by the history of the discovery of the vitamins.

2. THE PROCESS OF DISCOVERY IN NUTRITIONAL SCIENCE

Empiricism and Experiment

History demonstrates that the process of scientific discovery begins with the synthesis of general ideas about the natural world from observations of particulars within it—i.e., an *empirical phase*. In the discovery of the vitamins, this initial phase was characterized by the recognition of associations between diet and human diseases, namely night blindness, scurvy, beriberi, rickets, and pellagra, each of which was long prevalent in various societies. The next phase in the process of discovery involved the use of these generalizations to form hypotheses that could be tested experimentally—i.e., the *experimental phase*. In the discovery of the vitamins, this phase necessitated the development of two key tools of modern experimental nutrition: the **animal model** and the **purified diet**. The availability of both of these tools proved to be necessary for the discovery of each vitamin; in cases where an animal model was late to be developed (e.g., for pellagra), the elucidation of the identity of the vitamin was substantially delayed.

3. THE EMPIRICAL PHASE OF VITAMIN DISCOVERY

The major barrier to entering the empirical phase of nutritional inquiry proved to be the security provided by prescientific attitudes about foods that persisted through the 19th century. Many societies had observed that human populations in markedly contrasting parts of the world tended to experience similar health standards despite the fact that they subsisted on very different diets. These observations were taken by 19th-century physiologists to indicate that health was not particularly affected by the kinds of foods consumed. Foods were thought important as sources of the only nutrients known at the time: *protein*, *available energy*, and *ash*. While the "chemical revolution," led by

the French scientist Antoine Lavoisier,[2] started probing the elemental components and metabolic fates of these nutrients, the widely read ideas of the German chemist Justus von Liebig[3] resulted in protein being recognized as the only essential nutrient, supporting both tissue growth and repair as well as energy production. In the middle part of the century, attention was drawn further from potential relationships of diet and health by the major discoveries of Pasteur,[4] Liebig,[5] Koch,[6] and others in microbiology. For the first time, several diseases, first anthrax and then others, could be understood in terms of a microbial etiology. By the end of the century, germ theory, which proved to be of immense value in medicine, directed hypotheses for the etiologies of most diseases. The impact of this understanding as a barrier to entering the inductive phase of nutritional discovery is illustrated by the case of the Dutch physician Christiaan Eijkman,[7] who found a water-soluble factor from rice bran to prevent a beriberi-like disease in chickens (now known to be the vitamin thiamin) and concluded that he had discovered a "pharmacological antidote" against the beriberi "microbe" presumed to be present in rice.

Diseases Linked to Diet

Nevertheless, while they appeared to have little effect on the prevailing views concerning the etiology of human disease, by the late 1800s empirical associations had been made between diet and the diseases scurvy, rickets, pellagra, and night blindness.

Scurvy has been known that scurvy, the disease involving apathy, weakness, sore gums, painful joints, and multiple hemorrhages, could be prevented by including in the diet green vegetables or fruits. Descriptions of cases in such sources as the Eber papyrus (c.1150 BCE) and writings of Hippocrates (c.420 BCE) are often cited to indicate that scurvy was prevalent in those ancient populations. Indeed, signs of the disease are said to have been found in the skeletal remains of primitive humans. Scurvy was common in northern Europe during the Middle Ages, a time when local agriculture provided few sources of vitamin C that lasted through the winter. In northern Europe, it was treated by eating cresses and spruce leaves. Scurvy was very highly prevalent among seamen, particularly those on ocean voyages to Asia during which they subsisted for months at a time on dried and salted foods. The Portuguese explorer Vasco da Gama reported losing more than 60% of his crew of 160 sailors in his voyage around the Cape of Good Hope in 1498. In 1535–1536, the French explorer Jacques Cartier reported that signs of scurvy were present in all but three of his crew of 103 men (25 of whom died) during his second Newfoundland expedition. In 1595–1597, the first Dutch East Indies fleet lost two-thirds of its seamen due to scurvy. In 1593, the British admiral Richard Hawkins wrote that, during his career, he had seen some 10,000 seamen die of the disease.

The link between scurvy and preserved foods was long evident to seafarers. The first report of a cure for the disease appears to have been Cartier's description of the rapidly successful treatment of his crew with an infusion of the bark of Arborvitae (*Thuja occidentalis*) prepared by the indigenous Hurons of Newfoundland. By 1601, the consumption of berries, vegetables, scurvy-grass (*Cochlearia officinalis*, which contains as much ascorbic acid as orange juice), and citrus fruits or juices was recognized as effective in preventing the disease. In that year, the English privateer Sir James Lancaster introduced regular issues of lemon juice (three spoonfuls each morning) on one of his found ships, finding significantly less scurvy among treated sailors. Nevertheless, the prestigious London College of Physicians viewed scurvy as a "putrid" disease in which affected tissues became alkaline and stated that other acids could be as effective as lemon juice in treating the disease. Accordingly, in the mid-1600s British ship's surgeons were supplied with vitriol (dilute sulfuric acid).

Against this background, in 1747, James Lind, a Scottish physician serving in the British Royal Navy, conducted what has been cited as the first controlled clinical trial to compare various therapies recommended for scurvy in British sailors at sea. Lind's report, published 6 years later, described 12 sailors with scurvy whom he assigned in pairs to 2-week regimens including either lemons and oranges, vitriol, vinegar, or other putative remedies. His results were

2. Antoine-Laurent de Lavoisier (1743–1794) is often considered the "father of modern chemistry", as his work changed that science from a qualitative to a quantitative one. He is best known for his discovery of oxygen and its role in combustion.

3. In his widely read book, *Animal Chemistry, or Organic Chemistry in its Application to Physiology and Pathology*, Liebig argued that the energy needed for the contraction of muscles, in which he was able to find no carbohydrate or fat, must come only from the breakdown of protein. Protein, therefore, was the only true nutrient.

4. Louis Pasteur (1822–1895) was a French pioneering microbiologist. He disproved the doctrine of "spontaneous generation" of microbial life and advanced "germ theory." He discovered the principles of vaccination, fermentation and developed the process of heat-killing of microbes in liquids is now called "pasteurization".

5. Justus von Liebig (1803–1873) was a German chemist who made major contributions to agricultural and biological chemistry, elucidated the importance of nitrogen in plant nutrition, and introduced laboratory experience in teaching chemistry.

6. Robert Koch (1843–1910) was a German physician who identified the causative agents of tuberculosis, cholera and anthrax, and formulated the general principles ("Koch's Postulates") for linking specific microorganisms to specific diseases. In 1905, he received the Nobel Prize for Physiology or Medicine.

7. Christiaan Eijkman (1858–1930) was trained in the Netherlands and served as a medical officer in the Dutch Indies. After contracting malaria in 1885, he returned to Amsterdam where he worked in the laboratories of Forster and, then, Kock (Berlin). In Koch's laboratory he met another Dutch physician C.A. Pekelharing whom he assisted in a second period of service in the Indies investigating beriberi. They proposed establishing a medical laboratory of which Eijkman was named director and Director of the Javanese Medical School, which ultimately became the University of Indonesia.

clear: the pair treated with lemons and oranges recovered almost completely within 6 days; whereas, no other treatment resulted in any improvement. In 1753, he published his now-classic work "A Treatise on Scurvy," which had great impact on the medical thought of the time, as it detailed past work on the subject (most of which was anecdotal) and also presented the results of his experiments. Lind believed that citrus contained "a saponaceous, attenuating and resolving virtue" that helped free skin perspiration that had become clogged by sea air; however, his results were taken as establishing the value of fresh fruits in treating the disease. Still, it was not until the 1790s that the British Navy had made it a regular practice to issue daily rations of lemon juice to all seamen—a measure that gave rise to the term "limey"[8] as a slang expression for a British seaman. In the early part of the 19th century, there remained no doubt of a dietary cause and cure of scurvy; even so, it would be more than a century before its etiology and metabolic basis would be elucidated. Outbreaks of scurvy continued in cases of food shortages: in British prisons, during the California gold rush, among troops in the Crimean War, among prisoners in the American Civil War, among citizens during the Siege of Paris in 1871, and among polar explorers in the early 20th century.

It is said that signs consistent with **beriberi** (e.g., initial weakness and loss of feeling in the legs leading to heart failure, breathlessness, and, in some cases, edema) are described in ancient Chinese herbals (~2600 BCE). Certainly, beriberi was an historic disease prevalent in many Asian populations subsisting on diets in which polished (i.e., "white" or dehulled) rice is the major food. For example, in the 1860s, the Japanese navy experienced the disease affecting 30–40% of its seamen. Interesting clinical experiments conducted in the 1870s with sailors by Dr Kanehiro Takaki, a British trained surgeon who later became Director General of the Japanese Naval Medical Service, first noted an association between beriberi and diet: Japanese sailors were issued lower protein diets than their counterparts in European navies, which had not experienced the disease. Takaki conducted an uncontrolled study at sea in which he modified sailors' rations to increase protein intake by including more meat, condensed milk, bread, and vegetables at the expense of rice. This cut both the incidence and severity of beriberi dramatically, which he interpreted as confirmation of the disease being caused by insufficient dietary protein. The adoption of Takaki's dietary recommendations by the Japanese navy was effective—eliminating the disease as a shipboard problem by 1880—despite the fact that his conclusion, reasonable in the light of contemporaneous knowledge, later proved to be incorrect.

Rickets, the disease of growing bones, presents in children as deformations of the long bones (e.g., bowed legs, knock knees, and curvatures of the upper and/or lower arms), swollen joints, and/or enlarged heads. It is generally associated with the urbanization and industrialization of human societies. Its appearance on a wide scale was more recent and more restricted geographically than that of either scurvy or beriberi. The first written account of the disease is believed to be that of Daniel Whistler,[9] who wrote on the subject in his medical thesis in 1645. A complete description of the disease was published shortly thereafter (in 1650) by the Cambridge professor Francis Glisson,[10] so it is clear that by the middle of the 17th-century rickets had become a public health problem in England. However, rickets appears not to have affected earlier societies, at least not on such a scale. Studies in the late 1800s by the Scottish physician T.A. Palm[11] showed that the mummified remains of Egyptian dead bore no signs of the disease. By the latter part of the century, the incidence of rickets among children in London exceeded one-third; by the turn of the century, estimates of prevalence were as high as 80% and rickets had become known as the "English disease." Noting the absence of rickets in southern Europe, Palm in 1888 was the first to point out that rickets was prevalent only where there is relatively little sunlight (e.g., in the northern latitudes). He suggested that sunlight exposure prevented rickets, but others held that the disease had other causes—e.g., heredity or syphilis. Through the turn of the century, much of the Western medical community remained either unaware or skeptical of a food remedy that had long been popular among the peoples of the Baltic and North Sea coasts, and that had been used to treat adult rickets in the Manchester Infirmary by 1848: cod liver oil. Not until the 1920s would the confusion over the etiology of rickets become clear.

Pellagra, the disease characterized by lesions of the skin and mouth, and by gastrointestinal and mental disturbances, also became prevalent in human societies fairly recently. There appears to have been no record of the disease, even in folk traditions, before the 18th century. Its first documented description, in 1735, was that of the Spanish physician Gaspar Casal. His observations were disseminated by the French physician François Thiery, whom he met some years later after having been appointed as physician to the court of King Philip V. In 1755, Thiery published a brief account of Casal's observations in the *Journal de Vandermonde*; this became the first published report on the

8. That lemons were often called *limes* has been a source of confusion to many writers on this topic.

9. Whistler (1619–1684) was an English physician. His thesis at the Royal College of Physicians was the first printed book on rickets.

10. Francis Glisson (1599–1677) was a British physician and anatomist who wrote a text on pediatric rickets.

11. Theobold A. Palm (1849–?) was a Scottish physician born to missionary parents in Ceylon. After studying medicine at Edinburgh University, he served as a medical missionary in Japan, where he noted the absence of rickets, in marked contrast to the prevalence of that condition he found in Britain on his return in 1884. In 1888, he commented on Britain's "want of light" in a letter to the British Medical Journal in which he went on to recommend "the systematic use of sunbaths" as a rickets therapy.

disease. Casal's own description was included in his book on the epidemic and endemic diseases of northern Spain, *Historia Natural y Medico de el Principado de Asturias*, which was published in 1762, i.e., 3 years after his death. Casal regarded the disease, popularly called *mal de la rosa*, as a peculiar form of leprosy. He associated it with poverty and with the consumption of spoiled corn (maize).

In 1771, a similar dermatological disorder was described by the Italian physician Francesco Frapolli. In his work *Animadversiones in Morbum Volgo Pelagrum*, he reported the disease to be prevalent in northern Italy. In that region corn, recently introduced from America, had become a popular crop, displacing rye as the major grain. The local name for the disease was "pelagra," meaning rough skin. There is some evidence that it had been seen as early as 1740. By 1784 the prevalence of pelagra (now spelled pellagra) in that area was so great that a hospital was established in Legano for its treatment. Success in the treatment of pellagra appears to have been attributed to factors other than diet—e.g., rest, fresh air, water, and sunshine. Nevertheless, the disease continued to be associated with poverty and the consumption of corn-based diets.

Following the finding of pellagra in Italy, the disease was reported in France in 1829 by the French physician Jean-Marie Hameau. It was not until 1845 that another French physician Théophile Roussel associated pellagra with Casal's *mal de la rosa* and proposed that these diseases, including a similar disease called *flemma salada*,[12] were related or identical. To substantiate his hypothesis, Roussel spent 7 months of 1847 in the area where Casal had worked in northern Spain[13] investigating *mal de la rosa* cases; on his return, he presented to the French Academy of Medicine evidence in support of his conclusion. Subsequently, pellagra, as it had come to be called, was reported in Romania by Theodari in 1858, and in Egypt by the British physician Pruner-Bey in 1874. It was a curiosity, not to be explained for years, that pellagra was never endemic in the Yucatán Peninsula, where the cultivation of corn originated. The disease was not reported there until 1896.

It is not known how long pellagra had been endemic in the United States; however, it became common early in the 20th century. In 1912, American physician J.W. Babcock examined the records of the state hospital of South Carolina and concluded that the disease had occurred there as early as 1828. It is generally believed that pellagra also appeared during or after the American Civil War (1861–1865), in association with food shortages in the southern states. It is clear from George Searcy's 1907 report to the American Medical Association that the disease was endemic at least in Alabama.[14] By 1909, it had been identified in more than 20 states, several of which had impaneled Pellagra Commissions, and a national conference on the disease was held in South Carolina.

Since it first appeared, pellagra was associated with poverty and with the dependence on corn as the major staple food. Ideas were proffered that it was caused by a toxin associated with spoiled corn, yet by the turn of the century other hypotheses were also popular. These included the suggestion of an infectious agent with, perhaps, an insect vector.

Night blindness, the inability to see under low levels of light, was one of the first recorded medical conditions. Writings of Ancient Greek, Roman, and Arab physicians show that animal liver was known to be effective in both the prevention and cure of the disease. The Eber papyrus (c.1550 BCE)[15] described its treatment by the squeezing of liquid from a lamb's liver (now known to be a good source of vitamin A in well-nourished animals) directly into the eyes of the affected patient. The use of liver for the prevention of night blindness became a part of the folk cultures of most seafaring communities. In the 1860s, the French physicians, Hubbenet and, later, Bitot, each noted the presence of small, foamy white spots on the outer aspects of the conjunctiva of patients with night blindness—those lesions have become known as "Bitot's spots." Corneal ulceration, now known to be a related condition resulting in permanent blindness, was recognized in the 18th and 19th centuries in association with protein energy malnutrition as well as such diseases as meningitis, tuberculosis, and typhoid fever. In Russia, it occurred during long Lenten fasts. In the 1880s, cod liver oil was found to be effective in curing both night blindness and early corneal lesions; by the end of the century, cod liver oil, meat, and milk were used routinely in Europe to treat both conditions. It was not until the early 1900s, however, that the dietary nature of night blindness, and the corneal lesions that typically ensued, was understood—not until the "active lipid" was investigated, i.e., the factor in cod liver oil that supported growth and prevented night blindness and xerophthalmia in the rat.

Ideas Prevalent by 1900

Thus, by the beginning of the 20th century, four different diseases had been linked with certain types of diet. Further,

12. Literally meaning "salty phlegm," this condition involved gastrointestinal signs, delirium, and a form of dementia. It did not, however, occur in areas where maize was the major staple food; this, and disagreement over the similarities of symptoms, caused Roussel's proposal of a relationship between these diseases to be challenged by his colleague Arnault Costallat. From Costallat's letters describing *flemma salada* in Spain in 1861, it is apparent that he considered it to be a form of acrodynia, then thought to be due to ergot poisoning.

13. Casal practiced in the town of Oviedo in the Asturias of northern Spain.

14. Sercy, a physician at the Mount Vernon Insane Hospital in Mobile, Alabama, reported 88 cases of pellagra at that institution in 1906.

15. The Eber Papyrus, named for the German egyptologist who discovered it, is among the oldest extant medical papyri of ancient Egypt. Written in c.1550 BCE, the 20 m long scroll is thought to be copied from earlier texts. It is housed at the University of Liepzig.

TABLE 2.1 Diet–Disease Relationships Recognized by 1900

Disease	Associated Diet	Recognized Prevention
Scurvy	Salted (preserved) foods	Fresh fruits, vegetables
Beriberi	Polished rice-based	Meats, vegetables
Rickets	Few "good" fats	Eggs, cod liver oil
Pellagra	Corn-based	None
Night blindness	None	Cod liver oil

by 1900, it was apparent that at least two, and possibly three, could be cured by changes in diet (Table 2.1).

Other diseases, in addition to those listed in Table 2.1, had been known since ancient times to respond to what is now called diet therapy. Unfortunately, much of this knowledge was overlooked, and its significance was not fully appreciated by a medical community galvanized by the new germ theory of disease. Alternative theories for the etiologies of these diseases were popular. Thus, as the 20th century began, it was widely held that scurvy, beriberi, and rickets were each caused by a bacterium or bacterial toxin rather than by the simple absence of something required for normal health. Some held that rickets might also be due to hypothyroidism, while others thought it to be brought on by lack of exercise or excessive production of lactic acid. These theories died hard and had lingering deaths. In explanation of the lack of interest in the clues presented by the diet–disease associations outlined above, Harris (1955) mused: "Perhaps the reason is that it seems easier for the human mind to believe that ill is caused by some positive evil agency, rather than by any mere absence of any beneficial property."

Limitations of Empiricism

In actuality, the process of discovery of the vitamins had moved about as far as it could in its empirical phase. Further advances in understanding the etiologies of these diseases would require the rigorous testing of the various hypotheses—i.e., entrance into the deductive phase of nutritional discovery. That movement, however, required tools for productive scientific experimentation—tools that had not been available previously.

4. THE EXPERIMENTAL PHASE OF VITAMIN DISCOVERY

In a world where one cannot examine all possible cases (i.e., use strictly inductive reasoning), natural truths can be learned only by inference from premises already known to be true (i.e., through deduction). Both the inductive and deductive approaches may be linked; that is, probable conclusions derived from observation may be used as hypotheses for testing deductively in the process of scientific experimentation.

Requirements of Nutrition Science

For scientific experimentation to yield informative results, it must be both **repeatable** and **relevant**. The value of the first point, **repeatability**, should be self-evident. Inasmuch as natural truths are held to be constant, nonrepeatable results cannot be construed to reveal them. The value of the second point, **relevance**, becomes increasingly important when it is infeasible to test a hypothesis in its real-world context. In such circumstances, it becomes necessary to employ a representation of the context of ultimate interest—a construct known in science as a **model**. Models are born of practical necessity, but they must be developed carefully to serve as analogs of situations that cannot be studied directly.

Defined Diets Provided Repeatability

Repeatability in nutrition experimentation became possible with the use of **diets of defined composition**. The most useful type of defined diet that emerged in nutrition research was the **purified diet**. Diets of this type were formulated using highly refined ingredients (e.g., isolated proteins, refined sugars and starches, refined fats) for which the chemical composition could be tested and quantified. It was the use of defined diets that facilitated experimental nutrition; such diets could be prepared over and over by the same or other investigators to yield comparable results. Results obtained through the use of defined diets were repeatable and, therefore, predictable.

Appropriate Animal Models Provided Relevance

Relevance in nutrition research became possible with the identification of **animal models**[16] appropriate to diseases of interest in human medicine or to physiological processes of

16. In nutrition and other biomedical research, an animal model consists of the experimental production in a conveniently managed animal species of biochemical and/or clinical changes that are comparable to those occurring in another species of primary interest but that may be infeasible, unethical, or uneconomical to study directly. Animal models are, frequently, easily managed and rapidly growing species with small body weights (e.g., rodents, chicks, rabbits); however, they may also be larger species (e.g., monkeys, sheep), depending on the target problem and species they are selected to represent. In any case, background information on the biology and husbandry should be available. The selection and/or development of an animal model should be based primarily on representation of the biological problem of interest without undue consideration of the practicalities of cost and availability.

interest in human medicine or animal production. The first of these was discovered quite by chance by keen observers studying human disease. Ultimately, the use of animal models would lead to the discovery of each of the vitamins, as well as to the elucidation of the nutritional roles and metabolic functions of each of the approximately 40 nutrients. The careful use of appropriate animal models made possible studies that would otherwise be infeasible or unthinkable in human subjects or in other animal species of interest.

Major Forces in the Emergence of Nutritional Science

- Recognition that certain diseases were related to diet
- Development of appropriate animal models
- Use of defined diets

An Animal Model for Beriberi

The analytical phase of vitamin discovery, indeed modern nutrition research itself, was entered with the finding of an animal model for beriberi in the 1890s. In 1886, Dutch authorities sent a commission led by Cornelius Pekelharing to their East Indian colony (now Indonesia) to find the cause of beriberi, which had become such a problem among Dutch soldiers and sailors as to interrupt military operations in Atjeh, Sumatra. Pekelharing took an army surgeon stationed in Batavia (now Jakarta), Christiaan Eijkman, whom he had met when each was on study leave (Pekelharing from his faculty post at the University of Utrecht, and Eijkman as a medical graduate from the University of Amsterdam) in the laboratory of the great bacteriologist, Robert Koch. The team, unaware of Takaki's work, expected to find a bacterium as the cause, and was therefore disappointed, after 8 months of searching, to uncover no such evidence. They concluded, "Beriberi has been attributed to an insufficient nourishment and to misery: but the destruction of the peripheral nervous system on such a large scale is not caused by hunger or grief. The true cause must be something coming from the outside, but is it a poison or an infection?"

However, looking for a poison, they observed, would be very difficult, whereas they had techniques for looking for a microorganism that had been successful for other diseases. Thus, they tried to culture organisms from blood smears from patients and to create the disease in monkeys, rabbits, and dogs by inoculations of blood, saliva, and tissues from patients and cadavers. When single injections produced no effects, they used multiple injection regimens. Despite the development of abscesses at the point of some injections, it appeared that multiple inoculations could produce some nerve degeneration in rabbits and dogs. Pekelharing concluded that beriberi was indeed an infectious disease, but an unusual one requiring repeated reinfection of the host. Before returning to Holland, Pekelharing persuaded the Dutch military to allow Eijkman to continue working on the beriberi problem.

The facilities used by the Commission at the Military Hospital Batavia became a new Laboratory for Bacteriology and Pathology of the colonial government, and Eijkman was named as director, with one assistant. His efforts in 1888 to infect rabbits and monkeys with Pekelharing's *micrococcus* were altogether unsuccessful, causing him to posit that beriberi must require a long time before the appearance of signs. The following year, he started using chickens as his animal model. Later in the year, he noted that many, regardless of whether they had been inoculated, lost weight, and started walking with a staggering gait. Some developed difficulty standing and died. Eijkman noted on autopsy no abnormalities of the heart, brain, or spinal cord, but microscopic degeneration of the peripheral nerves, particularly in the legs. The latter were signs he had observed in people dying of beriberi. He was unable, though, to culture any consistent type of bacteria from the blood of affected animals. It would have been easy for Eijkman to dismiss the thought that this avian disease, which he called "polyneuritis," might be related to beriberi.

Serendipity or a Keen Eye?

After persisting in his flock for some 5 months, the disease suddenly disappeared. Eijkman reviewed his records and found that in June, shortly before the chickens had started to show paralysis, a change in their diet had been occasioned by failure of a shipment of feed grade brown (unpolished) rice to arrive. His assistant had used, instead, white (polished) rice from the hospital kitchen. It turned out that this extravagance had been discovered a few months earlier by a new hospital superintendent, who had ordered it stopped. When Eijkman again fed the chickens brown rice, he found affected animals recovered completely within days.

With this clue, Eijkman immediately turned to the chicken as the animal model for his studies. He found chicks showed signs of **polyneuritis** within days of being fed polished rice, and that their signs disappeared even more quickly if they were then fed unpolished rice. It was clear that there was something associated with rice polishings that protected chickens from the disease. After discussing these results, Eijkman's colleague Adolphe Verdeman, the physician inspector of prisons in the colony, surveyed the use of polished and unpolished rice and the incidence of beriberi among inmates. His results (Table 2.2), later confirmed by others in similar epidemiological investigations, demonstrated the advantage enjoyed by prisoners eating unpolished rice: they were much less likely to contract beriberi. This information, in conjunction with his experimental findings with chickens, allowed Eijkman to investigate, by means of bioassay, the beriberi-protective factor apparently associated with rice husks.

TABLE 2.2 Beriberi in Javanese Prisons c.1890

Diet	Population	Cases	Prevalence (Cases/10,000 People)
Polished rice	150,266	4200	279.5
Partially polished rice	35,082	85	24.2
Unpolished rice	96,530	86	8.9

Antiberiberi Factor Is Announced

Eijkman used this animal model in a series of investigations in 1890–1897 and found that the antipolyneuritis factor could be extracted from rice hulls with water or alcohol, that it was dialyzable, but that it was rather easily destroyed with moist heat. He concluded that the water-soluble factor was a "pharmacological antidote" to the "beriberi microbe," which, although still not identified, he thought to be present in the rice kernel proper. Apparently, Gerrit Grijns,[17] who continued that work after Eijkman returned to Holland, came to interpret these findings somewhat differently. Grijns went on to show that polyneuritis could be prevented by including mung bean (*Vigna radiata*) in the diet; this led to mung beans being found effective in treating beriberi. In 1901, Grijns suggested, for the first time, that beriberi-producing diets "lacked a certain substance of importance in the metabolism of the central nervous system." Subsequently, Eijkman came to share Grijn's view; in 1906, the two investigators published a now-classic paper in which they wrote, "There is present in rice polishings a substance different from protein, and salts, which is indispensable to health and the lack of which causes nutritional polyneuritis."

5. THE VITAMINE THEORY

Defined Diets Revealed Needs for Accessory Factors

The announcement of the antiberiberi factor constituted the first recognition of the concept of the vitamin, although the term itself was yet to be coined. At the time of Eijkman's studies, but a world removed and wholly separate, others were finding that animals would not survive when fed "synthetic" or "artificial" diets formulated with purified fats, proteins, carbohydrates, and salts—i.e., containing all of the nutrients then known to be constituents of natural foods. Such a finding was first reported by the Russian surgeon Nikolai Lunin, in 1888, who found that the addition of milk to a synthetic diet supported the survival of mice. Lunin concluded, "A natural food such as milk must, therefore, contain besides these known principal ingredients small quantities of other and unknown substances essential to life."

Lunnin's finding was soon confirmed by several other investigators. By 1912, Rhömann in Germany, Socin in Switzerland, Pekelharing in The Netherlands, and Hopkins in England had each demonstrated that the addition of milk to purified diets corrected the impairments in growth and survival that were otherwise produced in laboratory rodents. The German physiologist Wilhelm Stepp took another experimental approach. He found it possible to extract, from bread and milk, factors required for animal growth. Although Pekelharing's 1905 observations, published in Dutch, lay unnoticed by many investigators, his conclusions about what Hopkins had called the accessory factor in milk alluded to the modern concept of a vitamin: "If this substance is absent, the organism loses the power properly to assimilate the well known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of foodstuffs, both of vegetable and animal origin."

Perhaps the most important of the early studies with defined diets were those of the Cambridge biochemist Frederick Gowland Hopkins.[18] His studies demonstrated that the growth-promoting activities of accessory factors were independent of appetite, and that such factors prepared from milk or yeast were biologically active in very small amounts.

Two Lines of Inquiry

Therefore, by 1912, two independently developed lines of inquiry had revealed that foods contained beneficial factor(s) in addition to the nutrients known at the time. That these factor(s) were present and active in minute amounts was apparent from the fact that almost all of the mass of food was composed of the known nutrients.

Two Lines of Inquiry Leading to the Discovery of the Vitamins

- The study of substances that prevent deficiency diseases
- The study of accessory factors required by animals fed purified diets.

17. Grijns (1865–1944) was a Dutch physician trained at the University of Utrecht. He assisted Eijkman in Batavia and continued that work when Eijkman, having contracted malaria, returned to Holland in 1896.

18. Sir Frederick Gowland Hopkins (1861–1947), is known for his work at Cambridge University, which involved not only classic work on accessory growth factors (for which he shared, with Christiaan Eijkman, the 1929 Nobel Prize in Medicine or Physiology), but also the discoveries of glutathione and tryptophan.

Comments by Hopkins in 1906 indicate that he saw connections between the accessory factors and the deficiency diseases. On the subject of the accessory growth factors in foods, he wrote, "No animal can live on a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied the animal still cannot flourish. The animal is adjusted to live either on plant tissues or the tissues of other animals, and these contain countless substances other than protein, carbohydrates and fats. In diseases such as rickets, and particularly scurvy, we have had for years knowledge of a dietetic factor; but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure … They are, however, certainly of the kind which comprises these minimal qualitative factors that I am considering."

Hopkins demonstrated the presence of a factor(s) in milk that stimulated the growth of animals fed diets containing all of the then-known nutrients (Fig. 2.1).

The Lines Converge

The discovery by Eijkman and Grijns had stimulated efforts by investigators in several countries to isolate the antiberiberi factor in rice husks. Umetaro Suzuki, of Imperial University Agricultural College in Tokyo, succeeded in preparing a concentrated extract from rice bran for the treatment of polyneuritis and beriberi. He called the active fraction "oryzanin" but could not achieve its purification in crystalline form. Casimir Funk,[19] a chemist at the Lister Institute in London, concluded from the various conditions in which it could be extracted and then precipitated that the antipolyneuritis factor in rice husks was an organic base and, therefore, nitrogenous in nature. When he appeared to have isolated the factor, Funk coined a new word for it, with the specific intent of promoting the new concept in nutrition to which Hopkins had alluded. Having evidence that the factor was an organic base, and therefore an *amine*, Funk chose the term **vitamine**[20] because it was clearly *vital*, i.e., pertaining to life.

Funk's Theory

In 1912, Funk published his landmark paper presenting the **vitamine theory**; in it he proposed, in what some have referred to as a leap of faith, four different vitamines. That the concept was not a new one, and that not all of these factors later proved to be amines (hence, the change to **vitamin**[21]) are far less important than the focus the newly coined term gave to the diet–health relationship. Funk was not unaware of the importance of the term itself; he wrote, "I must admit that when I chose the name "vitamine" I was well aware that these substances might later prove not all to be of an amine nature. However, it was necessary for me to use a name that would sound well and serve as a 'catch-word.'"[22]

Funk's Vitamines

- Antiberiberi vitamine
- Antirickets vitamine
- Antiscurvy vitamine
- Antipellagra vitamine

Impact of the New Concept

The vitamine theory opened new possibilities in nutrition research by providing a new intellectual construct for interpreting observations of the natural world. No longer was the elucidation of the etiologies of diseases to be constrained by the germ theory. Thus, Funk's greatest contribution involves not the data generated in his laboratory, but rather the theory produced from his thoughtful review of information already in the medical literature of the time. This fact caused Harris (1955) to observe, "The interpreter may be as useful to science as the discoverer. I refer here to any man[23] who is able to take a broad view of what has already been done by others, to collect evidence and discern through it all some common connecting link."

The real impact of Funk's theory was to provide a new concept for interpreting diet-related phenomena. As the educational psychologist Novak[24] observed more recently, "As our conceptual and emotional frameworks change, we see different things in the same material."

Still, it was not clear by 1912 whether the accessory factors were the same as the vitamines. In fact, until 1915, there was a considerable debate concerning whether the growth factor for the rat was a single or multiple entity (it was already clear that there was more than one vitamine). Some investigators were able to demonstrate it in yeast and not butter; others found it in butter and not yeast. Some showed it to be identical with the antipolyneuritis factor; others showed that it was clearly different.

19. Funk (1884–1957) was born in Poland and studied in Switzerland, Paris and Berlin.
20. Harris (1955) reported that the word *vitamine* was suggested to Funk by his friend, Dr Max Nierenstein, Reader in Biochemistry at the University of Bristol.
21. The dropping of the *e* from *vitamine* is said to have been the suggestion of J.C. Drummond.
22. Funk, C. (1912). The etiology of the deficiency diseases. *J. State Med.* 20, 341–368.
23. Harris's word choice reveals him as a product of his times. Because it is clear that the process of intellectual discovery to which Harris refers does not recognize gender, it is more appropriate to read this word as *person*.
24. Novak, J.D. (1977) "A Theory of Education," Cornell University Press, Ithaca, NY.

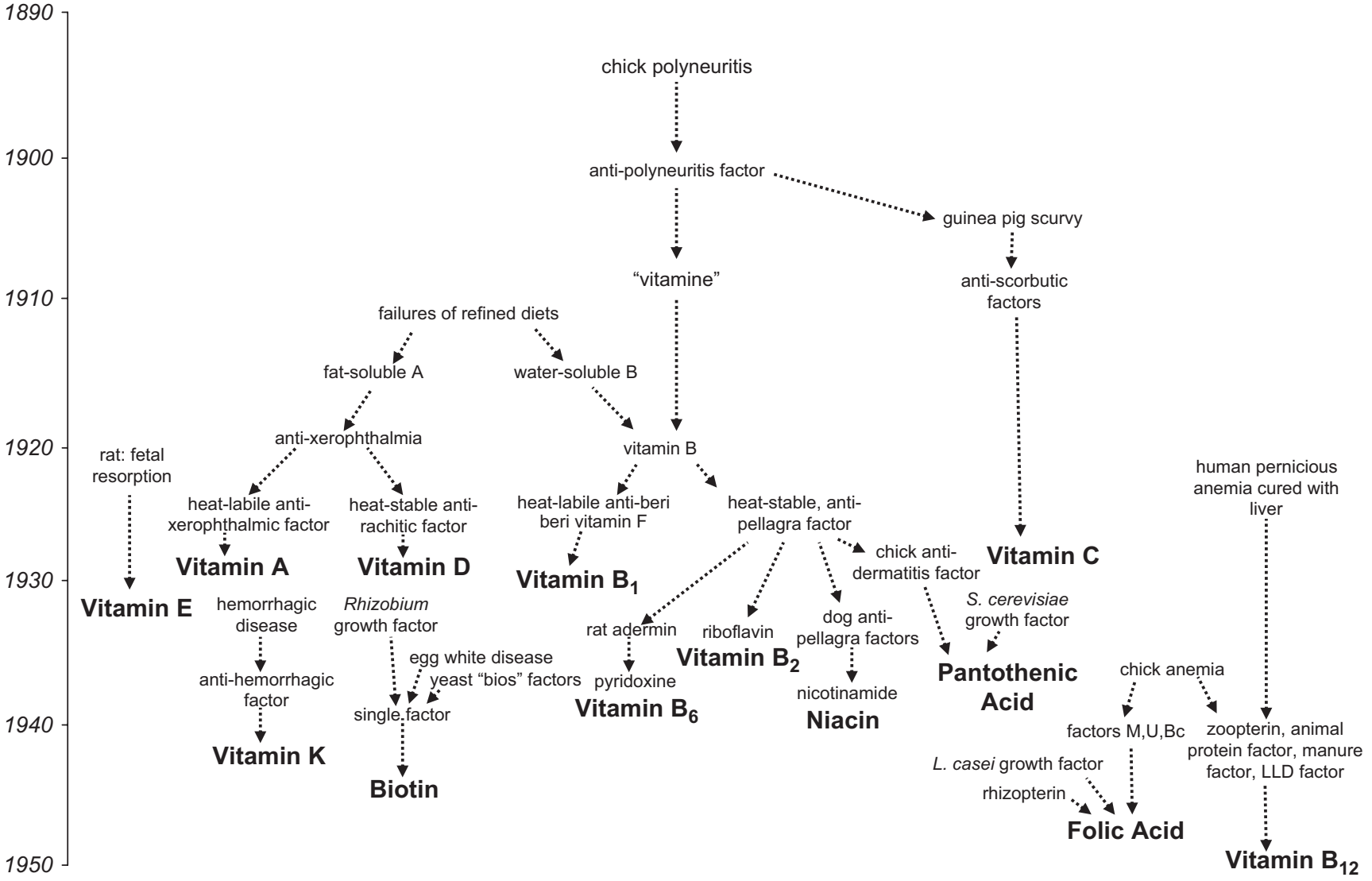

FIGURE 2.1 The cascade of vitamin discovery.

TABLE 2.3 McCollum's Rat Growth Factors

Factor	Found in	Not Found in
Fat-soluble A	Milk fat, egg yolk	Lard, olive oil
Water-soluble B	Wheat, milk, egg yolk	Polished rice

There Is More Than One Accessory Factor

The debate was resolved by the landmark studies of the American biochemist Elmer McCollum[25] and his volunteer assistant Marguerite Davis[26] at the University of Wisconsin in 1913–1915. Using diets based on casein and lactose, they demonstrated that at least two different additional growth factors were required to support normal growth of the rat. One factor could be extracted with ether from egg or butterfat (but not olive or cottonseed oils) but was nonsaponifiable; it appeared to be the same factor shown earlier by Wilhelm Stepp,[27] and by Thomas Osborne[28] and Lafayette Mendel[29] in the same year, to be required to sustain growth of the rat. The second factor was extractable with water and prevented polyneuritis in chickens and pigeons. McCollum called these factors **fat-soluble A** and **water-soluble B**, respectively (Table 2.3).

Accessory Factors Prevent Disease

Subsequent studies conducted by McCollum's group showed that the ocular disorders (i.e., **xerophthalmia**[30]) that developed in rats, dogs, and chicks fed fat-free diets could be prevented by feeding them cod liver oil, butter, or preparations of fat-soluble A, which then became known as the **antixerophthalmic factor**. Shortly, it was found that the so-called water-soluble B material was not only required for normal growth of the rat but also prevented polyneuritis in the chick. Therefore, it was clear that water-soluble B was identical to or at least contained Funk's antiberiberi vitamine; hence, it became known as **vitamine B**.

Accessory Factors Are the Same as Vitamines

With these discoveries, it became apparent that the biological activities of the accessory factors and the vitamines were likely to be due to the same compounds. The concept of a vitamine was thus generalized to include nonnitrogenous compounds, and the antipolyneuritis vitamine became **vitamin B**.

Elucidation of the Vitamines

So it was, through the agencies of several factors, a useful new intellectual construct, the use of defined diets, and the availability of appropriate animal models, that nutrition emerged as a scientific discipline. By 1915, thinking about diet and health had been forever changed, and it was clear that the earlier notions about the required nutrients had been incomplete. Therefore, it should not be surprising to find, by the 1920s, mounting interest in the many questions generated by what had become sound nutritional research. That interest and the further research activity it engendered resulted, over the brief span of only five decades, in the development of a fundamental understanding of the identities and functions of about 40 nutrients, one-third of which are considered vitamins.

Crooked Paths to Discovery

The paths leading to the discovery of the vitamins wandered from Java with the findings of Eijkman in the 1890s, to England with Funk's theory in 1912, to the United States with the recognition of fat-soluble A and water-soluble B in 1915. By that time the paths had already branched, and for the next four decades they would branch again and again as scientists from many laboratories and many nations would pursue many unexplained responses to diet among many types of animal model. Some of these pursuits appeared to fail; however, in aggregate, all laid the groundwork of understanding on which the discoveries of those factors now recognized to be vitamins were based. When viewed in retrospect, the path to that recognition may seem deceptively straight—but it most definitely was not. The way was branched and crooked; in many cases, progress was made by several different investigators traveling in apparently different directions. The following recounts the highlights of the exciting search for the elucidation of the vitamins.

25. Elmer Verner McCollum (1879–1967) received his doctorate at Yale and worked on dietary protein quality with Osborne and Mendel there. In 1909, he joined the faculty of the University of Wisconsin, where his work on growth-promoting factors in deproteinized milk led to the recognition of vitamin A. In 1917, he moved to Johns Hopkins University. McCollum opposed Funk's term "vitamine" on the basis that all essential nutrients were vital.
26. Marguerite Davis (1887–1967) was a graduate student with McCollum. When she and McCollum had shown that water-soluble B was not a single compound, she gave the components letter names, thus, starting that tradition of naming vitamins.
27. Wilhelm Stepp (1882–1964) was a professor of medicine at several German Universities (Strassburg, Jena, Breslau and Munich).
28. Thomas Burr Osborne (1859–1929) was a professor of chemistry who spent his career at the Connecticut Agricultural Experiment Station studying protein quality and nutritional requirements. His collaboration with Mendel led to the recognition of the essentiality of amino acids.
29. Lafayette Benedict Mendel (1872–1935) was a professor of physiological chemistry at Yale University who worked with Osborne to determine why rats could not survive on diets of only purified carbohydrates, fats and proteins.
30. Xerophthalmia, from the Greek *xeros* ("dry") and *ophthalmos* ("eye"), involves dryness of the eyeball owing to atrophy of the periocular glands, hyperkeratosis of the conjunctiva, and, ultimately, inflammation and edema of the cornea, which leads to infection, ulceration, and blindness.

6. ELUCIDATION OF THE VITAMINS

New Animal Model Reveals New Vitamin: "C"

Eijkman's report of polyneuritis in the chicken and an animal model for beriberi stimulated researchers Axel Holst and Theodor Frölich at the University of Christiana in Oslo, who were interested in **shipboard beriberi**, a common problem among Norwegian seamen. Working with pigeons, they found a beriberi diet to produce the polyneuritis described by Eijkman; however, they considered that condition very different from the disease of sailors. In 1907, they attempted to produce the disease in another experimental animal species: the common Victorian household pet, the guinea pig. Contrary to their expectations, they failed to produce, by feeding that species a cereal-based diet, anything resembling beriberi; instead, they observed the familiar signs of scurvy. Eijkman's work suggested to them that, like beriberi, scurvy too might be due to a dietary deficiency. Having discovered, quite by chance, one of the few possible animal species in which scurvy could be produced,[31] Holst and Frölich had produced something of tremendous value—an animal model of scurvy[32]—showing that lesions could be prevented by feeding apples, (unboiled) cabbage, potatoes, and lemon juice.

This finding led Henriette Chick and Ruth Skelton of the Lister Institute, in the second decade of the 20th century, to use the guinea pig to develop a bioassay for the determination of the antiscorbutic activity in foods, and S.S. Zilva and colleagues (also at the Lister Institute) to isolate from lemons the crude factor that had come to be known as **vitamin C**. It was soon found that vitamin C could reduce the dye 2,6-dichloroindophenol, but the reducing activity determined with that reagent did not always correlate with the antiscorbutic activity determined by bioassay. Subsequently, it was found that the vitamin was reversibly oxidized, but that both the reduced and oxidized forms had antiscorbutic activity.

In 1932, Albert Szent-Györgi, a Hungarian scientist working in Hopkins' laboratory at Cambridge University, and Glen King at the University of Pittsburgh established that the antiscorbutic factor was identical with the reductant *hexuronic acid*,[33] now called **ascorbic acid**. Szent-Györgi had isolated it in crystalline form from adrenal cortex, while King had isolated it from cabbage and citrus juice.[34] After Szent-Györgi returned to Hungary to take a professorship, he was joined by an American-born Hungarian, J. Svirbely, who had been working in King's laboratory. Szent-Györgi had isolated c.500 grams of crystalline hexuronic acid from peppers, and then 25 g of the vitamin from adrenal glands, making samples available to other laboratories. On April 1, 1932, King and Waugh reported that their crystals protected guinea pigs from scurvy; 2 weeks later, Svirbely and Szent-Györgi reported virtually the same results. The following year, the chemical structure of ascorbic acid was elucidated by the groups of Haworth in Birmingham and Karrer in Zurich, both of which also achieved its synthesis.

Fat-Soluble A: Actually Two Factors

Pursuing the characterization of fat-soluble A, by 1919 McCollum's group[35] and others had found that, in addition to supporting growth for the rat, the factor also prevented xerophthalmia and night blindness in that species. In 1920, Drummond called the active lipid **vitamin A**.[36] This factor was present in cod liver oil, which at the turn of the century had been shown to prevent both xerophthalmia and night blindness—which Bitot, some 40 years earlier, had concluded had the same underlying cause.

Vitamin A Prevents Rickets?

Undoubtedly influenced by the recent recognition of vitamin A, Edward Mellanby, who had worked with Hopkins, undertook to produce a dietary model of rickets. For this he used puppies, which the Scottish physician Findley found developed rickets if kept indoors.[37] Mellanby fed a low-fat diet based on oatmeal with limited milk intake to puppies that he kept indoors; the puppies developed the

31. Their finding was, indeed, fortuitous, as vitamin C is now known to be an essential dietary nutrient only for the guinea pig, primates, fishes, some fruit-eating bats, and some passeriform birds. Had they used the rat, the mouse or the chick in their study, vitamin C might have remained unrecognized for perhaps quite a while.

32. In fact, scorbutic signs had been observed in the guinea pig more than a decade earlier, when a U.S. Department of Agriculture pathologist noted in an annual report: "When guinea pigs are fed with cereals (bran and oats mixed), without any grass, clover or succulent vegetables, such as cabbage, a peculiar disease, chiefly recognizable by subcutaneous extravasation of blood, carries them off in four to eight weeks." That this observation was not published for a wider scientific audience meant that it failed to influence the elucidation of the etiology of scurvy.

33. It is said that when Szent-Györgi first isolated the compound, he was at a loss for a name for it. Knowing it to be a sugar, but otherwise ignorant of its identity, he proposed the name *ignose*, which was disqualified by an editor who did not appreciate the humor of the Hungarian chemist. Ultimately, the names **ascorbic acid** and **vitamin C**, by which several groups had come to refer to the antiscorbutic factor, were adopted.

34. The reports of both groups (King, C.G., Waugh, W.S., 1932. Science 75, 357–358; Svirbely, J.L., Szent-Györgi, A., 1932. Biochem. J. 26, 865–870) appeared within 2 weeks of one another in 1932. In fact, Svirbely had recently joined Szent-Györgi's group, having come from King's laboratory. In 1937, King and Szent-Györgi shared the Nobel Prize for their work in the isolation and identification of vitamin C.

35. In 1917, McCollum moved to the newly established School of Public Health at Johns Hopkins University.

36. In 1920, J.C. Drummond proposed the use of the names *vitamin A* and *vitamin B* for McCollum's factors, and the use of the letters C, D, etc., for any vitamins subsequently to be discovered.

37. Exposing infants to sunlight is a traditional practice in many cultures and had been a folk treatment for rickets in northern Europe.

marked skeletal deformities characteristic of rickets. When he found that these deformities could be prevented by feeding cod liver oil or butterfat without allowing the puppies outdoors, he concluded that rickets, too, was caused by a deficiency of vitamin A, which McCollum had discovered in those materials.

New Vitamin: "D"

McCollum, however, suspected that the antirachitic factor present in cod liver oil was different from vitamin A. Having moved to the Johns Hopkins University in Baltimore, he conducted an experiment in which he subjected cod liver oil to aeration and heating (100°C for 14 h), after which he tested its antixerophthalmic and antirachitic activities with rat and chick bioassays, respectively. He found that heating had destroyed the antixerophthalmic (vitamin A) activity, but that cod liver oil had retained antirachitic activity. McCollum called the heat-stable factor **vitamin D**.

β-Carotene, a Provitamin

At about the same time (1919), Steenbock in Wisconsin pointed out that the vitamin A activities of plant materials seemed to correlate with their contents of yellow pigments. He suggested that the plant pigment **carotene** was responsible for the vitamin A activity of such materials. Yet the vitamin A activity in organic extracts of liver was colorless. Therefore, Steenbock suggested that carotene could not be vitamin A, but that it may be converted metabolically to the actual vitamin. This hypothesis was not substantiated until 1929, when von Euler and Karrer in Stockholm demonstrated growth responses to carotene in rats fed vitamin A-deficient diets. Further, Moore in England demonstrated, in the rat, a dose–response relationship between dietary β-carotene and hepatic vitamin A concentration. This proved that β-carotene is, indeed, a **provitamin**.

Vitamin A Linked to Vision

In the early 1930s, the first indications of the molecular mechanism of the visual process were produced by George Wald, of Harvard University but working in Germany at the time, who isolated the chromophore **retinen** from bleached retinas.[38] A decade later, Morton in Liverpool found that the chromophore was the aldehyde form of vitamin A—**retinaldehyde**. Just after Wald's discovery, Karrer's group in Zurich elucidated the structures of both β-carotene and vitamin A. In 1937, Holmes and Corbett succeeded in crystallizing vitamin A from fish liver. In 1942, Baxter and Robeson crystallized *retinol* and several of its esters; in 1947, they crystallized the 13-*cis*-isomer. Isler's group in Basel achieved the synthesis of retinol in the same year and that of β-carotene 3 years later.

The Nature of Vitamin D

McCollum's discovery of the antirachitic factor he called vitamin D in cod liver oil, which was made possible through the use of animal models, was actually a *rediscovery*, as that material had been long recognized as an effective medicine for rickets in children. Still, the nature of the disease was the subject of considerable debate, particularly after 1919, when Huldschinsky, a physician in Vienna, demonstrated the efficacy of ultraviolet light in healing rickets. This confusion was clarified by the findings in 1923 of Goldblatt and Soames, who demonstrated that when livers from rachitic rats were irradiated with ultraviolet light, they could cure rickets when fed to rachitic, nonirradiated rats. The next year, Steenbock's group demonstrated the prevention of rickets in rats by ultraviolet irradiation of either the animals themselves *or* their food. Further, the light-produced antirachitic factor was associated with the fat-soluble portion of the diet.[39]

Vitamers D

The ability to produce vitamin D (which could be bioassayed using both rat and chick animal models) by irradiating lipids led to the finding that large quantities of the vitamin could be produced by irradiating plant sterols. This led Askew's and Windaus's groups, in the early 1930s, to the isolation and identification of the vitamin produced by irradiation of **ergosterol**. Steenbock's group, however, found that while the rachitic chick responded appropriately to irradiated products of cod liver oil or the animal sterol **cholesterol**, that animal did *not* respond to the vitamin D so produced from ergosterol. On the basis of this apparent lack of equivalence, Wadell suggested in 1934 that the irradiated products of ergosterol and cholesterol were different. Subsequently, Windaus's group synthesized 7-dehydrocholesterol and isolated a vitamin D-active product of its irradiation. In 1936, they reported its structure, showing it to be a side chain isomer of the form of the vitamin produced from plant sterols. Thus, two forms of vitamin D were found: **ergocalciferol** (from ergosterol), which was called vitamin D_2,[40] and **cholecalciferol** (from cholesterol), which

38. For this and other discoveries of the basic chemical and physiological processes in vision, George Wald was awarded, with Haldan K. Hartline (of the United States) and R. Grant (of Sweden), the Nobel Prize in Chemistry in 1967.

39. This discovery, i.e., that by ultraviolet irradiation it was possible to induce vitamin D activity in such foods as milk, bread, meats, and butter, led to the widespread use of this practice, which has resulted in the virtual eradication of rickets as a public health problem.

40. Windaus's group had earlier isolated a form of the vitamin he had called vitamin D_1, which had turned out to be an irradiation-breakdown product, **lumisterol**.

was called vitamin D_3. While it was clear that the vitamers D had important metabolic roles in calcification, insights concerning the molecular mechanisms of the vitamin would not come until the 1960s. Then, it became apparent that neither vitamer was metabolically active per se; each is converted in vivo to metabolites that participate in a system of calcium homeostasis that continues to be of great interest to the biomedical community. With this understanding, it became apparent that vitamin D_3 was actually a steroid hormone.[41]

Multiple Identities of Water-Soluble B

By the 1920s, it was apparent that the antipolyneuritis factor, called water-soluble B and present in such materials as yeasts, was not a single substance. This was demonstrated by the finding that fresh yeast could prevent both beriberi and pellagra. However, the antipolyneuritis factor in yeast was unstable to heat, while such treatment did not alter the efficacy of yeast to prevent **dermatitis** in rodents. This caused Goldberger to suggest that the then-called vitamin B was actually at least *two* vitamins: the antipolyneuritis vitamin and a new antipellagra vitamin.

In 1926, the heat-labile antipolyneuritis/beriberi factor was first crystallized by Jansen and Donath, working in the Eijkman Institute (which replaced Eijkman's simple facilities) in Batavia. They called the factor *aneurin*. Their work was facilitated by the use of the small rice bird (*Munia maja*) as an animal model in which they developed a rapid bioassay for antipolyneuritic activity.[42] Six years later, Windaus's group isolated the factor from yeast, perhaps the richest source of it. In the same year (1932), the chemical structure was determined by R.R. Williams, who named it **thiamin**—i.e., the vitamin containing sulfur (*thios*, in Greek). Noting that deficient subjects showed high blood levels of pyruvate and lactate after exercise, in 1936 Rudolph Peters of Oxford University used, for the first time, the term "biochemical lesion" to describe the effects of the dietary deficiency. Shortly thereafter, methods of synthesis were achieved by several groups, including those of Williams, Andersag and Westphal, and Todd. In 1937, thiamin diphosphate (thiamin pyrophosphate) was isolated by Lohmann and Schuster, who showed it to be identical to the *cocarboxylase* that had been isolated earlier by Auhagen. That many research groups were actively engaged in the research on the antipolyneuritis/beriberi factor is evidence of intense international interest due to the widespread prevalence of beriberi.

The characterization of thiamin clarified the distinction of the antiberiberi factor from the antipellagra activity. The latter was not found in maize (corn), which contained appreciable amounts of thiamin. Goldberger called the two substances the "A-N factor" (antineuritic) and the "P-P factor" (pellagra-preventive). Others called these factors vitamins F (for Funk) and G (for Goldberger), respectively, but these terms did not last.[43] By the mid-1920s the terms **vitamin B_1** and ***vitamin B_2*** had been rather widely adapted for these factors, respectively; this practice was codified in 1927 by the Accessory Food Factors Committee of the British Medical Research Council.

Vitamin B_2: A Complex of Several Factors

That the thermostable second nutritional factor in yeast, which by that time was called vitamin B_2, was not a single substance, and was not immediately recognized, giving rise to considerable confusion and delay in the elucidation of its chemical identity (identities). It should be noted that efforts to fractionate the heat-stable factor were guided almost exclusively by bioassays with experimental animal models. Yet, different species yielded discrepant responses to preparations of the factor. When such variation in responses among species was finally appreciated, it became clear that vitamin B_2 actually included *several* heat-stable factors. Vitamin B_2, as then defined, was indeed a complex.

Components of the Vitamin B_2 Complex

- The P-P factor (preventing pellagra in humans and pellagra-like diseases in dogs, monkeys, and pigs)
- A growth factor for the rat
- A pellagra-preventing factor for the rat
- An antidermatitis factor for the chick

Vitamin B_2 Complex Yields Riboflavin

The first substance in the vitamin B_2 complex to be elucidated was the heat-stable, water-soluble rat growth factor, which was isolated by Kuhn, György, and Wagner-Jauregg at the Kaiser Wilhelm Institute in 1933. Those investigators found that thiamin-free extracts of autoclaved yeast, liver, or rice bran prevented the growth failure of rats fed a thiamin-supplemented diet. Further, they noted that a yellow-green fluorescence in each extract promoted rat growth, and that

41. 1,25-dihydroxycholecalciferol meets the standard definition of a hormone in as much as it is produced and transported through the circulation to exert biological activity in distal organs.

42. The animals, which consumed only 2 grams of feed daily, showed a high (98+%) incidence of polyneuritis within 9–13 days if fed white polished rice. The delay of onset of signs gave them a useful bioassay of antipolyneuritic activity suitable for use with small amounts of test materials. This point is not trivial, inasmuch as there is only about a teaspoon of thiamin in a ton of rice bran. The bioassay of Jansen and Donath was sufficiently responsive for 10 µg of active material to be curative.

43. In fact, the name *vitamin F* was later used, with some debate as to the appropriateness of the term, to describe essential fatty acids. The name *vitamin G* has been dropped completely.

the intensity of fluorescence was proportional to the effect on growth. This observation enabled them to develop a rapid chemical assay that, in conjunction with their bioassay, they exploited to isolate the factor from egg white in 1933. They called it **ovoflavin**. The same group then isolated, by the same procedure, a yellow-green fluorescent growth-promoting compound from whey (which they called **lactoflavin**). This procedure involved the adsorption of the active factor on fuller's earth,[44] from which it could be eluted with base.[45] At the same time, Ellinger and Koschara, at the University of Düsseldorf, isolated similar substances from liver, kidney, muscle, and yeast, and Booher in the United States isolated the factor from whey. These water-soluble growth factors became designated as **flavins**.[46] By 1934, Kuhn's group had determined the structure of the so-called flavins. These substances were thus found to be identical; because each contained a ribose-like (ribotyl) moiety attached to an isoalloxazine nucleus, the term **riboflavin** was adopted. Riboflavin was synthesized by Kuhn's group (then at the University of Heidelberg) and by Karrer's group at Zurich in 1935. As the first component of the vitamin B_2 complex, it is also referred to as vitamin B_2; however, that should not be confused with the earlier designation of the P-P factor.

Vitamin B_2 Complex Yields Niacin

Progress in the identification of the P-P factor was retarded by two factors: the pervasive influence of the germ theory of disease and the lack of an animal model. The former made acceptance of evidence suggesting a nutritional origin of the disease a long and difficult undertaking. The latter precluded the rigorous testing of hypotheses for the etiology of the disease in a timely and highly controlled manner. These challenges were met by Joseph Goldberger, a U.S. Public Health Service bacteriologist who, in 1914, was put in charge of the Service's pellagra program.

Pellagra: An Infectious Disease?

Goldberger's first study[47] is now a classic. He studied a Jackson, Mississippi, orphanage in which pellagra was endemic. He noted that whereas the disease was prevalent among the inmates, it was absent among the staff, including the nurses and physicians who cared for patients; this suggested to him that pellagra was not an infectious disease. Noting that the food available to the professional staff was much different from that served to the inmates (the former included meat and milk not available to the inmates), Goldberger suspected that an unbalanced diet was responsible for the disease. He secured funds to supply meat and milk to inmates for a 2-year period of study. The results were dramatic: pellagra soon disappeared, and no new cases were reported for the duration of the study. However, when funds expired at the end of the study and the institution was forced to return to its former meal program, pellagra reappeared. While the evidence from this uncontrolled experiment galvanized Goldberger's conviction that pellagra was a dietary disease, it was not sufficient to affect a medical community that thought the disease likely to be an infection.

Over the course of two decades, Goldberger worked to elucidate the dietary basis of pellagra. Among his efforts to demonstrate that the disease was not infectious was the exposure, by ingestion and injection, of himself, his wife, and 14 volunteers to urine, feces, and biological fluids from **pellagrins**.[48] He also experimented with 12 male prisoners who volunteered to consume a diet (based on corn and other cereals, but containing no meat or dairy products) that he thought might produce pellagra: within 5 months half of the subjects had developed dermatitis on the scrotum, and some also showed lesions on their hands.[49] The negative results of these radical experiments, plus the finding that therapy with oral supplements of the amino acids cysteine and tryptophan was effective in controlling the disease, led, by the early 1920s, to the establishment of a dietary origin of pellagra. Further progress was hindered by the lack of an appropriate animal model. Although pellagra-like diseases had been identified in several species, most proved not to be useful as biological assays (indeed, most of these later proved to be manifestations of deficiencies of other vitamins of the B_2 complex and to be wholly unrelated to pellagra in humans).

The identification of a useful animal model for pellagra came from Goldberger's discovery in 1922 that maintaining dogs on diets essentially the same as those associated with human pellagra resulted in the animals developing a necrotic degeneration of the tongue called **black tongue disease**. This animal model for the disease led to the final solution of the problem.

44. Floridin, a nonplastic variety of kaolin containing an aluminum magnesium silicate. The material is useful as a decolorizing medium. Its name comes from an ancient process of cleaning or *fulling* wool, in which a slurry of earth or clay was used to remove oil and particulate dirt.

45. By this procedure, the albumen from 10,000 eggs yielded c.30 mg of riboflavin.

46. Initially, the term **flavin** was used with a prefix that indicated the source material; for example, ovoflavin, hepatoflavin, and lactoflavin designated the substances isolated from egg white, liver, and milk, respectively.

47. See the listing of papers of key historical significance, in Recommended Reading at the end of this chapter.

48. People with pellagra.

49. Goldberger conducted this study with the approval of prison authorities. As compensation for participation, volunteers were offered release at the end of the 6 mo. experimental period, which each exercised upon the conclusion of the study without evaluation. For that reason, Goldberger was unable to demonstrate to a doubting medical community that the unbalanced diet had, indeed, produced pellagra.

Impact of an Animal Model for Pellagra

This finding made possible experimentation that would lead rather quickly to an understanding of the etiology to the disease. Goldberger's group soon found that yeast, wheat germ, and liver would prevent canine black tongue and produce dramatic recoveries in pellagra patients. By the early 1930s, it was established that the human pellagra and canine black tongue curative factor was heat-stable and could be separated from the other B_2 complex components by filtration through fuller's earth, which adsorbed only the latter. Thus, the P-P factor became known as the *filtrate factor*. In 1937, Elvehjem isolated *nicotinamide* from liver extracts that had high antiblack tongue activity and showed that nicotinamide and **nicotinic acid** each cured canine black tongue. Both compounds are now called **niacin**. In the same year, several groups went on to show the curative effect of nicotinic acid against human pellagra.

It is ironic that the antipellagra factor was already well known to chemists of the time. Some 70 years earlier, the German chemist Huber had prepared nicotinic acid by the oxidation of nicotine with nitric acid. Funk had isolated the compound from yeast and rice bran in his search for the antiberiberi factor; however, because it had no effect on beriberi, nicotinic acid remained, for two decades, an entity with unappreciated biological importance. This view changed in the mid-1930s, when Warburg and Christiaan isolated nicotinamide from the hydrogen-transporting coenzymes I and II,[50] giving the first clue to its importance in metabolism. Within a year, Elvehjem had discovered its nutritional significance.

B_2 Complex Yields Pyridoxine

During the course of their work leading to the successful isolation of riboflavin, Kuhn and colleagues noticed an anomalous relationship between the growth-promoting and fluorescence activities of their extracts: the correlation of the two activities diminished at high levels of the former. Further, the addition of nonfluorescent extracts was necessary for the growth-promoting activity of riboflavin. They interpreted these findings as evidence for a second component of the heat-stable complex—one that was removed during the purification of riboflavin. These factors were also known to prevent dermatoses in the rat, an activity called **adermin**; however, the lack of a critical assay that could differentiate between the various components of the B_2 complex led to a considerable confusion.

In 1934, György proffered a definition of what he called **vitamin B_6 activity**[51] as the factor that prevented what had formerly been called **acrodynia** or **rat pellagra**, which was a symmetrical florid dermatitis spreading over the limbs and trunk, with redness and swelling of the paws and ears. His definition effectively distinguished these signs from those produced by riboflavin deficiency, which involves lesions on the head and chest, and inflammation of the eyelids and nostrils. The focus provided by György's definition strengthened the use of the rat in the bioassay of vitamin B_6 activity by clarifying its end point. Within 2 years, partial purification of **vitamin B_6** had been achieved by his group; and in 1938 (only 4 years after the recognition of the vitamin), the isolation of vitamin B_6 in crystalline form was achieved by five research groups. The chemical structure of the substance was quickly elucidated as 3-hydroxy-4,5-bis-(hydroxymethyl)-2-methylpyridine. In 1939, Folkers achieved the synthesis of this compound, which György called **pyridoxine**.

B_2 Complex Yields Pantothenic Acid

In the course of studying the growth factor called vitamin B_2, Norris and Ringrose at Cornell described, in 1930, a pellagra-like syndrome of the chick. The lesions could be prevented with aqueous extracts of yeast or liver, then recognized to contain the B_2 complex. In studies of B_2 complex-related growth factors for chicks and rats, Jukes and colleagues at Berkeley found positive responses to a thermostable factor that, unlike pyridoxine, was not adsorbed by fuller's earth from an acid solution. They referred to it as their **filtrate factor**.

At the same time, and quite independently, the University of Texas microbiologist R.J. Williams was pursuing studies of the essential nutrients for *Saccharomyces cerevisiae* and other yeasts. His group found a potent growth factor that they could isolate from a wide variety of plant and animal tissues.[52] They called it **pantothenic acid**, meaning "found everywhere," and also referred to the substance as **vitamin B_3**. Later in the decade, Snell's group found that several lactic and propionic acid bacteria require a growth factor that had the same properties. Jukes recognized that his filtrate factor, Norris's chick antidermatitis factor, and the unknown factors required by yeasts and bacteria were identical. He demonstrated that both his filtrate factor and pantothenic acid obtained from Williams could prevent dermatitis in the chick. Pantothenic acid was isolated and its chemical structure was determined by Williams's group in 1939. The chemical synthesis of the vitamin was achieved by Folkers the following year. The

50. Nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), respectively.

51. György defined vitamin B_6 activity as "that part of the vitamin B-complex responsible for the cure of a specific dermatitis developed by rats on a vitamin-free diet supplemented with vitamin B1, and lactoflavin."

52. The first isolation of pantothenic acid employed 250 kilograms of sheep liver. The autolysate was treated with fuller's earth; the factor was adsorbed to Norite and eluted with ammonia. Brucine salts were formed and were extracted with chloroform–water, after which the brucine salt of pantothenic acid was converted to the calcium salt. The yield was 3 grams of material with c. 40% purity.

TABLE 2.4 Factors Leading to the Discovery of Pantothenic Acid

Factor	Bioassay
Filtrate factor	Chick growth
Chick antidermatitis factor	Prevention of skin lesions and poor feather development in chicks
Pantothenic acid	Growth of *Saccharomyces cerevisiae* and other yeasts

various factors leading to the discovery of pantothenic acid are presented in Table 2.4.

A Fat-Soluble, Antisterility Factor: Vitamin E

Interest in the nutritional properties of lipids was stimulated by the resolution of fat-soluble A into vitamins A and D by the early 1920s. Several groups found that supplementation with the newly discovered vitamins A, C, and D and thiamin markedly improved the performance of animals fed purified diets containing adequate amounts of protein, carbohydrate, and known required minerals. However, H.M. Evans and Katherine Bishop, at the University of California, observed that rats fed such supplemented diets seldom reproduced normally. They found that fertility was abnormally low in both males (which showed testicular degeneration) and females (which showed impaired placental function and failed to carry their fetuses to term).[53] Dystrophy of skeletal and smooth muscles of the uterus was also noted. In 1922, these investigators reported that the addition of small amounts of yeast or fresh lettuce to the purified diet would restore fertility to females and prevent infertility in animals of both sexes. They designated the unknown fertility factor as *factor X*. Using the prevention of **gestation resorption** as the bioassay, Evans and Bishop found factor X activity in such unrelated materials as dried alfalfa, wheat germ, oats, meats, and milk fat, from which it was extractable with organic solvents. They distinguished the new fat-soluble factor from the known fat-soluble vitamins by showing that single droplets of wheat germ oil administered daily completely prevented gestation resorption, whereas cod liver oil, known to be a rich source of vitamins A and D, failed to do so.[54] In 1924, Sure, at the University of Arkansas, confirmed this work, concluding that the fat-soluble factor was a new vitamin, which he called **vitamin E**.

A Classic Touch in Coining Tocopherol

Soon, Evans was able to prepare a potent concentrate of vitamin E from the unsaponifiable lipids of wheat germ oil; others prepared similar vitamin E-active concentrates from lettuce lipids. By the early 1930s, Olcott and Mattill at the University of Iowa had found that such preparations, which prevented the gestation resorption syndrome in rats, also had chemical antioxidant properties that could be assayed in vitro.[55] In 1936, Evans isolated from unsaponifiable wheat germ lipids allophanic acid esters of three alcohols, one of which had very high biological vitamin E activity. Two years later, Fernholz showed that the latter alcohol had a phytyl side chain and a hydroquinone moiety and proposed the chemical structure of the new vitamin. Evans coined the term **tocopherol**, which he derived from the Greek words *tokos* ("childbirth") and *pherein* ("to bear");[56] he used the suffix *-ol* to indicate that the factor is an alcohol. He also named the three alcohols α-, β-, and γ-tocopherol. In 1938, synthesis of the most active vitamer, α-tocopherol, was achieved by the groups of Karrer, Smith, and Bergel. A decade later another vitamer, δ-tocopherol, was isolated from soybean oil; not until 1959 were the **tocotrienols** described.[57]

Antihemorrhagic Factor: Vitamin K

In the 1920s, Henrik Dam, at the University of Copenhagen, undertook studies to determine whether cholesterol was an essential dietary lipid. In 1929, Dam reported that chicks fed diets consisting of food that had been extracted with nonpolar solvents to remove sterols developed subdural, subcutaneous, or intramuscular **hemorrhages**, **anemia**, and abnormally long blood-clotting times. A similar syndrome in chicks fed ether-extracted fish meal was reported by McFarlane's group, which at the time was attempting to determine the chick's requirements for vitamins A and D. They found that nonextracted fish meal completely prevented the clotting defect. Holst and Holbrook found that cabbage prevented the syndrome, which they took as evidence of an involvement of vitamin C. By the mid-1930s, Dam had shown that the clotting

53. The vitamin E-deficient rat carries her fetuses quite well until a fairly late stage of pregnancy, at which time they die and are resorbed. This syndrome is distinctive; it termed **gestation resorption**.

54. In fact, Evans and Bishop found that cod liver oil actually increased the severity of the gestation resorption syndrome, a phenomenon now understood on the basis of the antagonistic actions of high concentrations of the fat-soluble vitamins.

55. Although the potencies of the vitamin preparations in the in vivo (rat gestation resorption) and in vitro (antioxidant) assays were not always well correlated.

56. Evans wrote in 1962 that he was assisted in the coining of the name for vitamin E by George M. Calhoun, Professor of Greek and a colleague at the University of California. It was Calhoun who suggested the Greek roots of this now-familiar name.

57. The tocotrienols differ from the tocopherols only by the presence of three conjugated double bonds in their phytyl side chains.

defect was also prevented by a fat-soluble factor present in green leaves and certain vegetables, and distinct from vitamins A, C, D, and E. He named the fat-soluble factor **vitamin K**.[58]

At that time, Herman Almquist and Robert Stokstad, at the University of California, found that the hemorrhagic disease of chicks fed a diet based on ether-extracted fish meal and brewers' yeast, polished rice, cod liver oil, and essential minerals was prevented by a factor present in ether extracts of alfalfa, and that was also produced during microbial spoilage of fish meal and wheat bran. Dam's colleague, Schønheyder, discovered the reason for prolonged blood-clotting times of vitamin K-deficient animals. He found that the clotting defect did not involve a deficiency of tissue thrombokinase or plasma fibrinogen, or an accumulation of plasma anticoagulants; he also determined that affected chicks showed relatively poor thrombin responses to exogenous thromboplastin. The latter observation suggested inadequate amounts of the clotting factor **prothrombin**, a factor already known to be important in the prevention of hemorrhages.

In 1936, Dam partially purified chick plasma prothrombin and showed its concentration to be depressed in vitamin K-deficient chicks. It would be several decades before this finding was fully understood.[59] Nevertheless, the clotting defect in the chick model served as a useful bioassay tool. When chicks were fed foodstuffs containing the new vitamin, their prothrombin values were normalized; hence, clotting time was returned to normal and the hemorrhagic disease was cured. The productive use of this bioassay led to the elucidation of the vitamin and its functions.

Vitamers K

Vitamin K was first isolated from alfalfa by Dam in collaboration with Paul Karrer at the University of Zurich in 1939. They found that the active substance, which was a yellow oil, was a quinone. The structure of this form of the vitamin (called **vitamin K_1**) was elucidated by Doisy's group at the University of St Louis, and by Karrer's, Almquist's and Feiser's groups in the same year. Soon, Doisy's group isolated a second form of the vitamin from putrified fish meal; this vitamer (called **vitamin K_2**) was crystalline. Subsequent studies demonstrated that this vitamer too differs from vitamin K_1 by having an unsaturated isoprenoid side chain at the 3-position of the naphthoquinone ring; in addition, putrified fish meal was found to contain several vitamin K_2-like substances with polyprenyl groups of differing chain lengths. Syntheses of vitamins K_2 were later achieved by Isler's and Folker's groups. A strictly synthetic analog of vitamers K_1 and K_2, consisting of the methylated head group alone (i.e., 2-methyl-1,4-naphthoquinone), was shown by Ansbacher and Fernholz to have high antihemorrhagic activity in the chick bioassay. It is, therefore, referred to as **vitamin K_3**.

Bios Yields Biotin

During the 1930s, independent studies of a yeast growth factor (called **bios IIb**[60]), a growth- and respiration-promoting factor for *Rhizobium trifolii* (called **coenzyme R**), and a factor that protected the rat against hair loss and skin lesions induced by raw egg white feeding (called **vitamin H**[61]) converged in an unexpected way. Kögl's group isolated the yeast growth factor from egg yolk and named it **biotin**. In 1940, György, du Vigneaud, and colleagues showed that vitamin H prepared from liver was remarkably similar to Kögl's egg yolk biotin.[62] The chemical structure of biotin was elucidated in 1942 by du Vigneaud's group at Cornell Medical College;[63] its complete synthesis was achieved by

58. Dam cited the fact that the next letter of the alphabet that had not previously been used to designate a known or proposed vitamin-like activity was also the first letter in the German or Danish phrase *koagulation facktor*, and was thus a most appropriate designator for the antihemorrhagic vitamin. The phrase was soon shortened to *K factor* and, hence, **vitamin K**.

59. It should be remembered that, at the time of this work, the biochemical mechanisms involved in clotting were incompletely understood. Of the many proteins now known to be involved in the process, only prothrombin and fibrinogen had been definitely characterized. It would not be until the early 1950s that the remainder of the now-classic clotting factors would be clearly demonstrated and that, of these, factors VII, IX, and X would be shown to be dependent on vitamin K. While these early studies effectively established that vitamin K deficiency results in impaired prothrombin activity, that finding would be interpreted as indicative of a vitamin K-dependent activation of the protein to its functional form.

60. **Bios IIb** was one of three essential growth factors for yeasts that had been identified by Wilders at the turn of the century in response to the great controversy that raged between Pasteur and Liebig. In 1860, Pasteur had declared that yeast could be grown in solutions containing only water, sugar, yeast ash (i.e., minerals), and ammonium tartrate; he noted, however, the growth-promoting activities of *albuminoid materials* in such cultures. Liebig challenged the possibility of growing yeast in the absence of such materials. Although Pasteur's position was dominant through the close of the century, Wilders presented evidence that proved that cultivation of yeast actually did require the presence of a little wort, yeast water, peptone, or beef extract. (Wilders showed that an inoculum the size of a bacteriological loopful, which lacked sufficient amounts of these factors, was unsuccessful, whereas an inoculum the size of a pea grew successfully.) Wilders used the term *bios* to describe the new activity required for yeast growth. For three decades, investigators undertook to characterize Wilders's bios factors. By the mid-1920s, three factors had been identified: *bios I*, which was later identified as meso-inositol; **bios IIa**, which was replaced by pantothenic acid in some strains and by β-alanine plus leucine in others; and **bios IIb**, which was identified as biotin.

61. György used the designation *H* after the German word *haut* (skin).

62. For a time, the factors obtained from egg yolk and liver were called α-biotin and β-biotin, respectively. They were reported as having different melting points and optical rotations. Subsequent studies, however, clearly demonstrated that such differences do not exist, nor do preparations from these sources exhibit different activities in microbiological systems.

63. du Vigneaud was to receive a Nobel Prize in Medicine for his work on the metabolism of methionine and methyl groups.

TABLE 2.5 Factors Leading to the Discovery of Biotin

Factor	Bioassay
Bios IIb	Yeast growth
Coenzyme R	*Rhizobium trifolii* growth
Vitamin H	Prevention of hair loss and skin lesions in rats fed raw egg white

Folkers in the following year. A summary of the factors leading to the discovery of biotin is presented in Table 2.5.

Antianemia Factors

The last discoveries that led to the elucidation of new vitamins involved findings of anemias of dietary origin. The first of these was reported in 1931 by Lucy Wills's group as a **tropical macrocytic anemia**[64] observed in women in Bombay, India, which was often a complication of pregnancy. They found that the anemia could be treated effectively by supplementing the women's diet with an extract of autolyzed yeast.[65] Wills and associates found that a macrocytic anemia could be produced in monkeys by feeding them food similar to that consumed by the women in Bombay. Further, the monkey anemia could be cured by oral administration of yeast or liver extract, or by parenteral administration of extract of liver; these treatments also cured human patients. The antianemia activity in these materials thus became known as the **Wills factor**.

Vitamin M?

Elucidation of the Wills factor involved the convergence of several lines of research, some of which appeared to be unrelated. The first of these came in 1935 from the studies of Day and colleagues at the University of Arkansas Medical School, who endeavored to produce riboflavin deficiency in monkeys. They fed their animals a cooked diet consisting of polished rice, wheat, washed casein, cod liver oil, a mixture of salts, and an orange; quite unexpectedly, they found them to develop anemia, leukopenia,[66] ulceration of the gums, diarrhea, and increased susceptibility to bacillary dysentery. They found that the syndrome did not respond to thiamin, riboflavin, or nicotinic acid; however, it could be prevented by daily supplements of either 10 grams of brewers' yeast or 2 grams of a dried hog liver–stomach preparation. Day named the protective factor in brewers' yeast **vitamin M** (for monkey).

Factors U and R, and Vitamin B_c

In the late 1930s, three groups (Robert Stokstad's at the University of California, Leo Norris's at Cornell, and Albert Hogan's at the University of Missouri) reported syndromes characterized by anemia in chicks fed highly purified diets. The anemias were found to respond to dietary supplements of yeast, alfalfa, and wheat bran. Stokstad and Manning called this unknown *factor U*; Baurenfeind and Norris at Cornell called it **factor R**. Shortly thereafter, Hogan and Parrott discovered an antianemic substance in liver extracts; they called it **vitamin B_c**.[67] At the time (1939), it was not clear to what extent these factors may have been related.

Yeast Growth Related to Anemia?

At the same time, the microbiologists Snell and Peterson, who were studying the bios factors required by yeasts, reported the existence of an unidentified water-soluble factor that was necessary for the growth of *Lactobacillus casei*. This factor was present in liver and yeast, from which it could be prepared by adsorption to and then elution from Norit;[68] for a while they called it the *yeast Norit factor*, but it quickly became known as the *L. casei factor*. Hutchings and colleagues at the University of Wisconsin further purified the factor from liver and found it to stimulate chick growth; this suggested a possible identity of the bacterial and chick factors. The factor from liver was found to stimulate the growth of both *Lactobacillus helveticus* and *Streptococcus fecalis* R.,[69] whereas the yeast-derived factor was twice as potent for *L. helveticus* as it was for *S. fecalis*. Thus, it became popular to refer to these as the "liver *L. casei* factor" and the "yeast (or fermentation) *L. casei* factor."

Snell's group found that many green leafy materials were potent sources of something with the microbiological effects of the **Norit eluate factor**—extracts promoted the growth of both *S. fecalis* and *L. casei*. They named the factor, by virtue of its sources, **folic acid**. In 1943, a fermentation product

64. A **macrocytic anemia** is one in which the number of circulating erythrocytes is below normal, but the mean size of those present is greater than normal (normal range, 82–92 μm^3). Macrocytic anemias occur in such syndromes as pernicious anemia, sprue, celiac disease, and macrocytic anemia of pregnancy. Wills' studies of the macrocytic anemia in her monkey model revealed megaloblastic arrest (i.e., failure of the large, nucleated, embryonic erythrocyte precursor cell type to mature) in the erythropoietic tissues of the bone marrow, and a marked reticulocytosis (i.e., the presence of young red blood cells in numbers greater than normal [usually <1%], occurring during active blood regeneration); both signs were eliminated coincidentally on the administration of extracts of yeast or liver.

65. Wills's yeast extract was not particularly potent, as they needed to administer 4 g two to four times daily to cure the anemia.

66. Leukopenia refers to any situation in which the total number of leukocytes (i.e., white blood cells) in the circulating blood is less than normal, which is generally c.5000 per mm^3.

67. Hogan and Parrott used the subscript *c* to designate this factor as one required by the chick.

68. A carbon-based filtering agent.

69. *Streptococcus fecalis* was then called *Streptococcus lactis* R.

was isolated that stimulated the growth of *S. fecalis* but not *L. casei*; this was called the **SLR factor** and, later, **rhizopterin**.

Who's on First?

It was far from clear in the early 1940s whether any of these factors were at all related, as folic acid appeared to be active for both microorganisms and animals, whereas concentrates of vitamin M, factors R and U, and vitamin B_c appeared to be effective only for animals. Clues to solving the puzzle came from the studies of Mims and associates at the University of Arkansas Medical School, who showed that incubation of vitamin M concentrates in the presence of rat liver enzymes caused a marked increase in the folic acid activity (i.e., assayed using *S. casei* and *Streptococcus lactis* R.) of the preparation. Subsequent work showed such "activation" enzymes to be present in both hog kidney and chick pancreas. Charkey, of the Cornell group, found that incubation of their factor R preparations with rat or chick liver enzymes produced large increases in their folic acid potencies for microorganisms. These studies indicated for the first time that at least some of these various substances may be related.

Derivatives of Pteroylglutamic Acid

The real key to solving what was clearly the most complicated puzzle in the discovery of the vitamins came in 1943 with the isolation of **pteroylglutamic acid** from liver by Stokstad's group at the Lederle Laboratories of American Cyanamid, Inc., and by Piffner's group at Parke-Davis, Inc. Stokstad's group achieved the synthesis of the compound in 1946. Soon it was found that pteroylmonoglutamic acid was indeed the substance that had been variously identified in liver as factor U, vitamin M, vitamin B_c, and the liver *L. casei* factor. The yeast *L. casei* factor was found to be the diglutamyl derivative (pteroyldiglutamic acid) and the liver-derived vitamin B_c was the hexaglutamyl derivative (pteroylhexaglutamic acid). Others of these factors (the *SLR factor*) were subsequently found to be single-carbon metabolites of pteroylglutamic acid. These various compounds thus became known generically as **folic acid**. A summary of the factors leading to the discovery of folic acid is presented in Table 2.6.

TABLE 2.6 Factors Leading to the Discovery of Folic Acid

Factor	Bioassay
Wills' factor	Cure of anemia in humans
Vitamin M	Prevention of anemia in monkeys
Vitamin B_c	Prevention of anemia in chicks
Factor R	Prevention of anemia in chicks
Factor U	Prevention of anemia in chicks
Yeast Norit factor	Growth of *Lactobacillus casei*
L. casei factor	Growth of *L. casei*
SLR factor	Growth of *Rhizobium* species
Rhizopterin	Growth of *Rhizobium* species
Folic acid	Growth of *Streptococcus fecalis* and *L. casei*

Antipernicious Anemia Factor

The second nutritional anemia that was found to involve a vitamin deficiency was the fatal condition of human patients that was first described by J.S. Combe in 1822 and became known as **pernicious anemia**.[70] The first real breakthrough toward understanding the etiology of pernicious anemia did not come until 1926, when Minot and Murphy found that lightly cooked liver, which the prominent hematologist G.H. Whipple had found to accelerate the regeneration of blood in dogs made anemic by exsanguination, was highly effective as therapy for the disease.[51,71,72] This indicated that liver contained a factor necessary for hemoglobin synthesis.

Intrinsic and Extrinsic Factors

Soon, studies of the antipernicious anemia factor in liver revealed that its enteric absorption depended on yet another factor in the gastric juice, which W.B. Castle, in 1928, called the **intrinsic factor**, to distinguish it from the *extrinsic factor* in liver. Biochemists then commenced a long endeavor to isolate the antipernicious anemia factor from liver. The isolation of the factor was necessarily slow and arduous for the reason that the only bioassay available was the hematopoietic response of human pernicious anemia patients, which was frequently not available. No animal model had been found, and a bioassay could not be replaced by a chemical reaction or physical method because, as is now known, this most potent vitamin is active at exceedingly low concentrations. Therefore, it was most important to the elucidation of the antipernicious anemia factor when, in 1947, Mary Shorb of the University of Maryland found that it

70. This condition has also been called **Addison's anemia** after T. Addison, who described it in great detail in 1949, and **Biemer's anemia**, after A. Biemier, who reported the disease in Zurich in 1872 and coined the term **pernicious anemia**.

71. Minot and Murphy treated 45 pernicious anemia patients with 120–240 g of lightly cooked liver per day. The patients' mean erythrocyte count increased from 1.47×10^6/mL before treatment to 3.4×10^6/mL and 4.6×10^6/mL after 1 and 2 mos. of treatment, respectively.

72. Whipple, Minot, and Murphy shared the 1934 Nobel Prize in Medicine for the discovery of whole liver therapy for pernicious anemia.

was also required for the growth of *Lactobacillus lactis* Dorner.[73] With Shorb's microbiological assay, isolation of the factor, by that time named **vitamin B_{12}** by the Merck group, proceeded rapidly.

Animal Protein Factors

At about the same time, animal growth responses to factors associated with animal proteins or manure were reported as American animal nutritionists sought to eliminate expensive and scarce animal by-products from the diets of livestock. Norris's group at Cornell attributed responses of this time to an **animal protein factor**; the factor in liver necessary for rat growth was called **factor X** by Cary and **zoopherin**[74] by Zucker and Zucker. It soon became evident that these factors were probably identical. Stokstad's group found the factor in manure and isolated an organism from poultry manure that would synthesize a factor that was effective both in promoting chick growth and in treating pernicious anemia. That the antipernicious anemia factor was produced microbiologically was important, in that it led to an economical means of industrial production of vitamin B_{12}.

Vitamin B_{12} Isolated

By the late 1940s, Combs[75] and Norris, using chick growth as their bioassay procedure, were fairly close to the isolation of vitamin B_{12}. However, in 1948, Folkers at Merck, using the *L. lactis* Dorner assay, succeeded in first isolating the antipernicious anemia factor in crystalline form. This achievement was accomplished in the same year by Lester Smith's group at the Glaxo Laboratories in England (who found their pink crystals to contain cobalt), assaying their material on pernicious anemia patients in relapse.[76] The elucidation of the complex chemical structure of vitamin B_{12} was finally achieved in 1955 by Dorothy Hodgkin's group at Oxford with the use of X-ray crystallography. In the early 1960s, several groups accomplished the partial synthesis of the vitamin; it was not until 1970 that the de novo synthesis of vitamin B_{12} was finally achieved by Woodward and Eschenmoser. A summary of the factors leading to the discovery of vitamin B_{12} is presented in Table 2.7.

TABLE 2.7 Factors Leading to the Discovery of Vitamin B_{12}

Factor	Bioassay
Extrinsic factor	Cure of anemia in humans
LLD factor	Growth of *L. lactis* Dorner
Vitamin B_{12}	Growth of *L. lactis* Dorner
Animal protein factor	Growth of chicks
Factor X	Growth of rats
Zoopherin	Growth of rats

TABLE 2.8 Timelines for the Discoveries of the Vitamins

Vitamin	Proposed	Isolated	Structure Determined	Synthesis Achieved
Thiamin	1906	1926	1932	1933
Vitamin C	1907	1926	1932	1933
Vitamin A	1915	1937	1942	1947
Vitamin D	1919	1932	1932 (D_2)	1932
			1936 (D_3)	1936
Vitamin E	1922	1936	1938	1938
Niacin	1926	1937	1937	1867[a]
Vitamin B_{12}	1926	1948	1955	1970
Biotin	1926	1939	1942	1943
Vitamin K	1929	1939	1939	1940
Pantothenic acid	1931	1939	1939	1940
Folate	1931	1939	1943	1946
Riboflavin	1933	1933	1934	1935
Vitamin B_6	1934	1936	1938	1939

[a]*Much of the chemistry of nicotinic acid was known before its nutritional roles were recognized.*

Vitamins Discovered in Only Five Decades

Beginning with the concept of a vitamin, which emerged with Eijkman's proposal of an antipolyneuritis factor in 1906, the elucidation of the vitamins continued through the isolation of vitamin B_{12} in potent form in 1948 (Table 2.8). Thus, the identification of the presently recognized vitamins was achieved within a period of only 42 years! For some vitamins (e.g., pyridoxine) for which convenient animal models were available, discoveries came rapidly; for others (e.g., niacin, vitamin B_{12}) for which animal models were late to be found, the pace of scientific progress was much slower (Fig. 2.1). These paths of discovery were marked by nearly a dozen Nobel Prizes (Table 2.9).

73. For a time, this was referred to as the **LLD factor**.
74. The term **zoopherin** carries the connotation: "to carry on an animal species."
75. Characterization of the animal protein factor was the subject of the senior author's father's doctoral thesis in Norris's laboratory at Cornell in the late 1940s.
76. Friedrich (1988) has pointed out that it should be no surprise that the first isolations of vitamin B_{12} were accomplished in industrial laboratories, as the task required industrial-scale facilities to handle the enormous amounts of starting material that were needed. For example, the Merck group used a ton of liver to obtain 20 mg of crystalline material.

TABLE 2.9 Nobel Prizes Awarded for Research on Vitamins

Year of Award	Recipients	Discovery
Prizes in Medicine and Physiology		
1929	Christiaan Eijkman and Frederick G. Hopkins	Discovery of the antineuritic vitamin; discovery of the growth-stimulating vitamins
1934	George H. Whipple, George R. Minot, and William P. Murphy	Discoveries concerning liver therapy against pernicious anemia
1937	Albert von Szent-Györgi and Charles G. King	Discoveries in connection with the biological combustion, with especial reference to vitamin C, and the catalysis of fumaric acid
1943	Henrik Dam and Edward ADoisy	Discovery of vitamin K; discovery of the chemical nature of vitamin K
1953	Fritz A. Lipmann	Discovery of coenzyme A and its importance in intermediary metabolism
1955	Hugo Theorell	Discoveries relating to the nature and mode of action of oxidizing enzymes
1964	Feodor Lynen and Konrad Bloch	Discoveries concerning the mechanism and regulation of cholesterol and fatty acid metabolism
Prizes in Chemistry		
1928	Adolf Windaus	Studies on the constitution of the sterols and their connection with the vitamins
1937	Paul Karrer and Walter N. Haworth	Research on the constitution of carotenoids, flavins, and vitamins A and B; researches into the constitution of carbohydrates and vitamin C
1938	Richard Kuhn	Work on carotenoids and vitamins
1957	Alexander Todd	Work on the structure of nucleotides (including vitamin B_{12})
1964	Dorothy C. Hodgkin	Elucidation of the structure of vitamin B_{12}
1965	Robert B. Woodward	Chemical synthesis of vitamin B_{12}
1967	George Wald, H.K. Hartline, and R. Grant	Discoveries of the basic chemical and physiological processes in vision

7. VITAMIN TERMINOLOGY

The terminology of the vitamins can be as daunting as that of any other scientific field. Many vitamins carry alphabetic or alphanumeric designations, yet the sequence of such designations has an arbitrary appearance by virtue of its many gaps and inconsistent application to all of the vitamins. This situation notwithstanding, the logic underlying the terminology of the vitamins becomes apparent when it is viewed in terms of the history of vitamin discovery. The familiar designations in use today are, in most cases, the surviving terms coined by earlier researchers on the paths to vitamin discovery. Thus, because McCollum and Davis used the letters A and B to distinguish the lipid-soluble antixerophthalmic factor from the water-soluble antineuritic and growth activity that was subsequently found to consist of several vitamins, such chemically and physiologically unrelated substances as thiamin, riboflavin, pyridoxine, and cobalamins (in fact, all water-soluble vitamins except ascorbic acid, which was designated before the vitamin B complex was partitioned) are all called B vitamins. In the case of folic acid, certainly the name survived its competitors by virtue of its relatively attractive sound (e.g., vs **rhizopterin**). Therefore, the accepted designations for the vitamins, in most cases, have relevance only to the history and chronology of their discovery and not to their chemical or metabolic similarities. The discovery of the vitamins left a path littered with designations of "vitamins," "factors," and other terms, most of which have been discarded (see Appendix A for a complete listing).

8. OTHER FACTORS SOMETIMES CALLED VITAMINS

Several other factors have, at various times or under certain conditions, been called vitamins. Many remain today only as historic markers of once incompletely explained phenomena, now better understood. Today, some factors would appear to satisfy, for at least some species, the operating definition of a vitamin; although in practice that term

TABLE 2.10 Vitamin-Like Factors

Substance	Biological Activity
Choline	Component of the primary membrane structural component phosphatidylcholine and the neurotransmitter acetylcholine; contributor to single-C metabolism; essential for normal growth and bone development in young poultry; can spare methionine in many animal species; and thus can be essential in diets that provide limited methyl groups.
Nonprovitamin A Carotenoids	
Flavonoids	Reported to reduce capillary fragility, and inhibit in vitro aldolase reductase (has a role in diabetic cataracts) and *o*-methyltransferase (inactivates epinephrine and norepinephrine)
Carnitine	Essential for transport of fatty acyl CoA from cytoplasm to mitochondria for β oxidation; synthesized by most species except some insects, which require a dietary source for growth
myo- i nositol	Component of phosphatidylinositol; prevents diet-induced lipodystrophies due to impaired lipid transport in gerbils and rats; essential for some microbes, gerbils, and certain fishes
Ubiquinones	Group includes a component of the mitochondrial respiratory chain; are antioxidants and can spare vitamin E in preventing anemia in monkeys, and in maintaining sperm motility in birds
Orotic Acid	
p-Aminobenzoic acid	Essential growth factor for several microbes, in which it functions as a provitamin of folic acid; reported to reverse diet- or hydroquinone-induced achromotrichia in rats and to ameliorate rickettsial infections
Lipoic acid	Cofactor in oxidative decarboxylation of α-keto acids; essential for growth of several microbes but inconsistent effects on animal growth
Pyrroloquinoline quinone	Component of certain bacterial and mammalian metallooxidoreductases; deprivation impairs growth, causes skin lesions in mice

is restricted to those factors required by higher organisms.[77] These vitamin-like factors (Table 2.10) are discussed in Chapter 19. At various times, of course, other factors have been represented as vitamins; however, no solid evidence has been sustained to support such claims (Table 2.11).

9. MODERN HISTORY OF THE VITAMINS

While the first half of the 20th century was an exciting period of vitamin discovery, the subsequent history of this field has been characterized by the generation of the huge amount of additional information needed to use the vitamins to improve human and animal health and to optimize the efficiency of producing food animals.[78] This work has revealed that some vitamins are widely underconsumed, that some may be associated with chronic disease, and that several are produced by the gut microbiome. Many recent studies have strived to elucidate advanced functional roles (such as transcriptional regulation) for the vitamins beyond those associated with their original discovery. Still, there are concerns about how best to assess vitamin intake, vitamin status, and the contents and stabilities of vitamin isomers in foods.

TABLE 2.11 Inactive Factors *Not* Considered Vitamins

Substance	Purported Biological Activity
Laetrile	A cyanogenic glycoside with unsubstantiated claims of antitumorigenicity
Gerovital	Unsubstantiated antiaging elixir
Orotic acid	Normal metabolic intermediate of pyrimidine biosynthesis with hypocholesterolemic activity
Pangamic acid	Ill-defined substance(s), originally derived from apricot pits, with unsubstantiated claims for a variety of health benefits

Have that all the vitamins have been discovered?[79] Perhaps, if one were to hold to the classical definition of "vitamin." Still, the field has already granted a considerable

77. Organic growth-promoting substances required only by microorganisms are frequently called **nutrilites**.

78. By the end of 2015, nearly 350,000 scientific papers on vitamins were listed in PubMed.gov (of the U.S. National Library of Medicine).

79. When the senior author (GFC) was an assistant professor (in the mid-1970s), he discussed with his Dad how unlikely it had become that a graduate student could be asked to undertake thesis research on "unidentified growth factors" (UGFs). He asked his Dad why, when he was a graduate student in the latter 1940s, he had thought it profitable to undertake such research. The senior Dr Combs pointed out that "Every UGF had proven to be an essential nutrient, so we had confidence that the 'animal protein factor' would also be one." He was correct, of course; his work in the Cornell laboratory of Prof. Leo Norris contributed directly to the elucidation of vitamin B_{12}.

TABLE 2.12 Contemporary Vitamin Research Needs

Area	Needs
Analytical and physical chemistry	• Better understandings of chemical and biological potencies and stabilities (to storage, processing, and cooking) of the vitamins, their various vitamers, and chemical derivatives • Better analytical methods for measuring vitamin contents of food
Biochemistry and molecular biology	• More complete understanding of the molecular mechanisms of vitamin action, including roles in gene expression • More complete elucidation of the pathways of vitamin metabolism • More complete understanding of the interactions with other nutrients and/or factors (e.g., disease, oxidative stress, genotype) that affect vitamin functions and needs • Understanding of the contributions of the gut microbiome to vitamin nutritional status and the effects of diet composition
Nutritional surveillance and epidemiology	• Development of informative biomarkers of status for underconsumed vitamins (e.g., vitamin A, riboflavin, folate) • Better understanding of vitamin intakes and status of populations and at-risk subgroups • Better understanding of the relationships of vitamin intake/status and disease risks
Nutrition and dietetics	• More complete understanding of the quantitative requirements for vitamins for individuals at all life stages • More complete understanding of genotypes with particular vitamin needs (e.g., choline) • More complete understanding of the role of the gut microbiome as a source of vitamins (e.g., vitamin K) for the host
Medicine	• More complete understanding of the roles of vitamins in etiology and/or management of chronic (e.g., cancer, heart disease), congenital (e.g., neural tube defects), and infectious diseases • More complete understanding of the needs for vitamins over the life cycle • More complete understanding of risks of supranutritional intakes of certain vitamins (e.g., vitamin A, vitamin D, folate)
Agriculture and international development	• Development of smallholder farming/gardening systems and other food-based approaches that support nutritional requirements with respect to vitamins (e.g., Vitamin A, Vitamin C, folate) and other nutrients • Development of foods with increased contents of underconsumed vitamins and other micronutrients
Food science and technology	• Development of food processing techniques that retain vitamins in food • Development of novel means of vitamin supplementation and fortification to enhance enteric absorption efficiency (e.g., nanoparticles)

latitude in that definition by admitting vitamin D and vitamin C to vitamin status. Why not choline? Or lycopene? In fact, it may not serve the field to be concerned as to whether these and other bioactive factors in foods can be called vitamins. It will be better to remain open to reinterpreting the notion "vitamin" in light of emerging knowledge of the metabolic roles of bioactive factors in foods with documentable roles in metabolism and health. Contemporary understanding of vitamins and their roles in nutrition and health is the subject of the following chapters. Those chapters will show the areas in which that understanding is incomplete. Those areas must be the foci of future research (Table 2.12).

10. STUDY QUESTIONS AND EXERCISES

1. How did the vitamin theory influence the interpretation of findings concerning diet and health associations?
2. For each vitamin, list the key empirical observations that led to its initial recognition.
3. In what general ways were animal models employed in the discovery of the vitamins? What ethical issues must be addressed in this type of research?
4. Which vitamins were discovered as results of efforts to use chemically defined diets for raising animals? How would you go about developing such a diet?
5. Which vitamins were discovered primarily through human experimentation? What ethical issues must be addressed in this type of research?
6. Prepare a concept map illustrating the interrelationships of the various prevalent ideas and the many goals, approaches, and outcomes that resulted in the discovery of the vitamins.

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Chapter 3

General Properties of Vitamins

Chapter Outline

Anchoring Concepts

1. The chemical composition and structure of a substance determine both its physical properties and chemical reactivity.
2. The physicochemical properties of a substance determine the ways in which it acts and is acted on in biological systems.
3. Substances tend to be partitioned between hydrophilic regions (plasma, cytosol, and mitochondrial matrix space) and hydrophobic regions (membranes, bulk lipid droplets) of biological systems on the basis of their relative solubilities; overcoming such partitioning requires actions of agents (micelles, binding, or transport proteins) that serve to alter their effective solubilities.
4. Isomers and analogs of a given substance may not have equivalent biological activities.

La vie est une fonction chimique.

A.L. Lavoisier[1]

LEARNING OBJECTIVES

1. To understand that the term **vitamin** refers to a family of compounds, i.e., structural analogs, with qualitatively similar biological activities but often quantitatively different potencies.
2. To become familiar with the chemical structures and physical properties of vitamins.
3. To understand the relationship between the physicochemical properties of vitamins and their stabilities, and how these properties affect their means of enteric absorption, transport, and tissue storage.
4. To become familiar with the general nature of vitamin metabolism.

VOCABULARY

Adenosylcobalamin
Ascorbic acid
β-Carotene
Binding proteins
Bioavailability
Biopotency
Biotin
Carotenoid
Cholecalciferol
6-Chromanol nucleus
Chylomicrons
Cobalamin
Corrin nucleus
Cyanocobalamin
Ergocalciferol
FAD
FMN
Folacin
Folic acid
HDL
LDL
Lipoproteins
Menadione
Menaquinone
Methylcobalamin
Micelle

1. Antoine-Laurent de Lavoisier (1743–94), while best known for his discovery of oxygen and its role in combustion, also discovered hydrogen and sulfur, recognized the constancy of mass in reactions that change the form of matter, and made important contributions to chemical nomenclature. A nobleman by birth and administrator of the Ferme Générale (profits from which funded his research) of the Ancien Régime, Lavoisier was sent to the guillotine at the height of the French Revolution.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00003-4

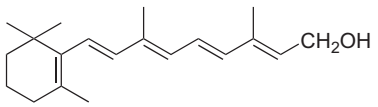
Microbiota
Microbiome
NAD
Naphthoquinone nucleus
Niacin
Nicotinamide
Nicotinic acid
NMN
Pantothenic acid
Phylloquinone
Portomicron
Pteridine
Pteroylglutamic acid
Pyridoxine
Pyridoxol
Retinal
Retinoic acid
Retinoid
Retinol
Riboflavin
Simon's metabolites
Steroid
Tetrahydrofolic acid
Thiamin
Thiamin pyrophosphate
Tocol
Tocopherol
Tocotrienol
Vitamin A
Vitamin B_2
Vitamin B_6
Vitamin B_{12}
Vitamin C
Vitamin D
Vitamin D_2
Vitamin D_3
Vitamin E
Vitamin K
Vitamin K_3
VLDL

1. VITAMIN NOMENCLATURE

The vitamins are organic, low molecular weight substances that have key roles in metabolism. Few of the vitamins are single substances; almost all are families of chemically related substances, i.e., **vitamers**, sharing qualitative (but not necessarily quantitative) biological activities. Thus, the vitamers comprising a vitamin family may vary in biopotency, and the common vitamin name is actually a generic descriptor for all of the relevant vitamers. Otherwise, vitamin families are chemically heterogeneous.

The nomenclature of the vitamins is in many cases complicated, reflecting both the terminology that evolved nonsystematically during the course of their discovery, as well as more recent efforts to standardize the vocabulary of the field. The standards for vitamin nomenclature policy were established by the International Union of Nutritional Sciences in 1978.[2] This policy distinguishes between generic descriptors used to describe families of compounds having vitamin activity (e.g., **vitamin D**) and to modify such terms as **activity** and **deficiency**, and trivial names used to identify specific compounds (e.g., **ergocalciferol**). These recommendations have been adopted by the Commission on Nomenclature of the International Union of Pure and Applied Chemists, the International Union of Biochemists, and the Committee on Nomenclature of the American Society for Nutrition. The latter organization publishes the policy every few years.[3] According to this accepted nomenclature, the vitamins are described as follows:

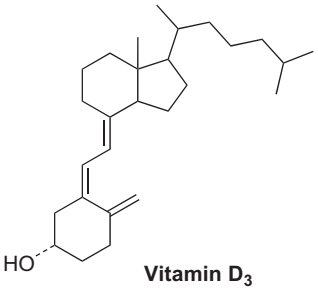
Vitamin A is the generic descriptor for compounds with the biological activity of retinol, formally derived from a monocyclic parent compound containing five C—C double bonds and a functional group at the terminus of the acyclic portion. Due to their structural similarities to retinol, they are called **retinoids**; those with vitamin A activity occur naturally in three forms: the alcohol **retinol**, the aldehyde **retinal** (also **retinaldehyde**), and the acid **retinoic acid**. β-Carotene and some other polyisoprenoid plant pigments (called **carotenoids** due to their relation to the carotenes) yield retinoids on metabolism and thus also have vitamin A activity; these are called **provitamin A carotenoids** and include β-carotene, a retinol dimer.



Retinol

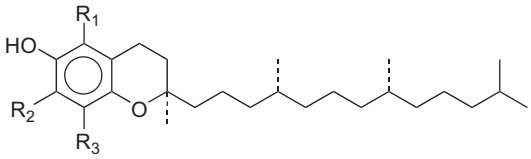
Vitamin D is the generic descriptor for all steroids qualitatively exhibiting qualitatively the biological activity of **cholecalciferol**. These compounds are derived in vivo by photolysis of the B ring of 7-dehydrocholesterol but retain the intact A, C, and D steroid rings. Vitamin D-active compounds with a 9-carbon side chain containing a single double bond are derivatives of **ergocalciferol**, also called **vitamin D_2**, which can be produced by photolysis of plant sterols. Vitamin D-active compounds with an 8-carbon side chain and no double bonds are derivatives of **cholecalciferol**, also called **vitamin D_3**, which is produced metabolically through a natural process of photolysis of 7-dehydrocholesterol on the surface of skin exposed to ultraviolet irradiation, e.g., sunlight.

2. Anonymous, 1978. Nutr. Abstr. Rev. 48, 831–835.
3. Anonymous, 1990. J. Nutr. 120, 12–19.



Vitamin D_3

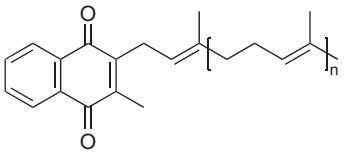
Vitamin E is the generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of **α-tocopherol**. These compounds are isoprenoid side chain derivatives of 6-chromanol, **tocols** with side chains consisting of three fully saturated isopentyl units, **tocopherols** comprising the mono-, di-, and trimethyl tocols; and t**ocotrienols** being 6-chromanol derivatives with a similar side chain containing three double bonds.



General Structure of the Tocopherols

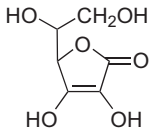
Vitamin K is the generic descriptor for 2-methyl-1,4-naphthoquinone and its derivatives exhibiting qualitatively the biological activity of phylloquinone. Three groups of vitamers occur, each consisting of variably substituted naphthoquinone ring compounds. The **phylloquinone**[4] group is synthesized by green plants; it includes forms with phytyl and further alkylated side chains having saturated isoprenoid units with a double bond only on the proximal isoprene unit. They are designated as *K-n,* n indicating the number of side chain isoprenoid units. The **menaquinone** group is synthesized by bacteria; it includes vitamers with side chains consisting of variable numbers of isoprenoid units each with a double bond. They are designated *MK*[5]*-n* to indicate side chain length. The synthetic compound 2-methyl-1,4-naphthoquinone (i.e., without a side chain) is called **menadione**.[6] It does not exist naturally but has biological activity by virtue of the fact that human and other animals can alkylate it to produce such metabolites as MK-*4*.

4. Formerly called **phytylmenaquinones**, or **vitamin K1**; the latter term is still encountered.
5. Formerly called **prenylmenaquinones**.
6. Formerly called **vitamin K3**.



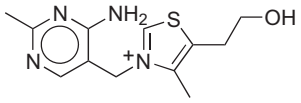
The Phylloquinones

Vitamin C is the generic descriptor for compounds exhibiting qualitatively the biological activity of ascorbic acid, i.e., 2,3-didehydro-l-threo-hexano-1,4-lactone.



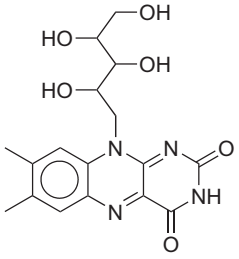
Ascorbic Acid

Thiamin is the trivial designation of the compound 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium.



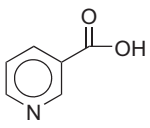
Thiamin

Riboflavin is the trivial designation of the compound 7,8-dimethyl-10-(1′-d-ribityl)isoalloxazine, formerly known as **vitamin B_2**, vitamin G, lactoflavin, or riboflavin.



Riboflavin

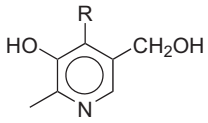
Niacin is the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.[7]



Nicotinic Acid

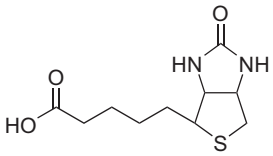
7. This compound is sometimes called niacinamide.

Vitamin B_6 is the generic descriptor for 3-hydroxy-2-methylpyridine derivatives exhibiting the biological activity of pyridoxine. The term **pyridoxine** is the trivial designation of the single vitamin B_6-active compound, 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine, formerly called **adermin** or **pyridoxol**.



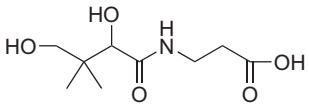
Pyridoxine

Biotin is the trivial designation of the compound *cis*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid, formerly known as vitamin **H** or **coenzyme R**.



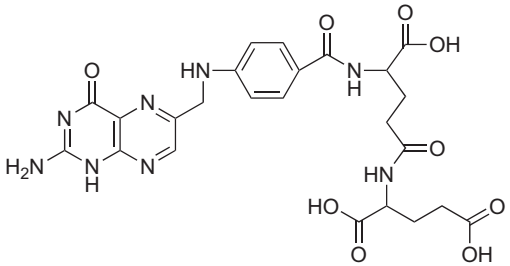
Biotin

Pantothenic acid is the trivial designation for the compound dihydroxy-β,β-dimethylbutyryl-β-alanine, formerly known as pantoyl-β-alanine.



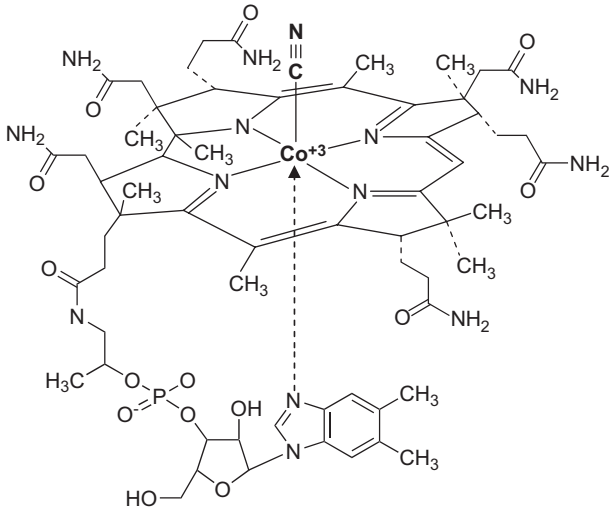
Pantothenic Acid

Folate is the generic descriptor for **folic acid** (pteroylmonoglutamic acid) and related compounds exhibiting the biological activity of folic acid. The terms **folacin**, **folic acids**, and **folates** are used only as general terms for this group of heterocyclic compounds based on the *N*-[(6-pteridinyl) methyl]-*p*-aminobenzoic acid skeleton conjugated with one or more L-glutamic acid residues. The reduced compound tetrahydropteroylglutamic acid is called **tetrahydrofolic acid**; its single carbon derivatives are named according to the specific carbon moiety bound.



Pteroylglutamic Acid

Vitamin B_{12} is the generic descriptor for cobalamins exhibiting the qualitative biological activity of **cyanocobalamin** (also called **cobalamin**). The cobalamins corrinoids (i.e., compounds containing a cyclic nucleus comprised of four pyrrole rings similar to the porphyrin) with a central cobalt atom that can bind small ligands and nucleotides. The unliganded, reduced form is **cob(I)alamin**. The two functional forms of the vitamin are **methylcobalamin** with a methyl group and a**denosylcobalamin** with a 5′-deoxyadenosyl grouping. Several synthetic vitamers are metabolically active by virtue of their being converted either of the functional forms; these include cyanocobalamin with a cyano group (CN^-), **aquacobalamin**[8] with a bound water molecule, **hydroxocobalamin**[9] with a hydroxo (OH) group, and **nitritocobalamin**[10] with a nitrite group.



Cyanocobalamin

Few of the vitamins are biologically active without metabolic conversion to another species and/or binding to a specific protein. Thus, any consideration of the vitamins in nutrition involves, for each vitamin group, a number of vitamers and metabolites; some of these are important in the practical sense for food and diet supplementation (Table 3.1); whereas, others are important in the physiological sense as they participate in metabolism.

2. CHEMICAL AND PHYSICAL PROPERTIES OF THE VITAMINS

It has been convenient to classify the vitamins on the basis of their physical properties (Table 3.2), as being either fat

8. Formerly, vitamin B_{12a}.
9. Formerly vitamin B_{12b}.
10. Formerly, vitamin B_{12c}.

TABLE 3.1 Relevant Forms of the Vitamins

Vitamin	Representative	Metabolically Active Forms	Important Dietary Forms
Vitamin A	Retinol	Retinol	Retinyl palmitate, retinyl acetate, provitamins (β-carotene, other carotenoids)
		Retinal	
		Retinoic acid	
Vitamin D	Cholecalciferol	25-OH-cholecalciferol	Cholecalciferol, ergocalciferol
		1,25-$(OH)_2$-cholecalciferol	
Vitamin E	α-Tocopherol	α-, β-, γ-, δ-Tocopherols	*R,R,R*-α-Tocopherol, all-*rac*-α-tocopheryl acetate
Vitamin K	Phylloquinone	Phylloquinones (K_n)	Phylloquinones (K_n), menaquinones (MK_n), menadione sodium bisulfite complex
		Menaquinones (MK_n)	
Vitamin C	Ascorbic acid	Ascorbic acid	L-ascorbic acid, sodium ascorbate
		Dehydroascorbic acid	
Thiamin	Thiamin	Thiamin pyrophosphate	Thiamin, thiamin pyrophosphate, thiamin disulfide, thiamin HCl, thiamin mononitrate
Riboflavin	Riboflavin	Flavin mononucleotide (FMN)	FMN, FAD, flavoproteins, riboflavin
		Flavin adenine dinucleotide (FAD)	
Niacin	Nicotinamide	Nicotinamide adenine dinucleotide (NAD)	NAD, NADP, nicotinamide, nicotinic acid
		Nicotinamide adenine dinucleotide phosphate (NADP)	
Vitamin B_6	Pyridoxine	Pyridoxal 5′-phosphate, pyridoxamine 5′-phosphate	Pyridoxal HCl, pyridoxal- 5′-phosphate, pyridoxamine-5′-phosphate
Biotin	*d*-Biotin	*d*-Biotin	Biocytin, *d*-biotin
Pantothenic acid	Pantothenic acid	Coenzyme A	Calcium pantothenate, coenzyme A, acyl CoAs
Folate	Pteroylglutamic acid	Pteroylpolyglutamates	Pteroyl poly- and monoglutamates
Vitamin B_{12}	Cyanocobalamin	Methylcobalamin5′-deoxyadenosylcobalamin	Cyano-, aqua-, hydroxo-, methyl-, and 5′-deoxyadenosylcobalamins

TABLE 3.2 Physical Properties of the Vitamins

Vitamin	Vitamer	MW	Solubility		Absorption Max	Molar Absorptivity		Fluorescence		Melting Point (°C)	Color/Form
			Organic[a]	H_2O		Absorptivity ε	$A^{1\%}_{1\ cm}$	Excitation (nm)	Emission (nm)		
Vitamin A	All-*trans*-retinol	286.4	+	–	325	52,300	1845	325	470	62–64	Yellow/crystal
	11-*cis*-retinol	286.4	+	–	319	34,900	1220				
	13-*cis*-retinol	286.4	+	–	328	48,300	1189				
	Retinal	284.4	+	–	373		1548			61–64	Orange/crystal
	All-*trans*-retinoic acid	300.4	+	sl[b]	350	45,300	1510			180–182	Yellow/crystal
	13-*cis*-Retinoic acid	300.4	+	sl[b]	354	39,800	1325			180–182	Yellow/crystal
	All-*trans*-retinyl acetate	312.0	+	sl[b]	326		1550			57–58	Yellow/crystal
	All-*trans*-retinyl palmitate	508.0	+	sl[b]	325–328		975			28–29	
Provitamin A	β-Carotene	536.9	+	–	453	2592	139			183	Purple/crystal
	α-Carotene	536.9	+	–	444	2800				187	Purple/crystal
Vitamin D	Vitamin D_2	396.7	+	–	264	18,300	459	No fluorescence		115–118	White/crystal
	Vitamin D_3	384.6	+	–	265	19,400	462	No fluorescence		84–85	White/crystal
	25(OH) vitamin D_3	400.7	+	–	265	18,000	449	No fluorescence			
	$1\alpha,25(OH)_2$vitamin D_3	416.6	+	–	264	19,000	418	No fluorescence			
Vitamin E	α-Tocopherol	430.7	+	–	292	3265	75.8	295	320	2.5	Yellow/oil
	β-Tocopherol	416.7	+	–	296	3725	89.4	297	322		Yellow/oil
	γ-Tocopherol	416.7	+	–	298	3809	91.4	297	322	–2.4	Yellow/oil
	δ-Tocopherol	402.7	+	–	298		91–92	297	322		Yellow/oil
	α-Tocopheryl acetate	472.8	+	–	286	1891–2080	40–44	290	323		Yellow/oil
	α-Tocopheryl succinate	530.8	+	–	286	2044	38.5	285	310		
	α-Tocotrienol	424.7	+	–	292	3652	86.0				
	β-Tocotrienol	410.6	+	–	296	3540	86.2	290	323		Yellow/oil
	γ-Tocotrienol	410.6	+	–	298	3737	91.0	290	324		Yellow/oil
	δ-Tocotrienol	396.6	+	–	298	3403	85.8	292	324		Yellow/oil

Vitamin K	Vitamin K_1	450.7	+	–	242	17,900	396	No fluorescence			Yellow/oil
					248	18,900	419				
					260	17,300	383				
					269	17,400	387				
					325	3100	68				
	Vitamin $K_{2(20)}$	444.7	+	–	248	19,500	439	No fluorescence		35	Yellow/crystal
	Vitamin $K_{2(30)}$	580.0	+	–	243	17,600	304	No fluorescence		50	Yellow/crystal
					248	18,600	320				
					261	16,800	290				
					270	16,900	292				
	Vitamin $K_{2(35)}$	649.2	+	–	243	18,000	278	No fluorescence		54	Yellow/crystal
					248	19,100	195				
					261	17,300	266				
					270	30,300	467				
					325–328	3100	48				
	Vitamin K_3	172.2	+	–						105–107	Yellow/crystal
Vitamin C	Ascorbic acid	176.1	–	+	245	12,200	695	No fluorescence		190–192	White/crystal
	Calcium ascorbate	390.3	–	+							White/crystal
	Sodium ascorbate	198.1	–	xs[e]						218[c]	White/crystal
	Ascorbyl palmitate	414.5	–	+							White/crystal
Thiamin	Thiamin disulfide	562.7	–	sl[b]				No fluorescence		177	Yellow/crystal
	Thiamin HCl	337.3	–	xs[e]						246–250	White/crystal
	Thiamin mononitrate	327.4	–	+						196–200[c]	White/crystal
	Thiamin monophosphate	344.3	–								
	Thiamin pyrophosphate	424.3	–							220–222[c]	
	Thiamin triphosphate	504.3	–							228–232[c]	

Continued

TABLE 3.2 Physical Properties of the Vitamins—cont'd

Vitamin	Vitamer	MW	Solubility		Absorption Max	Molar Absorptivity		Fluorescence		Melting Point (°C)	Color/Form
			Organic[a]	H_2O		Absorptivity ε	$A^{1\%}_{1cm}$	Excitation (nm)	Emission (nm)		
Riboflavin[d]	Riboflavin	376.4	–	+	260	27,700	736	360, 465	521	278[c]	Orange-yellow/ crystal
					375	10,600	282				
					450	12,200	324				
	Riboflavin-5′-phosphate	456.4	–	+	260	27,100	594	440–500	530		Orange-yellow/ crystal
	FAD	785.6	–	+	260	37,000	471	440–500	530		
					375	9300	118				
					450	11,300	144				
Niacin	Nicotinic acid	123.1	–	+	260	2800	227	No fluorescence		237	White/crystal
	Nicotinamide	122.1	–	xs[e]	261	5800	478	No fluorescence		128–131	White/crystal
Vitamin B_6	Pyridoxal HCl	203.6	–	+	390	200	9.8	330[f]	382	165[c]	White/crystal
					318	8128	399	310	365[g]		
	Pyridoxine	169.2			254	3891	23	320	380	160	
					324	7244	428	332	400		
	Pyridoxol HCl	205.6	–	+	253	3700	180			206–208	White/crystal
					290	8400	408				
					292	7720	375				
					325	7100	345				
	Pyridoxamine di-HCl	241.1	–	+	253	4571	190	320	370[g]	226–227	
					328	7763	322	337	400[h]		
	Pyridoxal 5′-phosphate	247.1	–	+	330	2500	101	365	423[h]		
								360	430[g]		
					388	4900	198	330	410[f]		

Biotin	*d*-Biotin	244.3	–	+	204	(Very weak)		No fluorescence		232–233	Colorless/crystal
Pantothenic acid	Pantothenic acid	219.2	–	xs[e]	204	(Very weak)		No fluorescence			Clear/oil
	Calcium pantothenate	467.5	–	–	No chromophore			No fluorescence		195–196[c]	White/crystal
	D-pantothenol	205.3		sl[b]	No chromophore			No fluorescence			Clear/oil
Folate	Folic acid	441.1			282	27,000	612	363	450–460[g]		
					350	7000	159				
	Tetrahyrdofolate	445.4			297	27,000	606	305–310	360[h]		
	10-Formyl FH_4	473.5			288	18,200	384	313	360[f]		
	5-Formyl FH_4	473.5			287	31,500	665	314	365[f]		
	5-Methyl FH_4	459.5			290	32,000	697				
	5-Formimino FH_4	472.5			285	35,400	749	308	360[f]		
	5,10-Methenyl FH_4	456.4			352	25,000	548	370	470[h]		
	5,10-Methylene FH_4	457.5			294	32,000	700				
Vitamin B_{12}	Cyanocobalamin	1355.4	–	xs[e]	278	8700	115	No fluorescence			Dark red/crystal
					261	27,600	204				
					551	8700	64				
	Hydroxylcobalamin (B_{12a})	1346.4	–	+	279	19,000	141	No fluorescence			Dark red/crystal
					325	11,400	85				
					359	20,600	153				
					516	8900	66				
					537	9500	71				
	Aquacobalamin (B_{12b})	1347.0	–	+	274	20,600	153				
					317	6100	45				
					351	26,500	197				
					499	8100	60				
	Nitrocobalamin (B_{12c})	1374.6	–	+	352	21,000	153				Red/crystal

Continued

TABLE 3.2 Physical Properties of the Vitamins—cont'd

Vitamin	Vitamer	MW	Solubility		Absorption Max	Molar Absorptivity		Fluorescence		Melting Point (°C)	Color/Form
			Organic[a]	H_2O		Absorptivity ε	$A^{1\%}_{1cm}$	Excitation (nm)	Emission (nm)		
					357	19,100	139				
					528	8400	60				
					535	8700	63				
	Methylcobalamin	1344.4	–	+	266	19,900	148				Red/crystal
					342	14,400	107				
					522	9400	70				
	Adenosylcobalamin cobamide	1579.6	–	+	288	18,100	115				Yellow-orange/ crystal
					340	12,300	78				
					375	10,900	60				
					522	800	51				

[a]*In organic solvents, fats, and oils.*
[b]*sl, Slightly soluble.*
[c]*Decomposes at this temperature.*
[d]*Fluoresces.*
[e]*xs, Freely soluble.*
[f]*Neutral pH.*
[g]*Alkaline pH.*
[h]*Acidic pH.*

soluble or water soluble.[11] In fact, this way of classifying the vitamins recapitulates the history of their discovery, calling to mind McCollum's "fat-soluble A" and "water-soluble B." The water-soluble vitamins tend to have one or more **polar** or ionizable groups (carboxyl, keto, hydroxyl, amino, or phosphate), whereas the fat-soluble vitamins have predominantly aromatic and aliphatic characters.

Fat-soluble vitamins—soluble in nonpolar solvents: Vitamins A, D, E, and K
Water-soluble vitamins—soluble in polar solvents: Vitamin C, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, biotin, folate, and vitamin B_{12}

The fat-soluble vitamins have some traits in common, in that each is composed either entirely or primarily of five-carbon **isoprenoid** units (i.e., related to *isoprene*, 2-methyl-1,3-butadiene) derived initially from acetyl CoA in those plant and animal species capable of their biosynthesis. In contrast, the water-soluble vitamins have, in general, few similarities of structure. The routes of their biosyntheses in capable species do not share as many common pathways.

Vitamin Stability

For the use of vitamins as food/feed additives, in dietary supplements, and as pharmaceuticals, stability is a prime concern. In general, the fat-soluble vitamins, vitamin C, thiamin, riboflavin, and biotin are poorly stable to oxidation. They must be protected from heat, oxygen, metal ions (particularly Fe^{2+} and Cu^{2+}), polyunsaturated lipids undergoing peroxidation, and ultraviolet light. Antioxidants are frequently used in formulations of these vitamins. For vitamins A and E, the more stable esterified forms are used for these purposes. Because of the instabilities of their naturally occurring vitamers, the amounts of the fat-soluble vitamins in natural foods and feedstuffs are highly variable, being greatly affected by the conditions of food production and processing. Niacin, vitamin B_6, pantothenic acid, folate, and vitamin B_{12} tend to be more stable under most practical conditions (Table 3.3). Some vitamins can undergo degradation by reacting to factors in foods during sample storage and/or preparation: ascorbic acid with plant ascorbic acid oxidase; thiamin with sulfites or with plant or microbial thiaminases; folates with nitrites; and pantothenic acid with microbial pantothenases.

3. PHYSIOLOGICAL UTILIZATION OF THE VITAMINS

Vitamin Absorption

The means by which the vitamins are absorbed are determined by their chemical and associated physical properties. The fat-soluble vitamins (and hydrophobic substances such as carotenoids and cholesterol), which are not soluble in the aqueous environment of the alimentary canal, are associated with and dissolved in other lipid materials. In the upper portion of the gastrointestinal tract, they are dissolved in the bulk lipid phases of the emulsions that are formed of dietary fats[12] by the mechanical actions of mastication and gastric churning. Emulsion oil droplets, however, are generally too large (e.g., 1000 Å) to gain the intimate proximity to the absorptive surfaces of the small intestine that is necessary to facilitate the diffusion of these substances into the hydrophobic environment of the brush border membranes of intestinal mucosal cells. However, **lipase**, which is present in the intestinal lumen following synthesis in and export from the pancreas via the pancreatic duct, binds to the surface of emulsion oil droplets, where it catalyzes the hydrolytic removal of the α and α′ fatty acids from triglycerides, which make up the bulk of the lipid material in these large particles. The products of this process (i.e., free fatty acids and β-monoglycerides) have strong polar regions or charged groups and, thus, will dissolve monomerically in this aqueous environment. However, they also have

11. The term solubility refers to the interactions of solutes and solvents. A soluble substance can disperse on a molecular level within a solvent. Solvents such as water, which can either donate or accept electrons, are said to be polar; whereas solvents (e.g., many organic solvents) incapable of such interactions are said to be nonpolar. Polar solvents such as water can either donate or accept electrons; whereas nonpolar solvents (e.g., many organic solvents) are incapable of such interactions. Compounds that are polar or that have charged or ionic character are soluble in polar solvents and are called hydrophilic; they are insoluble in nonpolar organic solvents and are, hence, also called lipophobic. Molecules that do not contain polar or ionizable groups tend to be insoluble in water (are hydrophobic) but soluble in nonpolar organic solvents (are lipophilic). Some large molecules (e.g., phospholipids, fatty acids, and bile salts) that have local areas of charge or ionic bond density, as well as other areas without charged groups, exhibit both polar and nonpolar characteristics. They are called amphipaths; having both hydrophilic and lipophilic internal regions, they tend to align along the interfaces of mixed polar/nonpolar phases. Amphipathic molecules are important in facilitating the dispersion of hydrophobic substances in aqueous environments; they do this by surrounding those substances, forming the submicroscopic structure called the mixed micelle.

12. It follows that fat-soluble vitamins are not well absorbed from low-fat diets; Studies have demonstrated markedly less absorption of carotenoids (Brown, M.J., Ferruzzi, M.G., Nguyen, M.L., Cooper, D.A., Eldridge, A.L., Schwartz, S.J., White, W.S., 2004. Am. J. Clin. Nutr. 80, 396–403) and vitamin D3 (Dawson-Huges, B., Harris, S.S., Lichtenstein, A.H., Dolnikowski, G., Palermo, N.J., Rasmussen, H., 2015. J. Acad. Nutr. Diet. 115, 225–230) from fat-free meals compared to fat-containing controls. However, the minimum amount of fat required is not clear. One study (Roodenburg, A.J., Leenen, R., van het Hof, K.H., Weststrate, J.A., Tilburg, L.B., 2000. Am. J. Clin. Nutr. 71, 1187–1193) found that as little as 3–5 g fat per meal may be sufficient for optimal absorption of provitamin A carotenoids, although the utilization of vitamins from plant foods is likely to require higher levels of fat to render accessible fat-soluble nutrients from the plant structures in which they are present.

TABLE 3.3 Stabilities of the Vitamins

Vitamin	Vitamer	Unstable to						To Enhance Stability
		UV	Heat[a]	O_2	Acid	Alkali	Metals[b]	
Vitamin A	Retinol	+		+	+		+	Keep in the dark, exclude O_2, use antioxidants
	Retinal			+	+		+	Exclude O_2, use antioxidants
	Retinoic acid							Good stability
	Dehydroretinol			+				Exclude O_2, use antioxidants
	Retinyl esters							Good stability
	β-Carotene	+		+				Keep in the dark, exclude O_2, use antioxidants
Vitamin D	D_2	+	+[c]	+	+		+	Keep cool, in the dark, exclude O_2, use antioxidants
	D_3	+	+[c]	+	+	+	+	Keep cool, in the dark, exclude O_2, use antioxidants
Vitamin E	Tocopherols		+	+	+	+	+	Keep cool, at neutral pH
	Tocopheryl esters				+	+		Good stability
Vitamin K	K	+		+		+	+	Avoid reductants[c], work in subdued light
	MK	+		+		+	+	Avoid reductants[c], work in subdued light
	Menadione	+				+	+	Avoid reductants[c], work in subdued light
Vitamin C	Ascorbic acid			+[b]		+	+	Exclude O_2, at neutral pH
Thiamin	Disulfide form		+	+	+	+	+	Keep at neutral pH[d]
	Hydrochloride[e]		+	+	+	+	+	Exclude O_2, at neutral pH[d]
Riboflavin	Riboflavin	+[f]	+			+	+	Keep in the dark, at pH 1.5–4[d]
Niacin	Nicotinic acid							Good stability
	Nicotinamide							Good stability
Vitamin B_6	Pyridoxal	+	+					Keep cool, work in subdued light
	Pyridoxol HCl			+		+		Good stability
Biotin	Biotin			+		+		Exclude O_2, at pH 4–9, use antioxidants, work in subdued light
Pantothenic acid	Free acid[g]	+		+		+		Cool, neutral pH
	Calcium salt[e]		+					Exclude O_2, at pH 5–7
Folate	FH_4	+	+	+	+[h]		+	Good stability[d]
Vitamin B_{12}	Cyano-B_{12}	+			+[i]		+[j]	Good stability[c] at pH 4–7

[a] *That is, 100°C.*
[b] *In solution with Fe^{3+} and Cu^{2+}.*
[c] *Isomerization to the previtamin form may be unavoidable, but tachysterol can also be formed under acid conditions in samples exposed to light.*
[d] *Unstable to reducing agents.*
[e] *Slightly hygroscopic.*
[f] *Especially in alkaline solution.*
[g] *Very hygroscopic.*
[h] *pH < 5.*
[i] *pH < 3.*
[j] *pH > 9.*

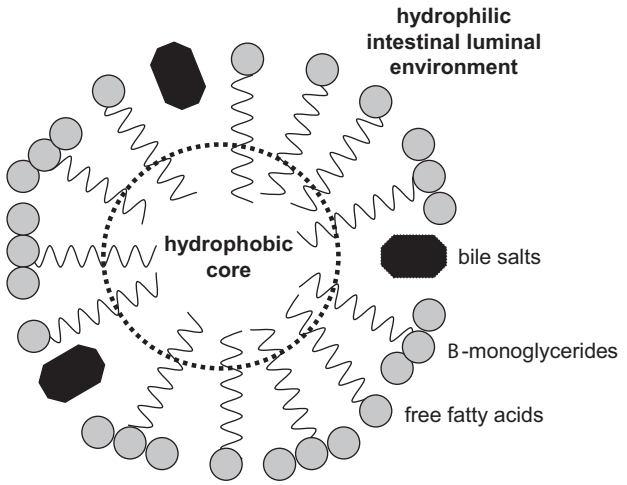


FIGURE 3.1 Mixed micelles form in the intestinal lumen by the spontaneous association of the products of triglyceride digestion, β-monoglycerides and free fatty acids, and bile salts. Their hydrophobic cores provide environments for the fat-soluble vitamins (A, D, E, and K) and other lipophilic dietary components. The absorption across the intestinal microvillar surface is facilitated by their very small size (10–50 Å diameter).

long-chain hydrocarbon nonpolar regions; therefore, when certain concentrations (**critical micellar concentrations**) are achieved, these species and bile salts, which have similar properties, combine spontaneously to form small particles called **micelles** (Fig. 3.1). Mixed micelles, thus, contain free fatty acids, β-monoglycerides, and bile salts in which the nonpolar regions of each are associated interiorly, and the polar or charged regions of each are oriented externally and are associated with the aqueous phase. The core of the mixed micelle is hydrophobic and serves to solubilize the fat-soluble vitamins and other nonpolar lipid substances. Because they are small (10–50 Å in diameter), mixed micelles can gain close proximity to microvillar surfaces of intestinal mucosa, facilitating the diffusion of their contents into and across those membranes. Because the enteric absorption of the fat-soluble vitamins depends on micellar dispersion, it is impaired under conditions of lipid malabsorption or very low dietary fat (<10 g/day).

The water-soluble vitamins, which are soluble in the polar environment of the intestinal lumen, can be taken up by the absorptive surface of the gut more directly. Some (vitamin C, thiamin, niacin, vitamin B_6, biotin, pantothenic acid, folate, and vitamin B_{12}) are absorbed as the result of passive diffusion; others are absorbed via specific carriers as a means of overcoming concentration gradients unfavorable to simple diffusion. Several (vitamin C, vitamin B_{12}, thiamin, niacin, and folate) are absorbed via carrier-dependent mechanisms at low doses[13] and by simple diffusion (albeit at lower efficiency) at high doses (Table 3.4).

13. These processes show apparent Km values in the range of 0.1–300 μM. [The Km parameter is a measure of the affinity of an enzyme for its substrate. Expressed as the substrate concentration at half-maximal velocity of the particular enzyme-catalyzed reaction, the Km is inversely related to the affinity of enzyme–substrate binding.]

The absorption of at least three water-soluble vitamins (vitamin C, riboflavin, and vitamin B_6) appears to be regulated in part by the dietary supply of the vitamin in a feedback manner. Thus, it has been questioned whether high doses of one vitamin/vitamer may antagonize the absorption of related vitamins. There is some evidence for such mutual antagonisms among the fat-soluble vitamins, as well as in the case of α-tocopherol, a high intake of which antagonizes the utilization of the related γ-vitamer.

Vitamin Transport

The mechanisms of postabsorptive transport of the vitamins also vary according to their particular physical and chemical properties (Table 3.5). The degree of solubility in the aqueous environments of the blood plasma and lymph is a major determinant of ways in which the vitamins are transported from the site of absorption (the small intestine) to the liver and peripheral organs. The fat-soluble vitamins, because they are insoluble in plasma and lymph, depend on carriers that are soluble in those aqueous environments. These vitamins, therefore, are associated with the lipid-rich **chylomicrons**[14] that are elaborated in intestinal mucosal cells, largely of reesterified triglycerides from free fatty acids and β-monoglycerides that have just been absorbed. As the lipids in these particles are transferred to other **lipoproteins**[15] in the liver, some of the fat-soluble vitamins (vitamins E and K) are also transferred to those carriers. Others (vitamins A and D) are transported from the liver to peripheral tissues by specific **binding proteins** of hepatic origin (Table 3.6). Some of the water-soluble vitamins are transported by protein carriers in the plasma and therefore are not found free in solution. Some (riboflavin, vitamin B_6) are carried via weak, nonspecific binding to albumin, and may thus be displaced by other substances (e.g., ethanol) that also bind to that protein. Others are tightly associated with certain immunoglobulins (riboflavin) or bind

14. Chylomicrons are the largest (~1 μm diameter) and the lightest of the blood lipids. They consist mainly of triglyceride with smaller amounts of cholesterol, phospholipid, protein, and the fat-soluble vitamins. They are normally synthesized in the intestinal mucosal cells and serve to transport lipids to tissues. In mammals, these particles are secreted into the lymphatic drainage of the small intestine (hence their name). However, in birds, fishes, and reptiles, they are secreted directly into the renal portal circulation; therefore, in these species they are referred to as **portomicrons**. In either case, they are cleared from the plasma by the liver, and their lipid contents are either deposited in hepatic stores (e.g., vitamin A) or released back into the plasma bound to more dense particles called **lipoproteins**.

15. As the name would imply, a lipoprotein is a lipid–protein combination with the solubility characteristics of a protein (i.e., soluble in the aqueous environment of the blood plasma) and hence involved in lipid transport. Four classes of lipoproteins, each defined empirically on the basis of density, are found in the plasma: chylomicrons/portomicrons, high-density lipoproteins **(HDLs)**, low-density lipoproteins **(LDLs)**, and very low-density lipoproteins **(VLDLs)**. The latter three classes are also known by names derived from the method of electrophoretic separation, i.e., α-, β-, and pre-β-lipoproteins, respectively.

TABLE 3.4 Enteric Absorption of the Vitamins[a]

Vitamer	Digestion	Site	Enterocytic Metabolism	Efficiency (%)	Conditions of Potential Malabsorption
Micelle-Dependent Diffusion					
Retinol	—	D, J	Esterification	80–90	Pancreatic insufficiency (pancreatitis, selenium deficiency, cystic fibrosis, cancer), β-carotene cleavage, biliary atresia, obstructive jaundice, celiac disease, very low-fat diet
Retinyl esters	Deesterified	D, J	Reesterification		
		D, J	Esterification	50–60	Pancreatic or biliary insufficiency
Vitamins D	—	D, J	—	~50	Pancreatic or biliary insufficiency
Tocopherols	—	D, J	—	20–80	Pancreatic or biliary insufficiency
Tocopherol esters	Deesterified[b]	D, J	—	20–80	Pancreatic or biliary insufficiency
MKs	—	D, J	—	10–70	Pancreatic or biliary insufficiency
Menadione	—	D, J	—	10–70	Pancreatic or biliary insufficiency
Active Transport					
Phylloquinone	—	D, J	—	~80	Pancreatic or biliary insufficiency
Ascorbic acid	—	I	—	70–80	D-isoascorbic acid
Thiamin	—	D	Phosphorylation		Pyrithiamin, excess ethanol
Thiamin di-P	Dephosphorylation[b]	D	Phosphorylation		Pyrithiamin, excess ethanol
Riboflavin	—	J	Phosphorylation		
FMN, FAD	Hydrolysis[b]	J	Phosphorylation		
Flavoproteins	Hydrolysis[b]	J	Phosphorylation		
Folylmono-glu	—	J	Glutamation		Celiac sprue
Folylpoly-glu	Hydrolysis[b]	J	Glutamation		Celiac sprue
Vitamin B_{12}	Hydrolysis[b]	I	Adenosinylation, methylation	>90	Intrinsic factor deficiency (pernicious anemia)
Facilitated Diffusion[c]					
Nicotinic acid	—	J		>90[d]	
Nicotinamide	—	J		~100[d]	
Niacytin	Hydrolysis[b]	J			
NAD(P)	Hydrolysis[b]	J			

Biotin	—	J			Biotinidase deficiency, consumption of raw egg white (avidin)
Biocytin	Hydrolysis[b]	J			Biotinidase deficiency, consumption of raw egg white (avidin)
Pantothenate	—				
Coenzyme A	Hydrolysis[b]				
Simple Diffusion					
Ascorbic acid[e]	—	D, J, I	—	<50	
Thiamin[e,f]	—	J	Phosphorylation		
Nicotinic acid	—	J	—		
Nicotinamide	—	J	—		
Pyridoxol	—	J	Phosphorylation		
Pyridoxal	—	J	Phosphorylation		
Pyridoxamine	—	J	Phosphorylation		
Biotin	—	D, J	—	>95	Consumption of raw egg white (avidin)
Pantothenate	—		—		
Folylmono-glu[e]	—	J	Glutamation		
Vitamin B_{12}[e]	—	D, J	Adenosinylation, methylation	~1	

[a]D, *Duodenum;* folylmono-glu, *folylmonoglutamate;* folylpoly-glu, *folylpolyglutamate;* I, *ileum;* J, *jejunum;* thiamin di-P, *thiamin diphosphate.*
[b]*Yields vitamin in absorbable form.*
[c]*Na^+-dependent saturable processes.*
[d]*Estimate may include contribution of simple diffusion.*
[e]*Simple diffusion important only at high doses.*
[f]*Symport with Na^+.*

TABLE 3.5 Postabsorptive Transport of Vitamins in the Body

Vehicle	Vitamin	Form Transported
Lipoprotein Bound		
Chylomicrons[a]	Vitamin A	Retinyl esters
	Vitamin A	β-Carotene
	Vitamin D	Vitamin D[b]
	Vitamin E	Tocopherols
	Vitamin K	K, MK, menadione
VLDL[c]/HDL[d]	Vitamin E	Tocopherols
	Vitamin K	Mainly MK-4
Associated Nonspecifically With Proteins		
Albumin	Riboflavin	Free riboflavin, FMN
	Vitamin B_6	Pyridoxal, pyridoxal phosphate
Immunoglobulins[e]	Riboflavin	Free riboflavin
Bound to Specific Binding Proteins		
Retinol BP (RBP)	Vitamin A	All-*trans*-retinol
Transcalciferin (vitamin D BP)	Vitamin D	D_2; D_3; 25-OH-D; 1,25-$(OH)_2$-D; 24,25-$(OH)_2$-D
Thiamin BP	Thiamin	Free thiamin
Riboflavin BP	Riboflavin	Riboflavin
Biotinidase	Biotin	Free biotin
Folate BP	Folate	Folate
Transcobalamin II	Vitamin B_{12}	Methylcobalamin
Transcobalamin III	Vitamin B_{12}	Vitamin B_{12}
Carried in Erythrocytes		
Erythrocyte membranes	Vitamin E	Tocopherols
Erythrocytes	Vitamin B_6	Pyridoxal phosphate
	Pantothenic acid	Coenzyme A
Free in Plasma		
—	Vitamin C	Ascorbic acid
—	Thiamin	Free thiamin, thiamin pyrophosphate
—	Riboflavin	FMN
—	Pantothenic acid	Pantothenic acid
—	Biotin	Free biotin
—	Niacin	Nicotinic acid, nicotinamide
—	Folate	Pteroylmonoglutamates[f]
Bound to Specific Intracellular Binding Proteins		
Cellular RBP (CRBP)	Vitamin A	All-*trans*-retinol
Cellular RBP, type II (CRBPII)	Vitamin A	All-*trans*-retinol
Interstitial RBP (IRBP)	Vitamin A	All-*trans*-retinol
Cellular retinal BP (CRALBP)	Vitamin A	All-*trans*-retinal

TABLE 3.5 Postabsorptive Transport of Vitamins in the Body—cont'd

Vehicle	Vitamin	Form Transported
Cellular retinoic acid BP (CRABP)	Vitamin A	All-*trans*-retinoic acid
Vitamin D receptor	Vitamin D	1,25-$(OH)_2$-D
Vitamin E BP	Vitamin E	Tocopherols
Flavoproteins	Riboflavin	FMN, FAD
Transcobalamin I	Vitamin B_{12}	Vitamin B_{12}

[a]*In mammals, lipids are absorbed into the lymphatic circulation, where they are transported to the liver and other tissues as large lipoprotein particles called* chylomicra *(singular,* chylomicron*); in birds, reptiles, and fishes, lipids are absorbed directly into the hepatic portal circulation and the analogous lipoprotein particle is called a* ***portomicron****.*
[b]*Representation of vitamin D without a subscript is meant to refer to both major forms of the vitamin: ergocalciferol (D_2) and cholecalciferol (D_3).*
[c]VLDL*, very low-density lipoprotein.*
[d]HDL*, high-density lipoprotein.*
[e]*For example, IgG, IgM, and IgA.*
[f]*Especially 5-CH_3-tetrahydrofolic acid.*

TABLE 3.6 Tissue Distribution of the Vitamins

Vitamin	Predominant Storage Form(s)	Depot(s)
Vitamin A	Retinyl esters (e.g., palmitate)	Liver
Vitamin D	D_3; 25-OH-D	Plasma, adipose, muscle
Vitamin E	α-Tocopherol	Adipose, adrenal, testes, platelets, other tissues
Vitamin K	K-4, MK-4	Liver
	MK-4	All tissues
Vitamin C	Ascorbic acid	Adrenals, leukocytes
Thiamin	Thiamin pyrophosphate[a]	Heart, kidney, brain, muscle
Riboflavin	FAD[b]	Liver, kidney, heart
Vitamin B_6	Pyridoxal phosphate[b]	Liver[c], kidney[c], heart[c]
Vitamin B_{12}	Methylcobalamin	Liver[d], kidney[c], heart[c], spleen[c], brain[c]
Niacin	No appreciable storage	—
Biotin	No appreciable storage[b]	—
Pantothenic acid	No appreciable storage	—
Folate	No appreciable storage	—

[a]*The amounts in the body are composed of the enzyme-bound coenzyme.*
[b]*Small amounts of the vitamin are found in these tissues.*
[c]*Predominant depot.*

to specific proteins involved in their transport (riboflavin, vitamins A, D, E, and B_{12}). Several vitamins (e.g., vitamin C, thiamin, niacin, riboflavin, pantothenic acid, biotin, and folate) are transported in free solution in the plasma.

Tissue Distribution of the Vitamins

The retention and distribution of the vitamins among the various tissues also vary according to their general physical and chemical properties (Table 3.6). In general, the fat-soluble vitamins are well retained; they tend to be stored in association with tissue lipids. For that reason, lipid-rich tissues such as adipose and liver frequently have appreciable stores of the fat-soluble vitamins. Storage of these vitamins means that animals may be able to accommodate widely variable intakes without consequence by mobilizing their tissue stores in times of low dietary intakes.

In contrast, the water-soluble vitamins tend to be excreted rapidly in the urine and not retained well. Few of these vitamins are stored to any appreciable extent. The notable exception is vitamin B_{12}, which, under normal circumstances, can accumulate in the liver in amounts adequate to satisfy the nutritional needs of the host for periods of years.

4. METABOLISM OF THE VITAMINS

Some Vitamins Have Limited Biosynthesis

By definition, the vitamins as a group of nutrients are obligate factors in the diet (i.e., the chemical environment) of an organism. Nevertheless, some vitamins do not quite fit that general definition because they may, in fact, be biosynthesized regularly by certain species, and under certain circumstances by other species. This is the "**vitamin caveat**" (Chapter 1). The biosynthesis of such vitamins (Table 3.7) thus depends on the availability, either from dietary or metabolic sources, of appropriate precursors. Examples include the following:

- adequate free tryptophan is required for niacin production in species capable of substantive tryptophan–niacin conversion;
- the presence of 7-dehydrocholesterol in the surface layers of the skin is required for its conversion to vitamin D_3 in individuals exposed to UV light; and
- flux through the gulonic acid pathway is needed to produce ascorbic acid in those (most nonprimate) species in which that pathway is intact.

Some vitamins can be synthesized by the **microbiota** of the hindgut. Until fairly recently, this source of vitamins has not been regarded as having immediate physiological relevance, as it was thought that vitamins synthesized by the gut microbiota were not absorbed in the large intestine. However, recent studies and a greater appreciation of earlier work with animals models[16] have changed that view, and it is now clear that the hindgut microbiota can contribute meaningful amounts of at least six vitamins: vitamin K[17], thiamin, riboflavin, pyridoxine, biotin, and folate. Metagenomic studies have shown that microbiomes relatively rich in *Bacteroides* spp. tend to have enriched capacities of the synthesis of biotin and riboflavin, while those relatively rich in *Prevotella* spp. tend to have enriched capacities for the synthesis of thiamin and folate. The microbial synthesis of these vitamins in the gut is also affected by the nature of an individual's diet; greatest synthesis can be expected when diets are rich in soluble fiber, e.g., diets rich in plant foods and whole grains.

16. In 1914, Cooper (J. Hygiene 14,12-22) showed that feeding a fecal extract could cure pigeons of their polyneuritis.
17. As menaquinones, MKn.

Most Vitamins Require Metabolic Activation

Only a few vitamins are directly metabolically active: vitamin E, some vitamers K (e.g., MK-4), and vitamin C. The others require metabolic activation or linkage to a cofunctional species (e.g., an enzyme) (Table 3.8). The transformation of dietary forms of the vitamins into their respective, metabolically active forms may involve substantive modification of a vitamin's chemical structure and/or its combination with another species. Thus, factors that affect the metabolic (i.e., enzymatic) activation of vitamins to their functional species can have profound influences on their nutritional efficacy.

Vitamin Binding to Proteins

Some vitamins, even some requiring metabolic activation, are biologically active only when bound to a specific protein (Table 3.9). This often occurs when the vitamin serves as the prosthetic group of an enzyme, remaining bound to the enzyme protein during catalysis. Vitamins of this type are properly called **coenzymes**. In other cases, vitamins participate in enzymatic catalysis but are not firmly bound to enzyme protein during the reaction; they are more properly called **cosubstrates**. This distinction, however, does not address the mechanism, but only the tightness, of binding.[18] Therefore, the term coenzyme has come to be used to describe enzyme cofactors of both types. Other vitamins bind to specific nuclear receptors to elicit transcriptional modulation of one or more protein products (Table 3.10).

Vitamin Excretion

In general, the fat-soluble vitamins, which tend to be retained in hydrophobic environments, are excreted with the feces via the enterohepatic circulation[19] (Table 3.11). Exceptions include vitamins A and E, which to some extent have water-soluble metabolites (e.g., short-chain derivatives of retinoic acid; and the so-called ***Simon's metabolites*** [carboxylchromanol metabolites] of vitamin E), and menadione, which can be metabolized to a polar salt; these vitamin metabolites are excreted in the urine. In contrast, the water-soluble vitamins are generally excreted in the urine, both in intact forms (riboflavin, pantothenic acid) and as water-soluble metabolites (vitamin C, thiamin, niacin, riboflavin, pyridoxine, biotin, folate, and vitamin B_{12}).

18. For example, the associations of NAD and NADP with certain oxidoreductases are weaker than those of FMN and FAD with the flavoprotein oxidoreductases.
19. These substances are discharged from the liver with the bile; the amounts that are not subsequently reabsorbed are eliminated with the feces.

TABLE 3.7 Vitamins That Can Be Biosynthesized by the Host

Vitamin	Precursor	Route	Conditions Increasing Dietary Need
Niacin	Tryptophan	Conversion to nicotinamide mononucleotide (NMN) via picolinic acid	Low 3-OH-anthranilic acid oxidase activity
			High picolinic acid carboxylase activity
			Low dietary tryptophan
			High dietary leucine[a]
Vitamin D_3	7-Dehydrocholesterol	UV photolysis	Insufficient sunlight/UV exposure
Vitamin C[b]	Glucose	Gulonic acid pathway	L-gulonolactone oxidase deficiency

[a]*The role of leucine as an effector of the conversion of tryptophan to niacin is controversial (Chapter 12).*
[b]*Humans and other higher primates, guinea pigs, the Indian fruit bat, and a few other species are capable of vitamin C biosynthesis.*

TABLE 3.8 Vitamins That Must Be Activated Metabolically[a]

Vitamin	Active Form(s)	Activation Step	Condition(s) Increasing Need
Vitamin A	Retinol	Retinal reductase; hydrolase	Protein insufficiency
	11-*cis*-Retinol	Retinyl isomerase	
	11-*cis*-Retinal	Alcohol dehydrogenase	Zinc insufficiency
Vitamin D	1,25-$(OH)_2$-D	Vitamin D 25-hydroxylase; 25-OH-D 1-hydroxylase	Hepatic failure
			Renal failure, lead exposure, estrogen deficiency, anticonvulsant drug therapy
Vitamin K	All forms	Dealkylation of Ks, MKs; alkylation of Ks, MKs, menadione	Hepatic failure
Thiamin	Thiamin-diP	Phosphorylation	High carbohydrate intake
Riboflavin	FMN, FAD	Phosphorylation, adenosylation	
Vitamin B_6	Pyridoxal-P	Phosphorylation; oxidation	High protein intake
Niacin	NAD(H)	Amidation (nicotinic acid)	Low tryptophan intake NADP(H)
Pantothenic acid	Coenzyme A	Phosphorylation; decarboxylation;	
		ATP condensation; peptide bonding	
	ACP	Phosphorylation; peptide bonding	
Folate	C_1-FH_4	Reduction; addition of C_1	
Vitamin B_{12}	Methyl-B_{12}	Cobalamin methylation	Folate deficiency
		CH_3 group insufficiency	
	5′-Deoxyadenosyl-B_{12}	Adenosylation	

[a]ACP, *acyl carrier protein;* C_1-FH_4, *tetrahydrofolic acid;* Thiamin-diP, *thiamin pyrophosphate.*

5. METABOLIC FUNCTIONS OF THE VITAMINS

Vitamins Serve Five Basic Functions

The 13 families of nutritionally important substances called *vitamins* comprise two to three times that number of practically important vitamers, which function in metabolism in five general, and not mutually exclusive ways:

- As **coenzymes**—metabolites that link to enzymes and are required for their catalytic activity.
- As **H^+/e^- donors/acceptors**—factors that can undergo changes in oxidation state in metabolism by being

TABLE 3.9 Vitamins That Must Be Linked to Enzymes and Other Proteins

Vitamin	Form(s) Linked
Biotin	Biotin
Vitamin B_{12}	Methylcobalamin, adenosylcobalamin
Vitamin A	11-*cis*-Retinal
Thiamin	Thiamin pyrophosphate
Riboflavin	FMN, FAD
Niacin	NAD, NADP
Vitamin B_6	Pyridoxal phosphate
Pantothenic acid	Acyl carrier protein
Folate	Tetrahydrofolic acid (FH_4)

TABLE 3.10 Vitamins That Have Nuclear Receptor Proteins

Vitamin	Form(s) linked	Receptor
Vitamin A	All-*trans*-retinoic acid	Retinoic acid receptors (RARs)
	9-*cis*-Retinoic acid	
	9-*cis*-Retinoic acid	Retinoid X receptors (RXRs)
Vitamin D	1,25-$(OH)_2$-Vitamin D_3	Vitamin D receptor (VDR)

oxidized (losing electrons to an acceptor) or reduced (accepting electrons to a donor acceptor) in metabolism.

- As **antioxidants**—factors that inhibit oxidative processes, which produce free radicals that can start chain reactions that damage lipids and proteins and affect cellular function. Antioxidants interrupt such processes by being oxidized themselves and thus removing free radicals.
- As **hormones**—metabolites released by cells or glands in one part of the body that affect cell function in another part of the body.
- As effectors of **gene transcription**—factors that affect the first step in gene expression, the process of "transcription" by which a complementary RNA copy of a DNA sequence is made.

The type of metabolic function of any particular vitamer or vitamin family is dependent on its tissue/cellular distribution and its chemical reactivity, both of which are direct or indirect functions of its chemical structure. For example, the antioxidative function of vitamin E reflects the ability of that vitamin to form semistable radical intermediates; its lipophilicity allows vitamin E to discharge this antioxidant function within the hydrophobic regions of biomembranes, thus protecting polyunsaturated membrane phospholipids. Similarly, the redox function of riboflavin is due to its ability to undergo reversible reduction/oxidation involving a radical anion intermediate. These functions (summarized in Table 3.12), and the fundamental aspects of their significance in nutrition and health, are the subjects of Chapters 6–18.

6. VITAMIN BIOAVAILABILITY

Several factors affect the biological activities of vitamins (Table 3.13). This includes multiple vitamers, which may differ in stability, accessibility from food matrices, and efficiency of enteric absorption and intrinsic metabolic potency. These factors can confound interpretations of the results of vitamin analyses of foods. For this reason, it is useful to distinguish between the intrinsic biological activity of a vitamer, i.e., its *biopotency*, and the actual extent to which that vitamer is absorbed and utilized at the cellular level, i.e., its *bioavailability*.[20] Considerations of relative bioavailability, which can be determined in bioassays that employ reference vitamers of known biopotency, have led to the expression of vitamin contents in foods using standardized unitage based on activities compared to those reference vitamers, e.g., international units, retinol equivalents, α-tocopherol equivalents.

Vitamin Bioavailability

This describes that fraction of ingested vitamin that is absorbed, retained, and metabolized through normal pathways in a form(s) that can be utilized for normal physiologic functions.

7. VITAMIN ANALYSIS

Vitamins are analyzed in foods/feedstuffs and biological specimens for different purposes:

- **Foods/feedstuffs**—for ascertaining potency for food labeling and nutrient database development, for evaluating storage stability, and for estimating vitamin intakes;
- **Biological specimens**—for determining vitamin bioavailability and for vitamin nutritional status.

Various methods are available for the quantitative determination of the vitamins (Table 3.14). Because many vitamers are bound to proteins (e.g., ε-pyridoxyllysine, niacytin) or other factors (e.g., pyridoxine-5′-β-D-glucoside) in foods or can be entrapped in food matrices (e.g., folates), their

20. Some authors have used the term **bioefficacy** with a similar connotation.

TABLE 3.11 Excretory Forms of the Vitamins

Vitamin	Urinary Form(s)	Fecal Form(s)
Vitamin A	Retinoic acid, acidic short-chain forms	Retinoyl glucuronides; intact-chain products
Vitamin D		25,26-$(OH)_2$-D; 25-$(OH)_2$-D-23,26-lactone
Vitamin E	Some carboxylchromanol metabolites	Tocopheryl quinone; tocopheronic acid and its lactone
Vitamin K K's and MK's		Vitamin K-2,3-epoxide; 2-CH_3-3(5´-carboxy-3′-CH_3-2´-pentenyl)-1,4-naphthoquinone; 2-CH_3-3(3´-carboxy-3´-methylpropyl)-1,4-napthoquinone; other unidentified metabolites
Menadione	Menadiol phosphate; menadiol sulfate	Menadiol glucuronide
Vitamin C[a]	Ascorbate-2-sulfate; oxalic acid; 2,3-diketogulonic acid	
Thiamin	Thiamin; thiamin disulfide; thiamin pyrophosphate; thiochrome	
	2-Methyl-4-amino-5-pyrimidine carboxylic acid; 4-methyl-thiazole-5-acetic acid	
	2-Methyl-4-amino-5-hydroxymethyl pyrimidine; 5-(2-hydroxyethyl)-4-methylthiazole	
	3-(2′-Methyl-4-amino-5´-pyrimidinylmethyl)-4-methylthiazole-5-acetic acid	
	2-Methyl-4-amino-5-formylaminomethylpyrimidine; other minor metabolites	
Riboflavin	Riboflavin; 7- and 8-hydroxmethylriboflavins; 8β-sulfonylriboflavin, riboflavinyl peptide ester; 10-hydroxyethylflavin, lumiflavin, 10-formyl-methylflavin; 10-carboxymethylflavin; lumichrome	
Niacin	N^1-methylnicotinamide; nicotinuric acid; nicotinamide-N^1-oxide; N^1-methylnicotinamide-N^1-oxide; N^1-methyl-4-pyridone-3-carboxamide; N^1-methyl-2-pyridone-5-carboxamide	
Vitamin B_6	Pyridoxol, pyridoxal, pyridoxamine, and respective phosphates; 4-pyridoxic acid and its lactone; 5-pyridoxic acid	
Biotin	Biotin; *bis-nor*-biotin; biotin *d*- and *l*-sulfoxide	
Pantothenic acid	Pantothenic acid	
Folate	Pteroylglutamic acid; 5-methyl-pteroylglutamic acid; 10-HCO-FH_4; pteridine acetamidobenzoylglutamic acid	Intact folates
Vitamin B_{12}	Cobalamin	Cobalamin

[a]*Substantial amounts are also oxidized to CO_2 and are excreted across the lungs.*

extraction necessitates disruption of those complexes and separation from interfering substances. This must be done in ways that are both quantitative and accommodate the intrinsic characteristics of each vitamer. Accordingly, conditions of sample extraction must stabilize the vitamin(s) of interest to yield accurate results. Chromatographic separations have proven useful for determining vitamins A, D, E, K, C, thiamin, riboflavin, niacin, and vitamin B_6. They depend on separation by phase partitioning (liquid–liquid[21] or gas–liquid[22]) of vitamers for specificity, ascertained by comparison to authentic standards, and a suitable means of detection (e.g., ultraviolet–visible absorption, fluorescence, electrochemical reactivity, and mass spectrometry)

21. High-performance liquid chromatography, HPLC.
22. Gas-liquid chromatography, GLC.

TABLE 3.12 Metabolic Functions of the Vitamins

Vitamin	Functions
Coenzymes	
Vitamin A	Rhodopsin conformational change following light-induced bleaching
Vitamin K	Vitamin K-dependent peptide-glutamyl carboxylase
Vitamin C	Cytochrome *P*-450-dependent oxidations (drug and cholesterol metabolism, steroid hydroxylations)
Thiamin	Cofactor of α-keto acid decarboxylases and transketolase
Niacin	NAD(H)/NADP(H) used by more than 30 dehydrogenases in the metabolism of carbohydrates (e.g., glucose-6-phosphate dehydrogenase), lipids (e.g., α-glycerol phosphate dehydrogenase), protein (e.g., glutamate dehydrogenase); Krebs cycle, rhodopsin synthesis (alcohol dehydrogenase)
Riboflavin	FMN: L-amino acid oxidase, lactate dehydrogenase, pyridoxine (pyridoxamine); 5´-phosphate oxidase
	FAD: D-amino acid and glucose oxidases, succinic and acetyl CoA dehydrogenases; glutathione, vitamin K, and cytochrome reductases
Vitamin B_6	Metabolism of amino acids (aminotransferases, deaminases, decarboxylases, desulfhydrases), porphyrins (δ-aminolevulinic acid synthase), glycogen (glycogen phosphorylase), and epinephrine (tyrosine decarboxylase)
Biotin	Carboxylations (pyruvate, acetyl CoA, propionyl CoA, 3-methylcrotonyl CoA carboxylases), and transcarboxylations (methylmalonyl CoA carboxymethyltransferase)
Pantothenic acid	Fatty acid synthesis/oxidation
Folate	Single-carbon metabolism (serine–glycine conversion, histidine degradation, purine synthesis, methyl group synthesis)
Vitamin B_{12}	Methylmalonyl CoA mutase, N^5-CH_3-FH_4:homocysteine methyltransferase
H^+/e^- Donors/Acceptors (Cofactors)	
Vitamin K	Converts the epoxide form in the carboxylation of peptide glutamyl residues
Vitamin C	Oxidizes dehydroascorbic acid in hydroxylation reactions
Niacin	Interconverts NAD^+/NAD(H) and $NADP^+$/NADP(H) couples in several dehydrogenase reactions
Riboflavin	Interconverts FMN/FMNH/$FMNH_2$ and FAD/FADH/$FADH_2$ systems in several oxidases
Pantothenic acid	Oxidizes coenzyme A in the synthesis/oxidation of fatty acids
Antioxidants	
Vitamin E	Protects polyunsaturated membrane phospholipids and other substances from oxidative damage via conversion of tocopherol to tocopheroxyl radical and, then, to tocopheryl quinone
Vitamin C	Protects cytosolic substances from oxidative damage
Hormones	
Vitamin A	Signals coordinate metabolic responses of several tissues
Vitamin D	Signals coordinate metabolism important in calcium homeostasis
Effectors of Gene Transcription	
Vitamin A	Bind nuclear retinoid receptors to signal transcription of multiple pathways
Vitamin D	Regulates transcription of some 50 genes associated with many aspects of metabolism

TABLE 3.13 Several Factors Affecting Vitamin Bioavailability

Factor	Examples
Extrinsic Factors	
Differing inherent biopotencies	Cholecalciferol (vitamin D_3) is nearly 10-fold more biopotent than ergocalciferol (vitamin D_2) for humans; the difference is greater for the chick
	All-*rac*-α-tocopherol has 50% of the biopotency of R,R,R-α-tocopherol
	Pyridoxine-5′-β-D-glucoside has half the biopotency of pyridoxine for humans
	α-Carotene, which yields upon central cleavage only a single mole of retinol, has half of the biopotency of β-carotene, which yield 2 mol of retinol
Losses	The vitamin C content of potatoes can drop by one-third within 1 month of storage
	NADH and NADPH appear to be unstable in the acidic conditions of the stomach
	Pyridoxine can bind to proteins during food processing/storage to form ε-pyridoxyllysine, which has half of the biopotency of the parent vitamer
	10-Formyltetrahyrdofolic acid is susceptible to oxidation to 10-formyldihydrofolic acid as well as interconversion to 5,10-methenyltetrahydrofolic acid under acidic conditions
Dietary/food effects	Vitamin A, provitamin A carotenoids, and vitamin D are poorly absorbed from very low-fat diets
	ε-Pyridoxyllysine, which has half of the biopotency of pyridoxine, is present in many plant tissues
	Niacin bound to proteins in some foods (e.g., maize, as niacytin) is not bioavailable unless hydrolyzed during food preparation/cooking to yield niacin
	Some folates in foods can be entrapped in their food matrix and be less biopotent than predicted based on chemical analysis
Intrinsic Factors	
Physiological effects	Provitamin A carotenoids can be less bioavailable than expected based on chemical analysis due to incomplete intestinal cleavage to retinol
	Folic acid (tetrahydrofolic acid) is generally better absorbed than most food forms of (polyglutamated) folate, which requires deconjugation to be absorbed
Health status	Folate absorption is impaired in patients with enteritis, e.g., sprue
	Absorption of vitamin B_{12} is reduced in individuals with digestive disorders, including many older persons who experience diminished gastric parietal cell function

TABLE 3.14 Methods of Vitamin Analysis

Vitamin	Sample Preparation	Instrumental Analysis		Immunological Analysis	Microbiological Assay
		Separation	Detection		
Vitamin A	Direct solvent extraction; alkaline hydrolysis[a], extraction into organic solvents	HPLC[b]	UV absorption, MS[c]	ELISA[d]	
Vitamin D	Alkaline hydrolysis with extraction into organic solvents	HPLC	UV, MS	ELISA	
Vitamin E	Alkaline hydrolysis with extraction into organic solvents	HPLC	Fluorescence, UV, MS	ELISA	
Vitamin K	Direct solvent extraction; supercritical fluid	HPLC	UV	ELISA	
	Extraction[e]; enzymatic hydrolysis				
Vitamin C	Acid hydrolysis	HPLC, IEC[f], MECC[g]	UV, MS		
Thiamin	Acid hydrolysis; enzymatic hydrolysis[h] absorption	IEC, GLC[i], HPLC	FID[k], MS	Fluorescence, UV, ELISA	*Lactobacillus viridescens* (12706)[j]
Riboflavin	Acid hydrolysis	HPLC, MECC	Fluorescence, UV, MS		*Lactobacillus casei* subsp. *rhamnosus* (7469)
					Enterococcus faecalis (10100)
Niacin	Alkaline hydrolysis	IEC, HPLC GLC	UV, FID		*Lactobacillus plantarum* (8014)[l]
		MECC			*Leuconostoc mesenteroides* subsp. *mesenteroides* (9135)
Vitamin B_6	Acid hydrolysis	HPLC, IEC, GLC	Fluorescence, UV	ELISA	*Saccharomyces carlsbergensis* (9080)
		MECC	FID, MS		*Kloeckera apiculata* (8714)
Pantothenic acid	Alkaline hydrolysis; enzymatic hydrolysis	GLC	FID	ELISA	*Lactobacillus plantarum* (8014)[l]

Folate	Enzymatic hydrolysis[o]	IEC	MS	ELISA	*Lactobacillus casei* subsp. *rhamnosus* (7469)[m]
					Enterococcus hirae (8043)[n]
Biotin	Acid hydrolysis; enzymatic hydrolysis[p]		MS	ELISA	*Lactobacillus plantarum* (8014)
Vitamin B_{12}	Direct solvent extraction	MECC	UV absorption	ELISA	*Lactobacilus delbrueckii*, subsp. *lactis* (4797)

[a]*Saponification.*
[b]*High-performance liquid chromatography (HPLC).*
[c]*Mass spectrometry(MS).*
[d]*Enzyme-linked immunosorbent assay (ELISA).*
[e]*Supercritical fluids are gases held above its critical temperature and critical pressure, which confers solvating properties similar to organic solvents with very low viscosities and very high diffusivities.*
[f]*Ion-exchange chromatography (IEC).*
[g]*Micellar electrokinetic capillary electrophoresis or chromatography (MECC).*
[h]*Thiaminase or other phosphatase.*
[i]*Gas–liquid chromatography (GLC).*
[j]*American Type Culture Collection number.*
[k]*Flame ionization detection (FID, used with GLC separation).*
[l]*Responds to nicotinic acid only.*
[m]*Responds to free vitamer only.*
[n]Responds to all vitamers; yields "total" folate activity.
[o]*Folyl conjugase.*
[p]*Papain.*

for sensitivity. Microbiological assays are available for thiamin, riboflavin, niacin, vitamin B_6, pantothenic acid, biotin, folate, and vitamin B_{12}. These methods are based on the absolute requirement of certain microorganisms for particular vitamins for multiplication, which can be measured turbidimetrically or by the evolution of CO_2 from substrate provided in the growth media. Some forms of vitamins A, E, and C can be measured by chemical colorimetric reactions; however, only the dye reduction methods for ascorbic acid have appropriate specificity and reliability to be recommended.[23] Competitive protein-binding assays have been developed for biotin, folate, and vitamin B_{12}.[24] Enzyme-linked immunosorbent assays have been developed for vitamins A, D, E, K, B_6, and B_{12}, as well as pantothenic acid, biotin, and folate. While microbiological and immunological methods are generally economical, they lack the capacity of liquid chromatography–tandem mass spectrometry (LC-MS/MS) for unequivocal identification of multiple specific vitamers based on characteristic patterns of ion daughters produced instrumentally.

8. STUDY QUESTIONS AND EXERCISES

1. Prepare a concept map of the relationships between the chemical structures, the physical properties, and the modes of absorption, transport, and tissue distributions of the vitamins.
2. For each vitamin, identify the key feature(s) of its chemical structure. How is/are this/these feature(s) related to the stability and/or biologic activity of the vitamin?
3. Discuss the general differences between the fat-soluble and water-soluble vitamins, and the implications of those differences in diet formulation and meal preparation.
4. Which vitamins would you suspect might be in shortest supply in the diets of livestock? in your own diet? Explain your answer in terms of the physicochemical properties of the vitamins.
5. Which vitamins would you expect to be stored well in the body? Which would you expect to be unstable in foods or feeds?
6. What factors would you expect to influence the absorption of specific vitamins?

23. Vitamin A: The Carr–Price method, based on the time-sensitive production of a blue complex of retinol and antimony trichloride, is no longer recommended due to its lack of specificity and negative bias. Vitamin E: The Fe^{2+}-dependent reduction of a fat-soluble dye such as bathophenanthroline by vitamin E is not recommended due to its lack of specificity, although many interfering substances can be partitioned into aqueous solvents during sample preparation. **Vitamin C:** The reaction of ascorbic acid with the dye 2,4-dinitrophenolindolphenol remains a useful method due to the fact that most interfering substances can be partitioned into organic solvents during sample preparation.

24. Biotin and avidin; folate and folate-binding protein; vitamin B12 and transcobalamin or R proteins.

RECOMMENDED READING

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Chapter 4

Vitamin Deficiency

Chapter Outline

Anchoring Concepts

1. A **disease** is an interruption or perversion of function of any of the organs with characteristic signs and/or symptoms caused by specific biochemical and morphological changes.
2. **Deficient intakes** of essential nutrients can cause disease.

These diseases … were considered for years either as intoxication by food or as infectious diseases, and twenty years of experimental work were necessary to show that diseases occur which are caused by a deficiency of some essential substance in the food.

C. Funk[1]

LEARNING OBJECTIVES

1. To understand the concept of **vitamin deficiency**.
2. To understand that deficient intakes of vitamins lead to **sequences of lesions** involving changes starting at the biochemical level, progressing to affect cellular and tissue function, and, ultimately, resulting in morphological changes.
3. To appreciate the range of possible morphological changes in organ systems that can be caused by vitamin deficiencies.
4. To get an overview of *specific clinical signs and symptoms* of deficiencies of each vitamin in animals, including humans, as background for further study of the vitamins.
5. To appreciate the relationships of clinical manifestations of vitamin deficiencies and lesions in the biochemical functions of those vitamins.

VOCABULARY

Achlorhydria
Achromotrichia
Acrodynia
Age pigments
Alopecia
Anemia
Anorexia
Arteriosclerosis
Ataxia
Beriberi
Bradycardia
Brown bowel disease
Brown fat disease
Cage layer fatigue
Capillary fragility
Cardiomyopathy
Cataract
Cervical paralysis
Cheilosis
Chondrodystrophy
Cirrhosis
Clinical signs
Clubbed down
Convulsion
Cornification
Curled toe paralysis
Dermatitis
Desquamation
Dystrophy
Edema
Encephalomalacia
Encephalopathy
Exudative diathesis
Fatty liver and kidney syndrome
Geographical tongue
Glossitis

1. Casimir Funk (1884–1967) was a Polish born chemist who worked at the Lister Institute in London and is credited with formulating the vitamin concept, which he called "vitamines," and later elucidated the chemical structure of thiamin.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00004-6

Hyperkeratosis
Hypovitaminosis
Inflammation
Keratomalacia
Leukopenia
Lipofuscin(osis)
Malabsorption
Mulberry heart disease
Myopathy
Necrosis
Nephritis
Neuropathy
Night blindness
Nyctalopia
Nystagmus
Opisthotonos
Osteomalacia
Osteoporosis
Pellagra
Perosis
Photophobia
Polyneuritis
Retrolental fibroplasia
Rickets
Scurvy
Steatitis
Stomatitis
Symptom
Vitamin deficiency
Wernicke–Korsakoff syndrome
White muscle disease
Xerophthalmia
Xerosis

1. THE CONCEPT OF VITAMIN DEFICIENCY

What Is Meant by the Term *Vitamin Deficiency*?

Because the gross functional and morphological changes caused by deprivation of the vitamins were the source of their discovery as important nutrients, these signs have become the focus of attention for many with interests in human and/or veterinary health. Indeed, freedom from clinical diseases caused by insufficient vitamin nutriture has generally been used as the main criterion by which vitamin requirements have been defined. The expression **vitamin deficiency** therefore simply refers to the basic condition of **hypovitaminosis**. Vitamin deficiency is distinct from but underlies the various biochemical changes, physiological and/or functional impairments, or other overt disease signs by which the need for a vitamin is defined.

> A vitamin deficiency is … the shortage of supply of a vitamin relative to its needs by a particular organism.

Vitamin Deficiencies Involve Cascades of Progressive Changes

The diseases associated with low intakes of particular vitamins are clinical manifestations of a progressive sequence of lesions that result from initial biochemical perturbations (e.g., diminished enzyme activity due to lack of a coenzyme or cosubstrate; membrane dysfunction due to lack of a stabilizing factor) that lead first to cellular and subsequently to tissue and organ dysfunction. Thus, the early stages of vitamin deficiency are subclinical and detectable only with biochemical indicators. If uncorrected, these marginal changes lead to characteristic clinical (observable) signs (Fig. 4.1). This cascade can be generalized in four stages.

The Four Stages of Vitamin Deficiency

Subclinical (marginal) deficiency	**Stage I Depletion of vitamin stores**, which leads to…
	Stage II Cellular metabolic changes, which lead to…
Clinical (observable) deficiency	**Stage III Functional defects**, which ultimately produce…
	Stage IV Morphological changes.

Marks[2] illustrated this point with the results of a study of thiamin depletion in human volunteers (Fig. 4.2). When subjects were fed a thiamin-free diet, no changes of any type were detected for 5–10 days, after which the first signs of decreased saturation of erythrocyte transketolase with its essential cofactor, thiamin pyrophosphate (TPP), were noted. Not until nearly 200 days of depletion—i.e., long after tissue thiamin levels and transketolase–TPP saturation had declined—were classic **clinical signs**[3] of thiamin deficiency (**anorexia**, weight loss, malaise, insomnia, hyperirritability) detected.

Marginal deficiencies of vitamins in which the impacts of poor vitamin status are not readily observed without chemical or biochemical testing are often referred to as **subclinical deficiencies** for that reason. Subclinical deficiencies involve depleted reserves or localized abnormalities without the presence of overt functional or morphological defects. The traditional perspective has been that the

2. Marks, J., 1968. The Vitamins in Health and Disease: A Modern Reappraisal. Churchill, London, UK.
3. A symptom is a change, whereas a clinical sign is a change detectable by a trained observer, e.g., a physician.

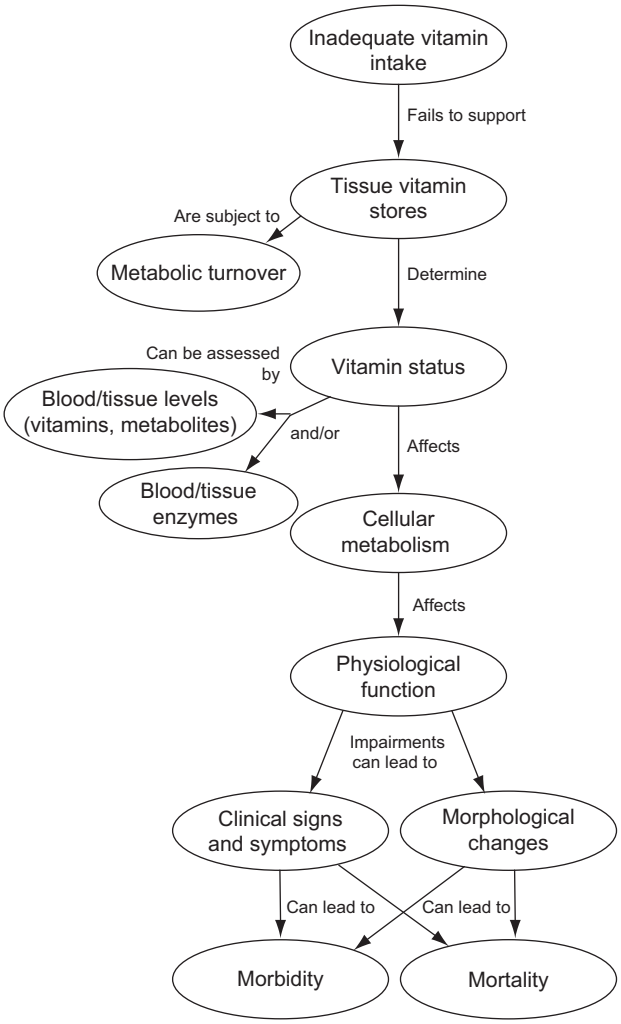

FIGURE 4.1 Concept map of effects of inadequate vitamin intake.

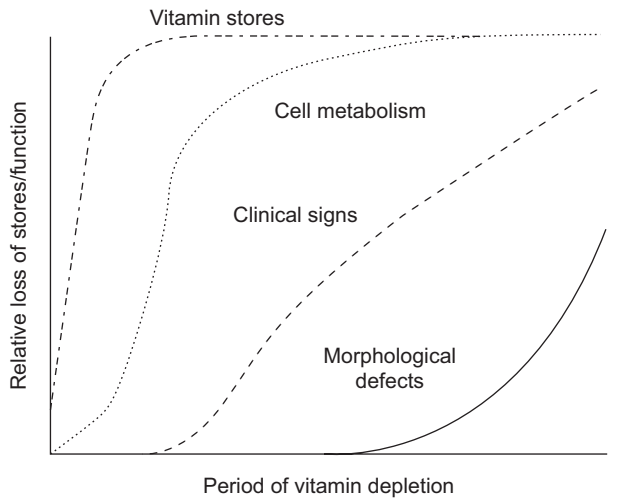

FIGURE 4.2 The four progressive stages of deficiency, starting with tissue depletion and ending with morphological changes. *From Marks, J., 1968. The Vitamins in Health and Disease: A Modern Reappraisal. Churchill, London, UK.*

absence of overt, clinical manifestations of deficiency constitutes good nutrition; this perspective ignores the importance of preventing the early functional impairments that can progress to overt clinical signs. Therefore, the modern view of nutritional adequacy must focus on the maintenance of normal metabolism and, in several cases, body reserves as criteria of adequate vitamin status.[4]

2. CLINICAL MANIFESTATIONS OF VITAMIN DEFICIENCIES

Many Organ Systems Can Be Affected by Vitamin Deficiencies

Every organ system of the body can be the target of a vitamin deficiency. Some vitamin deficiencies affect certain organs preferentially (e.g., vitamin D deficiency chiefly affects calcified tissues); others affect several or many organs in various ways. Because the diagnosis of a vitamin deficiency involves its differentiation from other potential causes of similar clinical signs, it is useful to consider the various morphologic lesions caused by vitamin deficiencies from an organ system perspective. After all, anatomical and/or functional changes in organs are the initial presentations of deficiencies of each of the vitamins. This point is illustrated in Table 4.1, which details the organ systems affected by vitamin deficiencies.

Manifestations of Biochemical Lesions

Relationships Between Biochemical Lesions and Clinical Diseases of Vitamin Deficiencies

The clinical signs and symptoms that characterize the vitamin deficiency diseases are manifestations of underlying impairments (i.e., *lesions*) in biochemical function that result from insufficient vitamin supply (Table 4.2). This is a fundamental concept in understanding the roles of the vitamins in nutrition and health. Hypovitaminosis of sufficient magnitude and duration is causally related to the morphological and/or functional changes associated with the latter stages of vitamin deficiency. While the validity of this concept may be apparent in the abstract, documentary evidence for it in the case of each of the vitamin deficiency diseases, that is, direct cause–effect linkages of specific biochemical lesions and clinical changes, is, for many vitamins, not complete.

Vitamin A offers a case in point of this fact. While the role of vitamin A in preventing nyctalopia (night blindness)

4. By such criteria, marginal vitamin status in the United States appears to be quite prevalent, even though the prevalence of clinically significant vitamin deficiencies appears to be very low. Estimates of marginal status with respect to one or more vitamins have been as high as 15% of adolescents, 12% of persons 65 years of age and older, and 20% of dieters.

TABLE 4.1 Organ Systems Affected by Vitamin Deficiencies in Humans and Other Animals

Organ Systems	Vitamin A	Vitamin D	Vitamin E	Vitamin K	Ascorbic Acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic Acid	Folate	Vitamin B_{12}
General													
Appetite	+	+	+		+[a]	+	+	+	+	+			
Growth	+	+	+	+	+[a]	+	+	+	+	+	+	+	+
Integument													
Skin	+						+	+					
Hair, nails, feathers	+						+			+			
Musculature													
Skeletal muscles			+										
Heart						+							
Gizzard			+										
Vascular System													
Vessels			+	+	+[a]				+				
Blood cells			+				+					+	+
Clotting system				+									
Gastrointestinal Tract													
Stomach						+		+					
Mouth							+						
Tongue								+					
Small intestine			+	+		+							
Colon							+	+					
Skeletal System													
Bone	+	+			+[a]		+	+					
Teeth		+			+[a]								
Vital organs													
Liver			+			+							

Kidney	+						+		+				
Thymus											+		
Adrenals							+				+		
Pancreas									+				
Adipose			+										
Ocular System													
Eye	+		+			+	+						
Reproductive System													
Vagina	+												
Uterus			+										
Ovary	+						+						
Egg		+											
Testes	+												
Fetus	+						+						
Nervous System													
General	+	+				+	+	+	+		+		
Spinal cord	+					+							
Brain			+										
Peripheral nerves			+										+
Psychological, emotional						+		+					

[a]Only human, higher primates, the guinea pig, and some birds are affected by ascorbic acid deprivation.

TABLE 4.2 The Underlying Biological Functions of the Vitamins

Vitamin	Active Form(s)	Deficiency Disorders	Important Biological Functions or Reactions
Vitamin A	Retinol, retinal, retinoic acid	Night blindness, xerophthalmia, keratomalacia, impaired growth	Photosensitive retinal pigment
			Regulation of epithelial cell differentiation
			Regulation of gene transcription
Vitamin D	1,25-$(OH)_2$-D	Rickets, osteomalacia	Promotion of intestinal calcium absorption, mobilization of calcium from bone, stimulation of renal calcium resorption, regulation of PTH secretion, possible function in muscle
Vitamin E	a-Tocopherol	Nerve, muscle degeneration	Antioxidant protector for membranes
Vitamin K	K_n, MK_n	Impaired blood clotting	Cosubstrate for γ-carboxylation of glutamyl residues of several clotting factors and other calcium-binding proteins
Vitamin C	Ascorbic acid, dehydroascorbic acid	Scurvy	Cosubstrate for hydroxylations in collagen synthesis, drug, and steroid metabolism
Thiamin	Thiamin pyrophosphate	Beriberi, polyneuritis, Wernicke–Korsakoff syndrome	Coenzyme for oxidative decarboxylation of 2-keto acids (e.g., pyruvate, 2-keto-glutarate); coenzyme for pyruvate decarboxylase and transketolase
Riboflavin	FMN, FAD	Dermatitis	Coenzymes for numerous flavoproteins that catalyze redox reactions in fatty acid synthesis/degradation, TCA cycle
Niacin	NAD(H), NADP(H)	Pellagra	Cosubstrates for hydrogen transfer catalyzed by many dehydrogenases, e.g., TCA cycle respiratory chain
Pyridoxine	Pyridoxal-5′-phosphate	Signs vary with species	Coenzyme for metabolism of amino acids, e.g., side chain, decarboxylation, transamination, racemization
Folate	Polyglutamyl tetrahydrofolates	Megaloblastic anemia	Coenzyme for transfer of single-carbon units, e.g., formyl and hydroxymethyl groups in purine synthesis
Biotin	1′-*N*-carboxybiotin	Dermatitis	Coenzyme for carboxylations, e.g., acetyl CoA/malonyl CoA conversion
Pantothenic acid	Coenzyme A	Signs vary with species	Cosubstrate for activation/transfer of acyl groups to form esters, amides, citrate, triglycerides, etc.
	Acyl carrier protein		Coenzyme for fatty acid biosynthesis
Vitamin B12	5′-deoxyadenosyl-B12	Megaloblastic anemia	Coenzyme for conversion of methylmalonyl CoA to succinyl CoA
	Methyl-B_{12}	Impaired growth	Methyl group transfer from 5-CH_3-FH_4 to homocysteine in methionine synthesis

5-CH_3-FH_4, 5-methyltetrahydrofolic acid; *CoA*, coenzyme A; *PTH*, parathyroid hormone; *TCA*, tricarboxylic acid cycle.

is clear from presently available knowledge of the essentiality of retinal as the prosthetic group of rhodopsin and several other photosensitive visual receptors in the retina, the amount of vitamin A in the retina, and thus available for visual function, is only about 1% of the total amount of vitamin A in the body. Further, it is clear from the clinical signs of vitamin A deficiency that the vitamin has other essential functions unrelated to vision, especially relating to the integrity and differentiation of epithelial cells. However, although evidence indicates that vitamin A is involved in the metabolism of mucopolysaccharides and other essential intermediates, present knowledge does not adequately explain the mechanism(s) of action of vitamin A in supporting growth and in maintaining epithelia. It has been said that 99% of our information about the mode of action of vitamin A concerns only 1% of the vitamin A in the body.

The ongoing search for a more complete understanding of the mechanisms of vitamin action is therefore largely based on the study of biochemical correlates of changes in physiological function or morphology effected by changes in vitamin status. Most of this knowledge has come from direct experimentation, mostly with animal models. Also edifying in this regard has been information acquired from observations of individuals with different rare, naturally occurring, hereditary anomalies involving

vitamin-dependent enzymes and transport proteins. Most of the documented inborn metabolic errors (Table 4.3) involve specific mutations manifest as either a loss or aberration in single factors in vitamin metabolism—a highly targeted situation not readily produced experimentally.[5]

Diagnosing Vitamin Deficiencies

Vitamin deficiencies can be diagnosed based on the organ system affected, the specific clinical signs and/or biochemical lesion, and reference to accepted biomarkers of status (Tables 4.3 and 4.4 can be helpful). This requires a three-step analysis:

1. Identify prospective vitamin deficiencies based on mapping of **signs/symptoms** to those reported in the scientific literature and considering relevant demographic and environmental predictors.
2. Use the appropriate **clinical biochemical markers** to exclude possibilities.
3. Determine the actual deficiency(ies) involved based on **responses to treatment.**

3. CAUSES OF VITAMIN DEFICIENCIES

Primary and Secondary Causes of Vitamin Deficiencies

The balance of vitamin supply and biological need of an individual is called **vitamin status**. Reductions in vitamin status can be produced either by reductions in effective vitamin supply or by increases in effective vitamin need. Vitamin deficiency occurs when vitamin status is reduced to the point of having metabolic impact (i.e., stage II); if not corrected, continued reductions in vitamin status lead inevitably to the observable stages of vitamin deficiency (stages III and IV), at which point serious clinical and morphological changes can manifest. When these changes occur as a result of the failure to ingest a vitamin in sufficient amounts to meet physiological needs, the condition is called a **primary deficiency**. When these changes come about as a result of the failure to absorb or otherwise utilize a vitamin owing to an environmental condition or physiological state, and not to insufficient consumption of the vitamin, the condition is called a **secondary deficiency**.

Causes of Vitamin Deficiencies in Humans

Many of the ways in which vitamin deficiencies can develop are interrelated. For example, poverty and disempowerment are often accompanied by lack of nutrition knowledge and result in a poor diet. People living alone, especially the elderly and others with chronic disease, tend to consume foods that require little preparation and that may not provide adequate nutrition. Despite these potential causes of vitamin deficiency, in most of the technologically developed parts of the world the general level of nutrition is high. In those areas, relative few persons can be expected to show signs of vitamin deficiency; those that do present such signs frequently have a potentiating condition that affects either their consumption of food or their utilization of nutrients. In low-income parts of the world, however, food insecurity is still the largest single cause of general malnutrition today, including deficiencies of multiple nutrients.

High-Risk Groups for Vitamin Deficiencies

- Pregnant women
- Infants and young children
- Elderly people
- Vegetarians
- Food-insecure people
- People with intestinal parasites or infections
- Dieters
- Smokers

Primary deficiencies in humans, therefore, tend to have psychosocial and technological causes:

- Poor food habits
- Poverty (i.e., low food-purchasing power)
- Ignorance (i.e., lack of sound nutrition information)
- Lack of total food (e.g., crop failure)
- Lack of vitamin-rich foods (e.g., consumption of highly refined foods)
- Vitamin destruction (e.g., during storage, processing, and/or cooking)
- Anorexia (e.g., homebound elderly, infirm, dental problems)
- Food taboos and fads (e.g., fasting, avoidance of certain foods)
- Apathy (lack of incentive to prepare adequate meals).

Whereas, secondary deficiencies in humans typically have biological causes:

- Poor digestion (e.g., **achlorhydria**—absence of stomach acid)
- **Malabsorption** (impaired intestinal absorption of nutrients; e.g., as a result of diarrhea, intestinal infection, parasites, and pancreatitis)
- Impaired metabolic utilization (e.g., certain drug therapies)
- Increased metabolic need (e.g., pregnancy, lactation, rapid growth, infection, and nutrient imbalance)
- Increased vitamin excretion (e.g., diuresis, lactation, and excessive sweating).

5. However, it is theoretically possible to produce transgenic animal models with similar metabolic anomalies.

TABLE 4.3 Vitamin-Responsive Inborn Metabolic Lesions

Curative Vitamin	Missing Protein or Metabolic Step Affected	Clinical Condition
Vitamin A	Apolipoprotein B	Abetalipoproteinemia; low tissue levels of retinoids
Vitamin D	Receptor	Unresponsive to $1,25(OH)_2$-D; osteomalacia
Vitamin E	Apolipoprotein B	Abetalipoproteinemia; low tissue levels of tocopherols
Thiamin	Branched-chain 2-oxoacid dehydrogenase	Maple syrup urine disease
	Pyruvate metabolism	Lactic acidemia; neurological anomalies
Riboflavin	Methemoglobin reductase	Methemoglobinemia
	Electron transfer flavoprotein	Multiple lack of acyl CoA dehydrogenations, excretion of acyl CoA metabolites, i.e., metabolic acidosis
Niacin	Abnormal neurotransmission	Psychiatric disorders, tryptophan malabsorption, abnormal tryptophan metabolism
Pyridoxine	Cystathionine β-synthase	Homocysteinuria
	Cystathionine γ-lyase	Cystathioninuria; neurological disorders
	Kynureninase	Xanthurenic aciduria
Folate	Enteric absorption	Megaloblastic anemia, mental disorder
	Methylene-FH_4-reductase	Homocysteinuria, neurological disorders
	Glutamate formiminotransferase	Urinary excretion of FIGLU[a]
	Homocysteine/methionine conversion	Schizophrenia
	Tetrahydrobiopterin–phenylalanine hydrolase	Mental retardation, PKU[b]
	Dihydrobiopteridine reductase	PKU, severe neurological disorders
	Tetrahydrobiopterin formation	PKU, severe neurological disorders
Biotin	Biotinidase	Alopecia, skin rash, cramps, acidemia, developmental disorders, excess urinary biotin, and biocytin
	Propionyl CoA carboxylase	Propionic acidemia
	3-Methylcrotonyl CoA carboxylase	3-Methylcrontonylglycinuria
	Pyruvate carboxylase	*Leigh disease*, accumulation of lactate and pyruvate
	Acetyl CoA carboxylase	Severe brain damage
	Holocarboxylase synthase	Lack of multiple carboxylase activities, urinary excretion of metabolites
Vitamin B_{12}	Intrinsic factor/enteric absorption	Juvenile pernicious anemia
	Transcobalamin	Megaloblastic anemia, growth impairment
	Methylmalonyl CoA mutase	Methylmalonic acidemia

[a]FIGLU, *formiminoglutamic acid.*
[b]PKU, *phenylketonuria.*

Causes of Vitamin Deficiencies in Animals

Many of the same primary and secondary causal factors that result in vitamin deficiencies in humans can also produce vitamin deficiencies in animals. In livestock, however, most of the serious cases of vitamin deficiency in animals are due to human errors involving improper or careless animal husbandry.

Primary deficiencies in animals typically have physical causes:

- Improperly formulated diet (i.e., error in vitamin premix formulation)
- Feed mixing error (e.g., omission of vitamin from vitamin premix)

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals

Signs and Symptoms by Organ System and Organ			Vitamin Deficiencies Possibly Involved	Humans Affected	Other Species Affected	Vitamin A	Vtiamin D	Vitamin E	Vitamin K	Ascorbic acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic acid	Folate	Vitamin B_{12}
System	Organ	Signs/Symptoms																
						Criteria for deficient/low status (humans)												
General		General weakness	Vitamin A		Cat													
			Vitamin D	+														
			Ascorbic acid	+														
			Thiamine	+	Rat													
			Riboflavin		Pig, dog, fox													
			Niacin	+	Chick													
			Pyridoxine		Rat, chick													
			Pantothenic acid	+	Chick													
		Reduced appetite	Vitamin A		Rat, chick, mouse, pig, calf													
			Vitamin D		Rat, chick, mouse, pig, calf													
			Vitamin K		Rat, chick, mouse, pig, calf													
			Ascorbic acid		Guinea pig													
			Thiamin[a]	+	Rat, chick, mouse, pig, calf													
			Riboflavin		Rat, chick, mouse, pig, calf													
			Niacin	+	Rat, chick, mouse, pig, calf													
			Pyridoxine		Rat, chick, mouse, pig, calf													
			Biotin		Rat, chick, mouse, pig, calf													
		Growth retardation	Ascorbic acid		Guinea pig													
			Other Vitamins		Rat, mouse, chick, dog, calf													
						Plasma retinol <10 µg/dl (<5 mos., >17 yrs); <20 µg/dl (5 mos.-17 yrs)	Plasma 25(OH)D3 <3 ng/ml	Plasma a-tocopherol <3.5 µg/ml	Clotting time >10 min	Plasma ascorbic acid <2 µg/ml; WBC ascorbic acid <8 µg/ml	RBC transketolase TPP-stimulation >25%; urine thiamin <40 µg/24 hr	RBC GSH reductase FAD-stimulation >40%; urine riboflavin <40 µg/24 hr	Urine N'-methyl-2-pyridone-5-carboxamide <1 µg/24 hr	Plasma pyridoxal phosphate <60 nM	Blood biotin <0.4 ng/ml; urine biotin <10 µg/24 hr	Plasma pantothenic acid <6 µg/dl; urine pantothenic acid <1 mg/24 hrs	Plasma folates <3 ng/ml; RBC folates <140 ng/ml	Plasma Vitamin B_{12} <100 pg/ml
Integument																		
Skin	Dermis	Scaly dermatitis[b]	Vitamin A		Cattle	+												
			Riboflavin		Pig							+						

Continued

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals—cont'd

Signs and Symptoms by Organ System and Organ			Vitamin Deficiencies Possibly Involved	Humans Affected	Other Species Affected	Vitamin A	Vtiamin D	Vitamin E	Vitamin K	Ascorbic acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic acid	Folate	Vitamin B_{12}
System	**Organ**	**Signs/Symptoms**																
			Pyridoxine		Rat (acrodynia[c])									+				
			Biotin		Rat, mouse, hamster, cat, mink, fox										+			
			Pantothenic acid		Rat										+			
		Cracking dermatitis	Niacin	+ (Pellagra)	Chick								+					
			Biotin		Pig, poultry, monkey										+			
			Pantothenic acid		Chick (feet)										+			
		Desquamation[d]	Riboflavin	+	Monkey, rat, chick, dog							+						
			Niacin	+	Chick								+					
			Pyridoxine	+										+				
			Biotin		Rat										+			
		Hyperkeratosis[e]	Riboflavin		Rat							+						
			Niacin	+ (Pellagra)	Chick								+					
			Biotin		Rat, mouse, hamster										+			
		Hyperpigmentation	Niacin	+ (Pellagra)									+					
		Photosensitization	Niacin	+ (Pellagra)									+					
Hair, nails, feathers		Rough	Vitamin A		Cattle, poultry (feathers)	+												
			Biotin		Poultry (feathers)													
		Achromatrichia[f]	Biotin		Rat, rabbit, cat, mink, fox, monkey										+			
			Pantothenic acid		Rat										+			
		Alopecia[g]	Riboflavin		Rat, pig, calf							+						
			Niacin		Rat, pig								+					
			Biotin		Rat, mouse, hamster, rabbit, pig, chick, cat, mink, fox: (spectacle eye)										+			
			Pantothenic acid		Rat										+			

		"Blood"-caked whiskers[h]	Pantothenic acid		Rat					+		
		Impaired growth	Biotin		Poultry					+		
			Folate		Poultry						+	
Musculature												
Skeletal muscles	Myopathy[i]		Vitamin E		Rat, guinea pig, pig, rabbit, chick, duck, calf, horse, goat, salmon, mink, catfish (white muscle dis,); lamb (stiff lamb dis.)	+						
			Ascorbic acid	+ (Scurvy)	Guinea pig		+					
			Thamin	+ (Beriberi)	Rat			+				
			Pantothenic acid		Pig					+		
Heart	Rhythm	Bradycardia	Thiamin	+ (Beriberi)				+				
	Muscle	Cardiomyopathy[j]	Thiamin	+ (Beriberi)	Rat			+				
			Vitamin E		Pig (mulberry heart dis.); guinea pig, rabbit, rat, dog, calf, lamb, goat	+						
Gizzard[k]		Myopathy	Vitamin E		Turkey poults, ducklings	+						
Vascular system												
Vessels	General	Arteriosclerosis[l]	Pyridoxine		Monkey				+			
	Capillary	Edema[m]	Vitamin E		Chick (exudative diathesis), pig (visceral edema)	+						
			Thiamin	+ (Beriberi)				+				
		Hemorrhage	Vitamin K		Poultry		+					
			Ascorbic acid	+ (Scurvy)	Guinea pig, monkey		+					
Blood cells	Erythrocyte	Hemolytic anemia[n]	Vitamin E	+	Pig, monkey	+						
		Hemorrhagic anemia[o]	Vitamin K		Rat, chick		+					
		Normocytic hypochromic anemia[p]	Riboflavin		Monkey, baboon				+			

Continued

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals—cont'd

Signs and Symptoms by Organ System and Organ			Vitamin Deficiencies Possibly Involved	Humans Affected	Other Species Affected	Vitamin A	Vtiamin D	Vitamin E	Vitamin K	Ascorbic acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic acid	Folate	Vitamin B_{12}
System	**Organ**	**Signs/Symptoms**																
		Megaloblastic anemia[q]	Folate	+	Rat, chick												+	
		Megaloblastic anemia	Vitamin B_{12}	+	Rat, chick													+
		Fragility	Vitamin E	+	Rat, pig, monkey			+										
	Leukocyte	Leukopenia[r]	Riboflavin	+								+						
			Folate		Rat, guinea pig												+	
	Platelet	Thrombocytosis[s]	Vitamin E		Rat			+										
		Excess aggregation	Vitamin E		Rat			+										
Clotting system	Prolonged clotting time		Vitamin K	+	Rat, chick, pig, calf				+									
Gastrointestinal tract																		
Stomach	Epithelium	Achlorhydria[t]	Niacin	+ (Pellagra)									+					
		Gastric distress	Thiamin	+ (Beriberi)							+							
Mouth		Stomatitis[u]	Riboflavin	+	Calf							+						
			Niacin	+ (Pellagra)									+					
			Biotin		Chick										+			
			Pantothenic acid		Chick										+			
		Cheliosis[v]	Riboflavin	+								+						
Tongue		Glossitis[w]	Niacin	+ (Pellagra)									+					
			Riboflavin	+	Rat							+						
			Niacin	+ (Pellagra)									+					
Small intestine	Mucosa	Inflammation	Thiamin		Rat						+							
			Riboflavin		Chick, dog, pig							+						
			Niacin		Chick								+					
		Ulcer	Thiamin		Rat						+							

	Enterocyte	Lipofuscinosis[x]	Vitamin E		Dog (brown bowel disease)			+										
		Hemorrhage	Vitamin K		Poultry				+									
			Thiamin		Rat						+							
			Niacin		Dog								+					
Colon		Diarrhea	Riboflavin		Chick, dog, pig, calf							+						
			Niacin	+ (Pellagra)	Dog, pig, poultry								+					
			Vitamin B_{12}		Young pigs													+
		Constipation	Niacin	+ (Pellagra)									+					
Skeletal system																		
Bone	Periosteum	Excessive growth	Vitamin A		Pig, dog, calf, horse, sheep	+												
	Epiphyses	Undermineralization malformations	Vitamin D	+ (Osteomalacia[y]: rickets in children)	Chick, dog, calf (rickets)		+											
			Ascorbic acid	Children						+								
	Cortical bone	Demineralization, increased fractures	Vitamin D	+ (Osteomalacia, osteoporosis[z])	Laying hen (caged layer fatigue)		+											
		Chondrodystrophy[aa]	Niacin		Chick, poult (perosis)								+					
			Biotin		Chick, poult (perosis)										+			
		Congenital deformities	Riboflavin		Rat							+						
			Pyridoxine		Rat									+				
Teeth	Dentin	Caries	Vitamin D	Children			+											
			Pyridoxine	+										+				
Vital organs																		
Liver	Hepatocyte	Necrosis	Vitamin E		Pig (hepatosis dietetica); rat, mouse			+										
		Steatosis[bb]	Thiamin		Rat						+							
			Biotin		Chick (fatty liver and kidney syndrome)										+			
			Pantothenic acid		Chick, dog										+			
		Cirrhosis[cc]	Choline		Rat, dog, monkey													

Continued

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals—cont'd

Signs and Symptoms by Organ System and Organ			Vitamin Deficiencies Possibly	Humans		Vitamin A	Vitamin D	Vitamin E	Vitamin K	Ascorbic acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic acid	Folate	Vitamin B_{12}
System	**Organ**	**Signs/Symptoms**	**Involved**	**Affected**	**Other Species Affected**													
Kidney	Nephron	Nephritis[dd]	Vitamin A	+		+												
		Steatosis	Riboflavin		Pig							+						
			Biotin		Chick										+			
		Hemorrhagic necrosis[ee]	Choline		Rat, mouse, pig, rabbit, calf													
		Calculi	Pyridoxine		Rat									+				
Thymus	Thymocyte	Necrosis	Pantothenic acid		Chick, dog										+			
Adrenals		Hypertrophy	Pantothenic acid		Pig										+			
		Hemorrhage	Riboflavin		Pig							+						
		Lymphoid necrosis	Riboflavin		Baboon							+						
			Pantothenic acid		Rat										+			
Pancreas	Eyelets	Insulin insufficiency	Pyrodoxine		Rat									+				
Adipose	Adipocyte	Lipofuscinosis	Vitamin E		Rat, mouse, hamster, cat, pig, mink (brown fat disease)			+										
Ocular system																		
Eye	Eyeball	Nystagmus[ff]	Thiamin	+ (Wernicke-Korsakoff synd.)							+							
	Retina	Photophobia	Riboflavin	+								+						
		Nyctalopia[gg]	Vitamin A	+	Rat, pig, cat, sheep	+												
		Pigmented retinopathy	Vitamin E	+	Rat, dog, cat, monkey			+										
	Cornea	Xerophthalmia[hh]	Vitamin A	+	Rat, calf	+												
		Keratomalacia[ii]	Vitamin A	+	Rat, calf	+												
	Lens	Cataract[jj]	Vitamin E		Rabbit, turkey embryo			+										

Reproductive system												
Vagina	Epithelium	Cornification[kk]	Vitamin A	+	Rat	+						
Uterus	Epithelium	Lipofuscinosis	Vitamin E		Rat		+					
Ovary	????	Degeneration	Vitamin A		Poultry	+						
	Estrus	Anestrus[ll]	Riboflavin		Rat				+			
		Low egg production	Vitamin A		Poultry	+						
			Riboflavin		Poultry				+			
			Pyridoxine		Poultry					+		
Egg	Shell	Thinning	Vitamin D		Poultry		+					
Testes	Germinal epithelium	Degeneration	Vitamin A		Rat, bull, cat	+						
			Vitamin E		Rat, rooster, dog, pig, guinea pig, hamster, rabbit, monkey		+					
Fetus		Developmental abnormalities	Riboflavin		Rat, chick (clubbed down)				+			
			Folate		Chick (parrot beak)						+	
		Death	Vitamin A		Poultry	+						
			Vitamin E		Rat		+					
			Riboflavin		Chick				+			
			Folate		Chick						+	
			Vitamin B_{12}		Poultry							+
Nervous system												
General		Ataxia[mm]	Vitamin A		Chick, pig, calf, sheep	+						
			Vitamin E	+	Chick		+					
			Thiamin	+ (Wernicke-Korsakoff synd.[nn])	Rat, mouse, chick, pig, rabbit, calf, monkey			+				
			Riboflavin		Rat, pig				+			
		Tremors	Niacin	+ (Pellagra)						+		
		Tetany	Vitamin D	+ (Rickets)	Chick, pig		+					
		Abnormal gait	Pantothenic acid		Pig, dog (goose-stepping)							+

Continued

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals—cont'd

Signs and Symptoms by Organ System and Organ			Vitamin Deficiencies Possibly Involved	Humans Affected	Other Species Affected	Vitamin A	Vtiamin D	Vitamin E	Vitamin K	Ascorbic acid	Thiamin	Riboflavin	Niacin	Pyridoxine	Biotin	Pantothenic acid	Folate	Vitamin B_{12}
System	**Organ**	**Signs/Symptoms**																
		Seizures	Pyridoxine	Infants	Rat									+				
		Paralysis	Riboflavin		Chick (curled toe paralysis); rat							+						
			Pyridoxine		Chick									+				
			Pantothenic acid		Poult (cervical paralysis); chick										+			
		Irritability	Thiamin	+ (Beriberi)							+							
			Niacin	+ (Pellagra)									+					
			Pyridoxine	+	Rat									+				
			Vitamin B_{12}		Pig													+
Spinal cord	Cerebro-spinal fluid	Excess pressure	Vitamin A		Chick, pig, calf	+												
		Opithotonus[oo]	Thiamin		Chick, pigeon (star gazing)						+							
Brain		Encephalopathy[pp]	Vitamin E		Chick (encephalomalacia[qq])			+										
			Thiamin	+ (Wernicke-Korsakoff synd.)							+							
Peripheral nerves		Neuropathy	Vitamin E		Rat, dog, duck, monkey			+										
			Thiamin	+ (Beriberi)	Chick (polyneuritis[rr])						+							
			Riboflavin	+								+						
			Niacin		Rat, pig								+					
			Pyridoxine		Rat									+				
			Pantothenic acid	+ (Burning feet)												+		
			Vitamin B_{12}	+														+

Psychological, emotional						
	Depression	Thiamin	+ (Beriberi)	+		
		Niacin	+ (Pellagra)		+	
	Anxiety	Thiamin	+ (Beriberi)	+		
	Dizziness	Niacin	+ (Pellagra)		+	
	Irritability	Thiamin	+ (Beriberi)	+		
		Niacin	+ (Pellagra)		+	
		Pyridoxine	+			+
	Dementia	Niacin	+ (Pellagra)		+	
	Psychosis	Thiamin	+ (Wernicke-Korsakoff synd.)	+		

[a]*Severe initiation of rapid onset.*
[b]*Inflammation of the skin.*
[c]*Swelling and* **necrosis** *(i.e., tissue and/or organ death) of the paws, tips of the ears and nose, and lips.*
[d]*Shedding of skin.*
[e]*Thickening of the stratum corneum.*
[f]*Loss of normal pigment from hair or feathers.*
[g]*Loss of hair or feathers.*
[h]*Whiskers accumulate porphyrins shed in tears.*
[i]*General term for disease of muscle.*
[j]*General term for disease of the heart muscle.*
[k]*Muscular portion of the forestomach of birds.*
[l]*General term for hardening, i.e., loss of elasticity, of medium or large arteries.*
[m]*Abnormal fluid retention.*
[n]*Abnormally low erythrocyte count due to their fragility, rupture, and clearance.*
[o]*Abnormally low erythrocyte count due to hemorhhage.*
[p]*Abnormally low hemoglobin content in otherwise normal erythrocytes.*
[q]*Abnormally low erythrocyte count due to impaired DNA synthesis, with erythroblast growth without division, i.e., forming macrocytes.*
[r]*Abnormally low white blood cell count.*
[s]*Abnormally high platelet count.*
[t]*Lack of gastric acid production due to dysfunction of gastric parietal cells.*
[u]*Inflammation of the oral mucosa (soft tissues of the mouth).*
[v]*Angular stomatitis, i.e., inflammatory lesions (cracks, fissures) at the labial commissure (corners of the mouth).*
[w]*Inflammation of the tongue.*
[x]*Accummulation of lipid oxidation products.*

Continued

TABLE 4.4 Diagnosis of Vitamin Deficiencies in Humans and Other Animals—cont'd

[y] *Demineralization leading to softening of bones.*
[z] *Progressive demineralization leading to thinning of bones, as in rickets in children.*
[aa] *Disorders of cartilaginous components of growing ends of bones.*
[bb] *Abnormal intracellular retention of lipids.*
[cc] *Chronic liver disease involving replacement of normal tissue with fibrosis and presence of regenerative nodules.*
[dd] *Inflammation of nephrons.*
[ee] *Cell death.*
[ff] *Involuntary eye movement.*
[gg] *Night blindness, i.e., difficulty seeing in low light.*
[hh] *Failure to produce tears due to dysfunction of lacrimal glands, resulting in dryness and thickening of the conjunctiva and cornea and leading to ulceration and blindness.*
[ii] *Drying and clouding of the cornea due to xerophthalmia.*
[jj] *Clouding of the lens.*
[kk] *Formation of an epidermal barrier in stratified squamous epithelial tissue by increased expression of keratin proteins.*
[ll] *Cessation of female ovulatory cycle.*
[mm] *Gross lack of muscular coordination.*
[nn] *Confusion, ataxia, nystagmus, and double vision due to damage in thalamus and hypothalamus (Wernicke's encephalopathy) progressing to loss of memory, confabulation, and hallucination (Korsokoff syndrome).*
[oo] *State of severe hyperextension and spasm of the axial muscles along the spinal column, causing an individual's head, neck, and spinal column to assume an arching position.*
[pp] *Term for global brain disease.*
[qq] *Degenerative disease of brain involving function: blindness, ataxia, circling, and terminal coma.*
[rr] *Neuropathy affecting multiple peripheral nerves.*

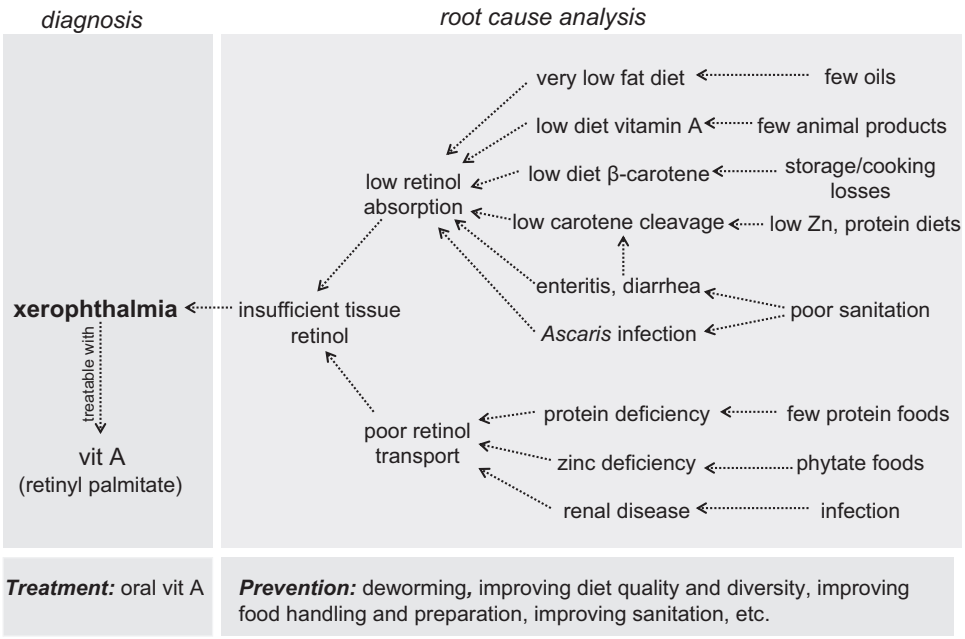

FIGURE 4.3 **Treatment** of nutritional deficiency and **prevention** of its occurrence call for different types of analyses. Cases demand a medical approach in which treatment is based on a diagnosis. Prevention calls for root cause analyses using systems approaches to identify and address the underlying factors.

- Vitamin losses (e.g., during pelleting, extrusion, and/or storage)
- Poor access to feed (e.g., competition for limited feeder space, improper feeder placement, and breakdown of feed delivery system).

Secondary deficiencies in animals tend have biological and social causes:

- Poor feed intake (e.g., inappetence, poor feed palatability, and heat stress)
- Other deficiencies (e.g., deficiencies of protein and/or zinc can impair retinol transport)
- Impaired digestion
- Malabsorption (e.g., diarrhea, parasites, and intestinal infection due to poor hygiene)
- Impaired postabsorptive utilization (e.g., certain drug therapies)
- Increased metabolic demand (e.g., infection, low environmental temperature, egg/milk production, rapid growth, pregnancy, and lactation).

Two Types of Vitamin Deficiencies

- *Primary deficiencies*… involve failures to ingest a vitamin in sufficient amounts to meet physiological needs.
- *Secondary deficiencies*… involve failures to absorb or otherwise utilize a vitamin postabsorptively.

Making Interventions Effective

The management of vitamin deficiencies[6] is no different from that of other diseases—treatment is generally most effective when administered during the early stages of cellular biochemical abnormality, rather than waiting for the manifestation of clinical signs.[7] For this reason, the early detection of insufficient vitamin status using biochemical indicators has been, and will continue to be, a very important activity in the clinical assessment of vitamin status.

Vitamin deficiency disorders are often treated using a medical/pharmacological approach involving a two-step analysis: (1) diagnosis of the deficiency and (2) treatment with an appropriate form of the relevant vitamin. This approach offers the advantages of speed and efficacy, which are often significant in the context of treating subjects in need. However, they typically do not address the multiple, underlying causes of deficiency; these must be addressed for the sustainable prevention of vitamin deficiency, especially in populations. This goal calls for considering the deficiency in the biological, social, demographic, and environmental contexts in which it occurs, to the end of identifying root causes—i.e., the underlying, contributing conditions, one or more of which will likely to be amenable to change. An example of a root cause analysis of xerophthalmia due to vitamin A deficiency is shown in Fig. 4.3.

6. This discussion, of course, is relevant to any class of nutrients.
7. Marks (1968) makes this point clearly with the example of diabetes, which should be treated once hypoglycemia is detected, thus reducing the danger of diabetic arteriosclerosis and retinopathy.

4. STUDY QUESTIONS AND EXERCISES

1. For a major organ system, discuss the means by which vitamin deficiencies may affect its function.
2. List the clinical signs that have special diagnostic value (i.e., are specifically associated with insufficient status with respect to certain vitamins) for specific vitamin deficiencies.
3. For a fat-soluble and a water-soluble vitamin, discuss the relationships between tissue distribution of the vitamin and organ site specificity of the clinical signs of its deficiency.
4. List the animal species and deficiency diseases that, because they show specificity for certain vitamins, might be particularly useful in vitamin metabolism research.
5. Develop a decision tree for determining whether lesions of a particular organ system may be due to insufficient intakes of one or more vitamins.
6. Detail differences between primary and secondary deficiencies and their causes citing specific examples in humans or animals.

RECOMMENDED READING

Marks, J., 1968. The Vitamins in Health and Disease. Churchill, London, 183 pp.

Ross, A.C., Caballero, B., Cousins, R.J., et al. (Eds.), 2012. Modern Nutrition in Health and Disease, eleventh ed. Lippincott Williams and Wilkins, Baltimore, 2069 pp.

Zemplini, J., Suttie, J.W., Gregory, J.F., et al., 2014. Handbook of Vitamins, fifth ed. CRC Press, New York, 592 pp.

Chapter 5

Vitamin Needs and Safety

Chapter Outline

Anchoring Concepts

1. The vitamins have many metabolic role(s) essential to normal physiological function; these roles can be compromised by quantitatively insufficient or temporarily irregular vitamin intakes.
2. Vitamin needs can be determined by monitoring responses of parameters related to the metabolic functions and/or body reserves of the vitamins.
3. Quantitative data are available describing vitamin contents of many common foods and feedstuffs.
4. Vitamins are frequently used in human feeding, in animal diets, and in treating certain clinical conditions at levels in excess of their requirements.
5. Several of the vitamins, most notably vitamins A and D, can produce adverse physiological effects when consumed in excessive amounts.

Nutriment is both food and poison. The dosage makes it either poison or remedy.

Paracelsus[1]

LEARNING OBJECTIVES

1. To understand the concepts of minimum requirement, optimal requirement, and allowance as used with respect to vitamins.
2. To understand the basis for establishing allowances for vitamins in human and animal feeding.
3. To understand the concept of upper safe use level with respect to the vitamins.
4. To understand the margins of safety above their respective requirements for intakes of each of the vitamins.
5. To understand the signs/symptoms of vitamin toxicities in humans and animals.

VOCABULARY

Adequate intake (AI)
Calcinosis
Carotenodermia
Daily values (DVs)
Dietary Guidelines for Americans (DGA)
Dietary reference intake (DRI)
Dietary standards
Estimated average requirement (EAR)
Estimated safe and adequate daily dietary intake (ESADDI)
Food and Agricultural Organization (FAO)
Food and Nutrition Board
Hypervitaminosis
Institute of Medicine (IOM)
Lowest observed adverse effect level (LOAEL)
Margin of safety
Metabolic profiling
Minimum requirement
National Academy of Sciences
National Research Council
No observed adverse effect level (NOAEL)
Nutrient allowances
Nutritional essentiality
Optimal requirement
Oxaluria
Protective nutrient intake
Range of safe intake
Recommended Daily Allowance (RDA)
Recommended Dietary Intake (RDI)

1. Paracelsus (1493–1541) was the Latinized name of the Swiss German philosopher, physician, botanist, and general occultist born Philippus Aureolus Theophrastus Bombastus von Hohenheim, who is generally credited as the founder of the field of Toxicology.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00005-8

Recommended Nutrient Intake (RNI)
Safety index (SI)
Tolerable upper intake limit (UL)
Toxic threshold upper limit (UL)
World Health Organization (WHO)

1. DIETARY STANDARDS FOR VITAMINS

Purposes of Dietary Standards

The need to formulate healthy diets for both humans and animals has stimulated the translation of current nutrition knowledge into a variety of **dietary standards** for the intakes of specific nutrients. As these are typically developed by committees of experts reviewing the pertinent scientific literature, they are frequently referred to as **dietary recommendations**. Formally, they may be called **Recommended Daily Allowances** (**RDAs**) or **Recommended Dietary Intakes** (**RDIs**).

Regardless of the ways they may be named, dietary standards differ from nutrient requirements, although they are derived from the latter. Dietary standards are relevant to populations; they describe the average amounts of particular nutrients that should satisfy the needs of almost all healthy individuals in defined groups. In contrast, **nutrient requirements** are relevant to individuals; they describe amounts of particular nutrients that satisfy certain criteria related to the metabolic activity of those nutrients or to general physiological function. Because recommended allowances and intakes are designed to satisfy the needs of groups of individuals whose nutrient requirements vary, by definition they exceed the average requirement.

Determining Nutrient Requirements

The nutrient requirement is a theoretical construct that describes the intake of a particular nutrient that supports a body pool of the nutrient and/or its metabolically active forms adequate to maintain normal physiological function. In practice, it is generally used in reference to the lowest intake that supports normal function, that is, the **minimum requirement**. Minimum requirements, while seemingly physiologically relevant, are difficult to define and impossible to measure with any reasonable precision. They can vary according to the criteria by which they are defined. This problem is illustrated by the widely varying estimates of the vitamin A requirement for calves; various estimates may be derived by different criteria (Table 5.1).

Therefore, to be relevant to the overall health of specific populations, estimates of minimum nutrient requirements must be based on responses of obvious physiological importance. For many nutrients (e.g., the indispensable amino acids), it may be appropriate to define minimum requirements on the basis of a fairly nonspecific parameter such as growth. For vitamins, however, it is appropriate to define minimum requirements on the basis of biomarkers more specifically related to their respective metabolic functions, such as enzyme activities and tissue concentrations, as these can reflect changes at the earlier stages of vitamin deficiencies. The most useful biomarkers of vitamin status are those that respond early to deprivation of the vitamin, as such changes can be used to detect suboptimal vitamin status at the early and most readily corrected stages.

TABLE 5.1 Vitamin A Requirements of Calves Based on Different Criteria

Criteria	Estimated Requirement (IU/day)
Prevention of nyctalopia	20
Normal growth	32
Normal serum retinol levels	40
Moderate hepatic retinyl ester reserves	250
Substantial hepatic retinyl ester reserves	1024

From Marks, J., 1968. The Vitamins in Health and Disease: A Modern Reappraisal. Churchill, London, p. 32.

Quantifying the minimum requirement, even with the use of an appropriate biomarker, is not straightforward. It generally requires an experimental approach that may be possible using an animal model, but is seldom feasible with human subjects.[2] In such studies with animals, test subjects are fed a basal diet constructed to be deficient in the nutrient of interest but otherwise adequate with respect to all known nutrients. Various treatment groups receive this diet supplemented with known amounts of the nutrient of interest. This may necessitate the use of uncommon foods/feedstuffs such that the diet bears little similarity to those used in practice; it often means that the test nutrient is provided in free form, which may not resemble its form in practical foods and feedstuffs.[3] Even with these caveats, the level of nutrient intake to be identified as the minimum requirement is not always clear, as the optimal value for that biomarker is usually a matter of judgment.

Most responses of specific nutrient-depleted animals to input of the relevant nutrient appear to be curvilinear (Fig. 5.1, right panel). However, in most nutrient requirement

2. Studies with human volunteers in which diet composition and level of consumption are both well controlled in reference to the caloric needs of individuals generally require fairly long observational periods and call for facilities (metabolic kitchen, clinical assessment, and in-house wards) that are available at relatively few institutions. As a result, they tend to be costly and not widely conducted. Instead, observational epidemiological approaches are often used to impute the levels of nutrient intake of apparently healthy people.

3. e.g., Menadione instead of phylloquinones; tetrahydrofolic acid instead of mixed folates.

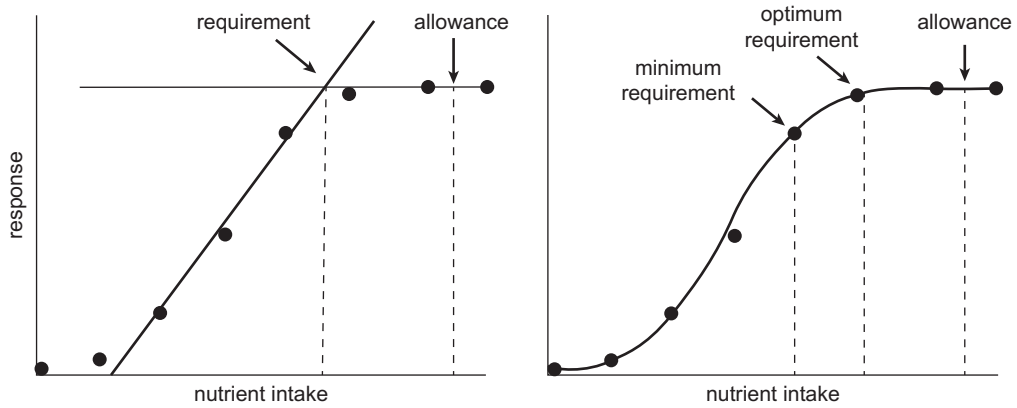

FIGURE 5.1 Requirements and allowances for nutrients are determined from the responses of physiologically meaningful parameters to the level of nutrient intake.

experiments, both rectilinear and curvilinear models usually fit equally well. For this reason, many investigators have used rectilinear models to impute requirements (e.g., the x value of the intercept of the two linear regressions of the observed data in broken-line regression analyses[4]; Fig. 5.1, left panel). Others, however, have used curvilinear models, which consider the variations in the experimental population of both the measured response and the nutrient need for maintenance (usually related to body size). From the proposition of curvilinearity, it follows that no value can be properly described as the "requirement" of the test populations. Nevertheless, the approach can be used to determine the risk of not fully satisfying the requirement for given proportions of the experimental population. The level of intake associated with acceptable risk of deficiency (a matter of judgment) is frequently called the "optimum requirement" or "level of optimum intake." In public health, such levels are determined on the basis of assumptions regarding putative health risks and in consideration of interindividual variation. In livestock production, where the cost of feeding accounts for as much as three-quarters of the total cost of production, it is necessary to determine intakes of the more costly nutrients (protein, limiting amino acids, energy) that optimize economic efficiency.

Factors Affecting Vitamin Needs

Many factors can affect nutrient requirements (Tables 5.2 and 5.3), such that those of individuals with the same general characteristics can vary substantially. For most nutrients the requirements of individuals in given populations appear to be normally distributed. For this reason, in the absence of clear information, it is reasonable to assume that the variations in vitamin requirements are similar to those typically observed in biological systems in being normally distributed with a coefficients of variation of 10–15%.

4. This approach offers the advantage of rendering a requirement value that is derived mathematically from the observed data; however, that value tends to be in the region of greatest variation in the input–response curve.

Developing Vitamin Allowances

Because nutrient requirements, even for the best cases, are quantitative estimates based on data of uncertain precision derived from a limited number of subjects, these values have limited practical usefulness. In practice, **nutrient allowances**, or recommended intakes (RIs), are far more useful. They are selected to meet the needs of those individuals with the greatest requirements. That is, an allowance is set at the right-hand tail of the natural distribution of requirements. An allowance exceeds the **estimated average requirement (EAR)** for the population by an increment sometimes referred to as a **margin of safety**. Allowances for vitamins, particularly in livestock feeding, have often been set on the basis of practical experience. Quantitative approaches have been used in establishing allowances for both animals and humans; such parameters are generally described in statistical terms relating to the proportion of the target population's requirements would be met by the recommended level of intake. For example, committees of the U.S. Food and Nutrition Board and WHO/FAO[5] have set allowances at 2 standard deviations (SD) above the EAR (Fig. 5.2), a decision to meet the needs of approximately 97.5% of the population.[6] This method has yielded satisfactory results, likely due in part to the generous estimates of EARs generally made by expert committees.

Allowances, therefore, are derived from estimates of EARs (of typical individuals) made from actual biological data, usually from nutritional experiments. Because they are used as standards for populations, allowances are developed in consideration of risk of nutrient deficiency. Therefore, allowances are relevant to specified populations with their

5. World Health Organization and Food and Agricultural Organization, respectively, of the United Nations.

6. A notable exception is in the setting of allowances for energy; these are typically set at the estimated average requirements of classes of individuals, for the reason that, unlike other nutrients, both intake and expenditure of energy appear to be regulated such that free-living individuals with free access to food maintain (at least very nearly) energy balance.

TABLE 5.2 Factors Affecting Vitamin Needs

Factor	Examples
Physiological determinants	Active growth
	Pregnancy
	Lactation
	Aging
	Intraindividual variation
	Level of physical activity
	Obesity
Hereditary conditions	Inborn vitamin-dependent diseases
	Polymorphisms of vitamin transporters, receptors, vitamin-dependent enzymes, and enzymes of vitamin metabolism
Conditions causing maldigestion/malabsoprtion	Pancreatitis
	Gastrorintestinal surgery
	Endocrine disorders (e.g., diabetes, hypoparathyroidism, congenital or acquired hemolytic, Addison's disease)
	Hepatobiliary disease
	Intestinal resection/bypass
	Pernicious anemia
	Regional ileitis
	Radiation injury
	Kwashiorkor
	Pellagra
	Gluten enteropathy
	Intestinal parasitism (e.g., hookworm, *strongyloides*, *Giardia lamblia*, *Dibothriocephalus latus*)
	Enteritis
	Cystic fibrosis
	Certain drug treatments
Hypermetabolic states	Thyrotoxicosis
	Pyrexial disease
	Various infections
Conditions causing decreased nutrient utilization	Chronic liver disease
	Chronic renal disease
Conditions involving increased cell turnover	Congenital and acquired hemolytic anemias
	Sickle cell disease
Conditions increasing nutrient turnover/loss	Extensive burns
	Bullous dermatoses
	Entheropathy
	Nephrosis
	Surgery
	Hemodialysis
	Smoking

characteristic food habits and inherent variations in nutrient requirements. For example, the RDAs established by the U.S. Food and Nutrition Board are implicitly intended to relate to the US population. These recommendations were originally developed to facilitate the wartime planning of food supplies but have become a key source of information for making food and health policy in the United States and elsewhere (Table 5.4).

TABLE 5.3 Physiologically Significant Drug–Vitamin Interactions

Vitamin	Drugs
Vitamin A	Diuretic: spironolactone
	Bile acid sequestrant: Cholestyramine, colestipol
	Laxative: Phenolphthalein, mineral oil
Vitamin D	Antibacterial: isoniazid
	Anticonvulsant: phenytoin, diphenylhydantoin, primidone
	Bile acid sequestrant: colestipol
	Laxative: phenolphthalein, mineral oil
Vitamin E	Smoking
Vitamin K	Anticoagulant: warfarin
	Anticonvulsant: phenytoin, diphenylhydantoin, primidone
	Bile acid sequestrant: colestipol
	Immunosuppressant: cyclosporins
	Laxative: mineral oil, phenolphthalein
Vitamin C	Antiinflammatory: aspirin
	Oral contraceptives
	Smoking
Thiamin	Alcohol
Riboflavin	Antibacterial: boric acid
	Tranquilizer: chlorpromazine
Niacin	Antibacterial: isoniazid
	Antiinflammatant: phenylbutazone
Vitamin B_6	Analytical reagent: thiosemicarbazide
	Antibacterial: isoniazid
	Anticholinergic, anti-parkinsonian: L-dopa
	Antihypertensive: hydralazine
	Chelating agents, antiarthritic: penicillamine
	Alcohol
	Oral contraceptives
	Smoking
Biotin	None reported
Pantothenic acid	None reported

Continued

TABLE 5.3 Physiologically Significant Drug–Vitamin Interactions—cont'd

Vitamin	Drugs
Folate	Antacid: sodium bicarbonate, aluminum hydroxide
	Antibacterial: sulfasalazine, trimethoprim
	Anticonvulsant: phenytoin
	Antiinflammatant: sulfasalazine, aspirin
	Antimalarial: pyrimethamine
	Antineoplastic: methotrexate
	Bile acid sequestrant: cholestyramine, colestipol
	Diuretic: triamterene
	Alcohol
	Oral contraceptives
Vitamin B_{12}	Antihyperglycemics: biguanides
	Antibacterials: *p*-aminosalicylic acid, neomycin
	Antihistaminic: cimetidine, ranitidine
	Antiinflammatant, gout suppressant: colchicine
	Bile acid sequestrant: cholestyramine, colestipol

A Member of the Food and Nutrition Board, Dr Alfred E. Harper, Observed:

There is not always agreement on the criteria for deciding when a requirement has been met. If the requirement is considered to be the minimal amount that will maintain normal physiological function and reduce the risk of impaired health from nutritional inadequacy to essentially zero, then we are left with questions such as: 'What is normal physiological function?; ' 'What is health?'; and 'What degree of reserve or stores of the nutrient is adequate?' Differences in judgment on such issues are to be expected.

Differences Between Requirements and Allowances

Confusion surrounds the allowances for the vitamins (and other nutrients) that have been developed by various expert committees. Some questions arise, particularly concerning dietary recommendations for livestock, because the rationales for such values are frequently not presented. A fairly common example is the mistaken impression, on the part of formulators of animal feeds, that vitamin allowances are requirements; this mistake can lead to vitamin overfortification of those feed vitamins. Other questions arise over the publication of differing recommendations by different committees of experts, all of whom consider the same basic data in their respective reviews of the pertinent literature. This situation results from the paucity of clear and compelling data on nutrient requirements; differences in environmental conditions and food supplies; and the lack of consensus on such issues as criteria for defining requirements, appropriate margins of safety, and whether standards should be based on intakes of food as consumed or as purchased. These considerations make the variable factor of scientific judgment important in estimating the nature of nutrient requirements. Thus, dietary recommendations are revised periodically[7] as new information becomes available.

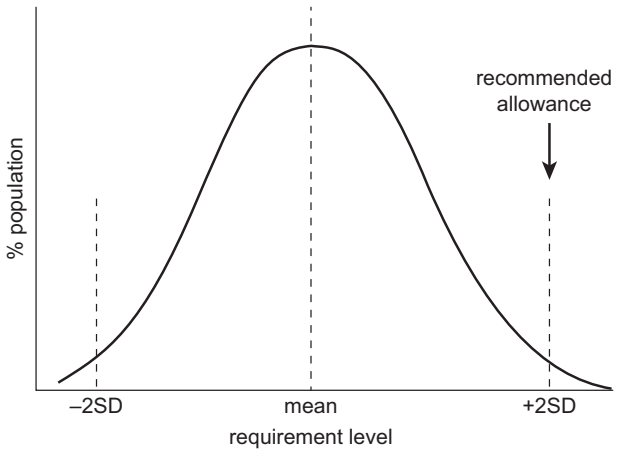

FIGURE 5.2 The "mean plus 2 SD" conversion algorithm for determining recommended daily allowances.

7. As evidence has grown, expert committees typically have reduced the levels of their recommendations for nutrient allowances. This likely reflects the basically conservative nature of the committee system used for these purposes, whereby the paucity of data tends to be handled by generously estimating quantitative needs.

TABLE 5.4 History of the Recommended Daily Allowances (RDAs) for Vitamins[a]

	1941	1948	1957	1968	1976	1980	1989	1997–2001	2010
Vitamin A (mg RE)	1000	1000	1000	1000	1000	1000	1000	900	[b]
Vitamin D (IU/μg)	—	—	—	400 IU	400 IU	5 μg	5 μg	[10][c]	[b]
Vitamin E (IU/mg)	—	—	—	30 IU	15 IU	10 IU	10 IU	15 mg	15 mg
Vitamin K (μg)	—	—	—	—	—	—	80	[120][c]	[b]
Vitamin C (mg)	75	75	70	60	45	60	60	90	[b]
Thiamin (mg)	2.3	1.5	0.9	1.3	1.4	1.4	1.5	1.2	[b]
Riboflavin (mg)	3.3	1.8	1.3	1.7	1.6	1.6	1.7	1.3	[b]
Niacin (mg)	23	15	15	17	18	18	19	16	[b]
Vitamin B_6 (mg)	—	—	—	2.0	2.0	2.2	2.0	1.7	[b]
Pantothenic acid (mg)	—	—	—	—	—	—	—	[5][c]	[b]
Biotin (mg)	—	—	—	—	—	—	—	[30][c]	[b]
Folate (μg)	—	—	—	400	400	400	200	400	[b]
Vitamin B_{12} (μg)	—	—	—	3.0	3.0	5.0	2.0	2.4	[b]

[a]Values shown are for males, 25–50 years of age or (for vitamin D in 2010) 31–50 years of age.
[b]Values set in 1997–2001 remain in use.
[c]RDAs not available for these vitamins; values shown are adequate intakes (AIs).

The RDA Concept

In one sense, the RDA construct is somewhat archaic in that it fails to pertain to biological functions of nutrients that may be nonspecific or nontraditional, in the context of being outside the known functions of nutrients. The conceptual framework upon which the RDA was derived is being replaced by a new, more individualistic view of nutrition that relates more broadly to health. This view is the basis of problems that have become apparent concerning the RDA. To retain the practical utility of the RDA, it will be necessary to reconstruct it; such reconstruction must be based on new paradigms for nutritional science that, informed by the "genomics revolution," explicitly consider individual metabolic characteristics.

The RDA was developed to facilitate food planning for the US population.[8] It is a product of the central concept of the field of nutrition, **nutritional essentiality**, which has been used to describe those factors in the external chemical environment that are specifically required for normal metabolic functions and, accordingly, those exogenous sources on which organisms depend for normal physiologic functions (e.g., growth, reproductive success, survival, and freedom from certain clinical/metabolic disorders). The vitamins are among the more than 40 such factors generally considered to be nutritionally essential, i.e., indispensable in the diets of animals and humans. Deprivation of any one of these is manifested by clinical signs that are usually specific in nature. Nutritional essentiality has been based on empirical findings that nutrients function to prevent ill health in very specific ways. Under this paradigm, nutrient deficiency diseases have contributed to the development of our knowledge of nutrition: their specific prevention has been used both to define nutrient essentiality and to quantify nutrient needs. Indeed, a nutrient has not been considered essential unless a clinical disease has been related specifically to its deprivation. Therefore, as the term has been used, nutritional essentiality clearly connotes the specific prevention of deficiency disease. This connotation is troublesome in dealing with issues of diet and health, as the essentiality paradigm does not pertain to functions of nutrients that are either nonspecific or nontraditional, i.e., outside the known functions of nutrients. This connotation, as expressed in the quantitative estimation of population-based nutrient needs, the RDAs, now serves to limit the essentiality paradigm as a conceptual framework. Modern nutrition is cast in a different context—one in which optimum health is more broadly conceptualized.

Questions about nutrient allowances for humans arise, owing to the application of those values to purposes for

8. The RDAs were originated in 1941 for use in planning U.S. food policy during World War II.

which they were not intended. The RDAs are now used for many other purposes: evaluating nutritional adequacy of diets; evaluating results of dietary surveys; setting standards for food assistance programs; institutional feeding programs, and food and nutrition regulations; developing food and nutrition education programs; formulating new food products and special dietary foods. Many of these uses have revealed limitations of the RDAs: not dealing with associations of diet and chronic and degenerative diseases; not including guidelines for appropriate intakes of fat, cholesterol, and fiber; and for not providing guidance for food selection and prevention of obesity. These limitations stem from that fundamental misunderstanding that, while the RDAs may be used in certain programs to implement sound public health policy, they are not intended to be policy recommendations per se. In fact, RDAs cannot serve as general dietary guidelines; by definition, they are reference standards dealing with nutrients, whereas dietary guidelines deal primarily with foods. The RDAs are, in fact, standards on which sound dietary guidelines, such as the **Dietary Guidelines for Americans (DGAs)**,[9] are to be based.

It should be kept in mind that the RDAs, like other nutrient allowances, are intended to relate to intakes of nutrients as part of normal diets[10] of specified population subgroups. They are intended to be average daily intakes based on periods as short as 3 days (for nutrients with fast turnover rates) to several weeks or months (for nutrients with slower turnover rates).

Questions Concerning RDAs:

- Which level of nutrient need should define a requirement—the level that supports all/some dependent enzymes at 50, 80, or 100% of maximal expression?
- Can a nutrient be *conditionally essential* (e.g., choline for individuals with low methionine intakes; glutamine for surgical patients)?
- How should an individual's varying nutrient requirements be described (e.g., effects of infection, oxidative stress)?
- Can nutrients be considered required for their nonspecific effects (e.g., antioxidants)?
- Can a nonnutrient be considered required (e.g., dietary fiber)?

9. The DGAs are issued every 5 years by the U.S. Departments of Agriculture (USDA) and Health and Human Services (HHS) based on expert consultations reviewing the state of nutrition understanding relative to the health of all Americans age 2 years and over.

10. The RDA subcommittee emphasized that the RDAs can typically be met or closely approximated by diets that are based on the consumption of different foods from diverse food groups that contain adequate energy.

Considering Nonclassical Functions of Nutrients

For some dietary factors, functions influencing the risk of chronic disease have been suggested by epidemiological and experimental animal model studies and, to a lesser extent, clinical trials. Reduced risks of such diseases have been associated with increased intakes and/or status of several vitamins. The metabolic bases of these linkages remain to be elucidated; indeed, these areas are among the most active in contemporary nutritional science:

- **Cancer** and foods containing vitamin A or vitamin C, intakes of riboflavin, and plasma levels or intakes of α-tocopherol, carotenoids, and 25-OH-vitamin D
- **Cardiovascular disease** and intakes of vitamin C, vitamin E, and β-carotene
- **Neural tube defects** and periconceptual folate intake
- **Diabetes and multiple sclerosis** and plasma 25-OH-vitamin D level
- **Psoriasis** response to vitamin D treatment.

The case of the apparent effects of antioxidants illustrates the limitation of the RDA construct. Current thinking is that antioxidant nutrients (vitamins E and C, selenium, and, perhaps, β-carotene) participate in a system of protection against the deleterious metabolic effects of free radicals. Because many diseases are thought to involve enhanced free-radical production, protection from oxidative stress is thought to be critical to normal physiologic function. According to this hypothesis, antioxidants would be expected to suppress radical-induced DNA damage involved in the initiation of carcinogenesis, to inhibit the oxidation of cholesterol in low-density lipoproteins (LDLs) in atherosclerosis, and to inhibit the oxidation of lens proteins in cataracts. Antioxidant nutrients have been shown to enhance immune functions, which may also contribute to reduced risks of cancer as well as infectious disease. Many of these antioxidant effects do not appear to have the specificity connoted by the essentiality paradigm. For example, the complementary natures of the antioxidant functions of vitamins E and C and selenium suggest that anyone may spare needs for the others in protecting against subcellular free-radical damage and LDL oxidation. It is likely that, through such biochemical mechanisms, the antioxidant nutrients may be modifiers of disease risk rather than primary agents in disease etiologies. However nonspecific they may be, such effects raise legitimate questions concerning nutrient need—questions not easily addressed under the essentiality paradigm or translated into RDAs.

New Paradigms for Nutrition

The term "essentiality" has become rather elastic in its application. Nutrients have come to be described as "required"

or "essential" for particular functions. Some are called "dispensable" or "indispensable" under specific conditions; several are recognized as "beneficial" at levels greater than those that are considered to be "required." Indeed, the translation of nutritional knowledge into dietary guidance requires such language. However, the emergence of this sort of terminology indicates that the essentiality paradigm is, in fact, being displaced by a new conceptualization of nutrition.

It is likely that new paradigms of nutrition will encompass an individualized view of organisms that recognizes both endogenous and exogenous conditions as determinants of the nature and amounts of factors available from the external chemical environment that must be obtained to support definable health outcomes. Accordingly, such factors will be considered as nutrients *if* and *when* their activities, in the metabolism of the host and/or the associated microflora, are beneficial to those outcomes. This view will recognize different outcomes as being appropriate for various individuals, both within and between a species/population. Thus, freedom from overt physiological dysfunction as well as reduced risk of chronic diseases will be important outcomes in human nutrition; whereas such outcomes as maximal growth rate, optimal efficiency of feed utilization, and minimal susceptibility to infection will be priorities in livestock nutrition.

The old paradigm is being outgrown at an increasing pace with progress in the modern field of molecular biology. It has now become clear that some nutrients function as gene regulators and that predisposition to disease can have genetic bases. The mapping of the human genome and human microbiome has led to the development of powerful tools to study individual metabolic characteristics. As **metabolic profiling** becomes more feasible, it will become possible to address individuals' nutritional needs on the basis of their peculiar metabolic characteristics. Not only clinicians, but also dieticians, will be able to ask such questions as whether an individual has sodium-sensitive hypertension, a cystathionine β-synthase mutation, or the methylenetetrahydrofolate reductase C677T/C genotype. The time is quickly approaching when it will be possible to identify disease predisposition, metabolic characteristics, and specific dietary needs of individuals based on rapid, genomic/metabolomic analyses. As that becomes practicable, the population-based paradigm will lose much of its value.

Reconstructing the RDA

This crisis in conceptualization was manifest in the lively discussion concerning the need for new approaches to the development of dietary recommendations that preceded the development of the **dietary reference intakes (DRIs)** in the 1990s. The challenge was to recreate the RDA as a useful construct under the emerging paradigm that addressed both the prevention of overt nutritional deficiencies as well as the maintenance of health. It became clear that DRIs must accommodate the possibility that a nutrient can have beneficial action at levels above those previously thought to be "required" for normal physiologic function.[11]

2. VITAMIN ALLOWANCES FOR HUMANS

The first nutrient allowances were published 50 years ago by the U.S. National Academy of Sciences. Based on available information, those RDAs have since been revised periodically. Since the first publication of RDAs, similar dietary standards have been produced by several countries and international organizations. For the reasons mentioned previously, the various recommendations tend to be similar but not always identical. For example, most are based on food as consumed; however, some are based on food as purchased, making them appear higher than those of other countries.

Actionable Information Needed for Any Nutrient:

- amount that prevents overt deficiency disease
- amount that can have other health benefits
- amount that can have specific health risks.

RDAs

The RDAs are probably the most widely referenced of the dietary standards, and the set that is most comprehensive with respect to the vitamins. It is worth noting that the RDAs for vitamins are still not complete; that is, quantitative recommendations on some (e.g., vitamin D, vitamin K, biotin, pantothenic acid) have not been made owing to a still-insufficient information base. In 1980, the problem of dealing with nutrients known to be essential for humans but for which insufficient data are available was handled by including provisional recommendations. The 9th and 10th editions of the RDAs included **Estimated Safe and Adequate Daily Dietary Intake (ESADDI)** ranges of daily dietary intakes for

11. The need is best illustrated by the case of the essential trace element selenium (Se). The RDAs for Se (now 55 μg for both women and men) were first established in 1989 based on the amount sufficient for maximal expression of Se-dependent glutathione peroxidase in young men. Since then, several clinical trials and a large number of animal tumor model studies have found antitumorigenic activities of higher intakes of Se, e.g., >200 μg/day (Clark, L.C., Combs Jr., G.F., Turnbull, B., et al., 1996. J. Am. Med. Assoc. 276 (1957)). While the RDA addresses the classical nutritional need for Se (for selenoprotein expression), it is mute of the prospect of cancer risk reduction.

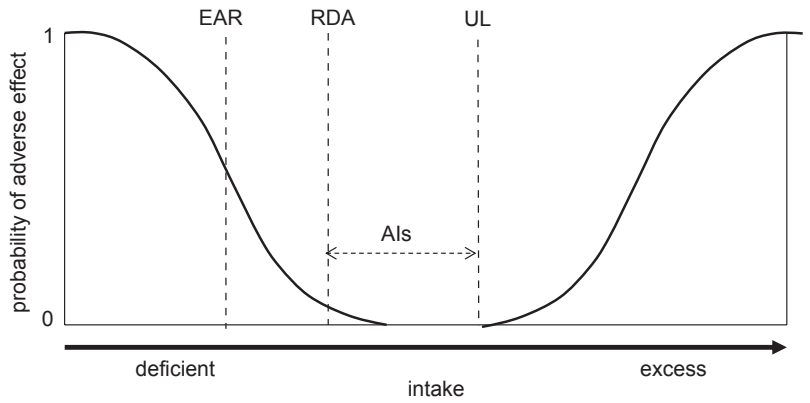

FIGURE 5.3 Conceptual basis for DRIs (dietary reference intakes).

such nutrients. This terminology is no longer used; instead, estimates of **adequate intakes (AIs)** are now used in cases where available data are judged to be insufficient for developing RDAs.

The setting of dietary allowances is an exercise of experts who evaluate published scientific literature. That different expert panels can reach different conclusions from the same body of published data is evidenced by the differences in national dietary allowances. That the growing body of relevant data also changes over time is evidenced by the changes in RDAs over the history of that institution (Table 5.4). For example, only in 1968 were RDAs established for vitamins D, E, C, and B_{12} and folate. In 1989, an RDA for vitamin K was first set; however that value, as well as that for vitamin D, were replaced with AI values in 2000. Only in 2010 were RDAs for vitamin D established.[12]

Accordingly, the setting of dietary allowances is a continuing process. There is a growing appreciation of the need and advantages of harmonizing these processes as carried out in different countries.[13]

Dietary Reference Intakes

The most recent (2010) edition of dietary allowances for vitamin D and calcium builds on the broad, previous (1997–2001) edition of dietary allowances (Table 21-5) produced by the U.S. Food and Nutrition Board. The former were developed in the 1990s by a series of workshops that addressed the conceptual framework upon which that work was based. This resulted in an expansion of the former RDAs with a system of DRIs.[14] This system involved four types of reference values (Fig. 5.3):

- **Estimated average requirements (EARs)**—Intakes to meet the requirements of half of the healthy individuals in each age–sex-specific demographic subgroup of the American population.
- **Recommended Daily Allowances (RDAs)**—Average daily intake level sufficient to meet the requirements of nearly all (97–98%) of the healthy individuals in each age–sex-specific demographic subgroup. The RDA is calculated from the EAR:

$$RDA = EAR + 2SD_{EAR}$$

where SD_{EAR} is the standard deviation of the EAR

While the RDA resembles the historic construct; in fact, it is different in that it assumes the SD_{EAR} to be 10% of the EAR, whereas a value to 15% had been used previously. For this reason, many of the more recent RDAs are lower than earlier ones.

- **Adequate intakes (AIs)**—Observed and/or experimentally determined approximations of nutrient intakes of groups of healthy individuals and extrapolated to each age–sex demographic subgroup. Used when data are

12. These were met with instant dispute by some researchers who thought they had been set too low: Hall, L.M., Kimlin, M.G., Aronov, P.A., et al., 2010. J. Nutr. 140 (542); Heaney, R.P., Holick, M.F., 2011. J. Bone Mineral Res. 26 (455).

13. Fairweather-Tait, S., Gurinović, M., van Ommen, B., et al., 2010. Eur. J. Clin. Nutr. 64 (S26).

14. Questions concerning the means of developing consistent and reliable standards led the 10th RDA Committee to review the scientific basis of the entire RDA table. The Committee recommended a lower RDA for vitamins A (reducing the RDA for men 1000–700 IU, and that for women from 800–600 IUand 600 IU) and vitamin C (reducing the RDA for men from 60 to 40 mg, that for women from 60 to 30 mg, and that for infants from 35 to 25 mg). It was reported that these reductions were resisted by the U.S. Food and Nutrition Board, which had been advised by another subcommittee to increase the intakes of these nutrients based on cancer risk reduction potential. In an unexpected move, the Board elected not to accept the recommendations of the RDA Committee. This move prompted lively discussion in the Nutrition community ultimately resulting in a rethinking of the RDA construct and the development of the DRIs.

judged to be insufficient for the estimation of an EAR and subsequent calculation of an RDA.

- **Tolerable upper intake limits (ULs)—**The highest level of daily intake that is likely to pose no risks of adverse health effects to almost all healthy individuals in a each age–sex-specific demographic subgroup. The use of ULs will facilitate the development of recommendations of nutrient intakes at what might be called "supranutritional" levels when such intakes have been shown to have health benefits. Pertinent to this consideration is emerging understanding of roles of at least several vitamins in reducing chronic disease risk.

This approach to the development of dietary allowances presumes the availability of empirical data for the distribution of individual nutrient requirements, necessary for calculating both the EAR and the SD_{EAR}. However, such data are available for very few nutrients. Thus, the DRI process involved a consensus opinion to assume that the distributions of individual nutrient requirements are each normal with a coefficient of variation (CV) of 10%, with only two exceptions: for vitamin A, CV = 20%; for niacin, CV = 15%.

International Standards

The FAO and WHO have established standards for energy, protein, calcium, iron, and eight of the vitamins (Table 5.6). This system of recommendations is intended for international use and, thus, to be relevant to varied population groups. It includes reference values similar to those used by the Food and Nutrition Board: Requirements similar to EARs, **Recommended Nutrient Intakes (RNIs),** similar to RDA; and Upper Tolerable Nutrient Intake Levels similar to the ULs. In addition, the FAO/WHO system also provides a value applicable for nutrients that may be protective against a specified nutritional or health risk of publish health relevance, **Protective nutrient intakes** (Table 5.5).

3. VITAMIN ALLOWANCES FOR ANIMALS

Public Versus Private Information

The development of livestock production for the economical production of human food and fiber has superimposed practical needs on the formulation of animal feeds that do not exist in human nutrition. Notably, this involves needs for accurate data for both nutrient requirements of animals and nutrient contents of feedstuffs. The availability of such data enables commercial animal nutritionists to formulate nutritionally balanced feeds using computer-based linear programming techniques. Often that capacity enables livestock enterprises to be competitive in a context where the cost of feeding can be the largest single cost of production.[15] Thus, research in food and animal nutrition has, over the past few decades, moved progressively out of the public sector and into the research divisions of agribusinesses with immediate interests in generating such data.[16] The result is that a diminishing proportion of practical animal nutrition data (particularly in the area of amino acid nutrition) remains in the public sector and is, thus, available to the scrutiny of experts. As a consequence, two types of dietary standards are in use. The first is the standard developed by review of open data available in the scientific literature; the second is the standard developed through in-house testing and/or practical experience by animal producers. Whereas the former data are in the public domain, the latter usually are not.

Public information on nutrient allowances is reviewed by expert committees in the United States, the United Kingdom, and several other countries under programs charged with the responsibility of establishing nutrient recommendations on the basis of the best available data. Perhaps the most widely used source of such recommendations is the Committee on Animal Nutrition of the U.S. National Research Council (Table 5.7). Through expert subcommittees, each dedicated to a particular species, the NRC maintains the periodic review of nutrient standards, many of which serve as the bases of recommendations for animal feed formulation throughout the world. Currently, many gaps remain in our knowledge of vitamin requirements. This is particularly true for ruminant species, for which the substantial ruminal destruction of vitamins appears to be compensated by adequate microbial synthesis, and for several nonruminant species that are not widely used for commercial purposes. Therefore, many of the standards for vitamins and other nutrients are imputed from available data on related species; in part for this reason, the requirements for some nutrients (e.g., selenium) appear to be very similar among many species.

4. USES OF VITAMINS ABOVE REQUIRED LEVELS

Typical Uses Exceed Requirements

Most normal diets that include varieties of foods can be expected to provide supplies of vitamins that meet those levels required to prevent clinical signs of deficiencies. In addition, most intentional uses of vitamins are designed to exceed those requirements for most individuals. Indeed, that is the principle by which vitamin allowances are set. Thus, the formulation of diets, the planning of meals, the vitamin fortification of foods, and the designing of vitamin supplements are all designed to provide vitamins at levels contributing to total intakes that exceed the requirements of

15. For example, feed costs for broiler chickens can comprise 60–70% of the total cost of producing poultry meat.

16. This is markedly different from human nutrition research, which continues to be seen as a public good such that the expansion of knowledge has come largely from public-sponsored research.

TABLE 5.5 Food and Nutrition Board Recommended Daily Allowances (RDAs) for Vitamins

Age–Sex Group	Vitamin A (μg)[a]	Vitamin D (μg)	Vitamin E (mg)[b]	Vitamin K (μg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)[c]	Vitamin B_6 (μg)	Pantothenic Acid (μg)	Biotin (μg)	Folate (μg)[d]	Vitamin B_{12} (μg)
Infants													
0–6 months	[400][e]	10	4	[2.0]	[40]	[0.2]	[0.3]	[2[b]]	[0.1]	[1.7]	[5]	[65]	[0.4]
7–11 months	[500]	10	5	[2.5]	[50]	[0.3]	[0.4]	[4]	[0.3]	[1.8]	[6]	[80]	[0.5]
Children													
1–3 years	300	15	6	[30]	15	0.5	0.5	6	0.5	2	[8]	[150]	0.9
4–8 years	400	15	7	[55]	25	0.6	0.6	8	0.6	3	[12]	[200]	1.2
Males													
9–13 years	600	15	11	[60]	45	0.9	0.9	12	1.0	4	[20]	[300]	1.8
14–18 years	900	15	15	[75]	75	1.2	1.3	16	1.3	5	[25]	[400]	2.4
19–30 years	900	15	15	[120]	90	1.2	1.3	16	1.3	5	[30]	[400]	2.4
31–50 years	900	15	15	[120]	90	1.2	1.3	16	1.3	5	[30]	[400]	2.4
51–70 years	900	15	15	[120]	90	1.2	1.3	16	1.7	5	[30]	[400]	2.4
>70 years	900	20	15	[120]	90	1.2	1.3	16	1.7	5	[30]	[400]	2.4
Females													
9–13 years	600	15	11	[60]	45	0.9	0.9	12	1.0	4	[20]	[300]	1.8
14–18 years	700	15	15	[75]	65	1.0	1.0	14	1.2	5	[25]	[400]	2.4
19–30 years	700	15	15	[90]	75	1.1	1.1	14	1.3	5	[30]	[400]	2.4
31–50 years	700	15	15	[90]	75	1.1	1.1	14	1.3	5	[30]	[400]	2.4
51–70 years	700	15	15	[90]	75	1.1	1.1	14	1.5	5	[30]	[400]	2.4
>70 years	700	20	15	[90]	75	1.1	1.1	14	1.5	5	[30]	[400]	2.4

Pregnancy													
≤18 years	750	15	15	[75]	80	1.4	1.4	18	1.9	6	[30]	[600]	2.6
19–30 years	770	15	15	[90]	85	1.4	1.4	18	1.9	6	[30]	[600]	2.6
31–50 years	770	15	15	[90]	85	1.4	1.4	18	1.9	6	[30]	[600]	2.6
Lactation													
≤18 years	1200	15	19	[75]	115	1.4	1.6	17	2.0	7	[35]	[550]	2.8
19–30 years	1300	15	19	[90]	120	1.4	1.6	17	2.0	7	[35]	[550]	2.8
31–50 years	1300	15	19	[90]	120	1.4	1.6	17	2.0	7	[35]	[550]	2.8

[a]*Retinol equivalents.*
[b]*α-Tocopherol.*
[c]*Niacin equivalents.*
[d]*Folate equivalents.*
[e]*Brackets indicate cases for which RDAs have not been set; AIs are given instead.*
From Food and Nutrition Board, 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. National Academy Press, Washington, DC, 432 pp.; Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.; Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, 506 pp.; Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC, 773 pp.; Food and Nutrition Board, 2010. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.

TABLE 5.6 FAO/WHO Recommended Nutrient Intakes (RNIs) for Vitamins[a]

Age-Sex Group	Vitamin A (μg)[b]	Vitamin D (μg)	Vitamin E (mg)	Vitamin K (μg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)[c]	Vitamin B_6 (μg)	Pantothenic Acid (μg)	Biotin (μg)	Folate (μg)	Vitamin B_{12} (μg)
Infants													
0–6 months	375	5	2.7	5	25	0.2	0.3	2[d]	0.1	1.7	5	80	0.4
7–11 months	400	5	2.7	10	30	0.3	0.4	4	0.3	1.8	6	80	0.5
Children													
1–3 years	400	5	5	15	30	0.5	0.5	6	0.5	2	8	160	0.9
4–6 years	450	5	5	20	30	0.6	0.6	8	0.6	3	12	200	1.2
7–9 years	500	5	7	25	35	0.9	0.9	12	1.0	4	20	300	1.8
Adolescents, 10–18 years													
Males	600	5	10	35–65	40	1.2	1.3	16	1.3	5	25	400	2.4
Females	600	5	7.5	35–55	40	1.1	1.0	16	1.2	5	25	400	2.4
Adults													
Males: 19–50 years	600	5	10	65	45	1.2	1.3	16	1.3	5	30	400	2.4
>50 years	600	10	10	65	45	1.2	1.3	16	1.7	5	30	400	2.4
Females: 19–50 years	500	5	7.5	55	45	1.1	1.1	14	1.3	5	30	400	2.4
51–65 years	500	10	7.5	55	45	1.1	1.1	14	1.5	5	30	400	2.4
Older Adults, >65 years													
Men[c]	600	15	10	65	45	1.2	1.3	16	1.7	5	–	400	2.4
Women[c]	600	15	7.5	55	45	1.1	1.1	14	1.5	5	–	400	2.4
Pregnancy	800	5	–	55	55	1.4	1.4	18	1.9	6	30	600	2.6
Lactation	850	5	–	55	70	1.5	1.6	17	2.0	7	35	500	2.8

[a]*Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, pp. 286.*
[b]*Retinol equivalents.*
[c]*Niacin equivalents.*
[d]*Preformed niacin.*

TABLE 5.7 Estimated Vitamin Requirements (units/kg Diet) of Domestic and Laboratory Animals

Species	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)[a]	Vitamin K (μg)[b]	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Folate (mg)	Pantothenate (mg)	Biotin (μg)	Vitamin B_{12} (μg)	Choline (g)
Birds														
Chickens														
Growing chicks	1500	200	10	0.5		1.8	3.6	27	2.5–3	0.55	10	0.1–0.15	3–9	0.5–1.3
Laying hens	4000	500	5	0.5		0.8	2.2	10	3	0.25	2.2	0.1	4	
Breeding hens	4000	500	10	0.5		0.8	3.8	10	4.5	0.25	10	0.15	4	
Ducks														
Growing	4000	220		0.4			4	55	2.6		11			
Breeding	4000	500		0.4			4	40	3		11			
Geese														
Growing	1500	200				2.5–4		35–55			15			
Breeding	4000	200				4	20							
Pheasants							3.5	40–60			10			1–1.5
Quail														
Growing bobwhite							3.8	30			13			1.5
Breeding bobwhite							4	20			15			1.0
Growing coturnix	5000	1200	12	1		2	4	40	3	1	10	0.3	3	2.0
Breeding coturnix	5000	1200	25	1		2	4	20	3	1	15	0.15	3	1.5
Turkeys														
Growing poults	4000	900	12	0.8–1		2	3.6	40–70	3–4.5	0.7–1	9–11	0.1–0.2	3	0.8–1.9
Breeding hens	4000	900	25	1		2	4	30	4	1	16	0.15	3	1.0
Cats	10,000	1000	80			5	5	45	4	1	10	0.5	20	2.0

Continued

TABLE 5.7 Estimated Vitamin Requirements (units/kg Diet) of Domestic and Laboratory Animals—cont'd

Species	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)[a]	Vitamin K (μg)[b]	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)	Folate (mg)	Pantothenate (mg)	Biotin (μg)	Vitamin B_{12} (μg)	Choline (g)
Cattle														
Dry heifers	2200	300												
Dairy bulls	2200	300												
Lactating cows	3200	300												
Beef cattle	2200	300												
Dogs	5000	275	50			1	2.2	11.4	1	0.18	10	0.1	22	1.25
Fishes														
Bream									5–6		30–50	1		4.0
Carp	10,000		300				7	28	5–6		10–20			
Catfish	2000	1000	30		60	1	9	14	3	5	40	1	20	3.0
Coldwater spp.	2500	2400	30	10	100	10	20	150	10					
Foxes	2440					1	5.5	9.6	1.8	0.2	7.4			
Goats	60[c]	12.9[c]												
Guinea pigs	23,333	1000	50	5	200	2	3	10	3	4	20	0.3	10	1.0
Hamsters	3636	2484	3	4		20	15	90	6	2	40	0.6	20	2.0
Horses														
Ponies	25[c]													
Pregnant mares	50[c]													
Lactating mares	55–65[c]													
Yearling	40[c]													
2-year olds	30[c]													
Mice	500	150	20	3		5	7	10	1	0.5	10	0.2	10	0.6

Mink	5930		27			1.3	1.6	20	1.6	0.5	8	0.12	32.6	
Primates[d]	15,000	2000	50		0.1		5	50	2.5	0.2	10	0.1		
Rabbits														
Growing	580		40					180	39					1.2
Pregnant	>1160		40	0.2										
Lactating			40											
Rats	4000	1000	30	0.5		4	3	20	6	1	8		50	1.0
Sheep														
Ewes														
Early pregnancy	26[c]	5.6[c]												
Late pregnancy/ lactating	35[c]	5.6[c]												
Rams	43[c]	5.6[c]												
Lambs														
Early weaned	35[c]	6.6[c]												
Finishing	26[c]	5.5[c]												
Shrimp					10		120			120	120			0.6
Swine														
Growing	2200	200	11	2		1.3	2.2–3	10–22	1.5	0.6	11–13	0.1	22	0.4–1.1
Bred gilt/ sow	4000	200	10	2			3	10	1	0.6	12	0.1	15	1.25
Lactating gilt/sow	2000	200	10	2			3	10	1	0.6	12	0.1	15	1.25
Boars	4000	200	10	2			3	10	1	0.6	12	0.1	15	1.25

[a]*α-Tocopherol.*
[b]*Menadione.*
[c]*Unlike almost all of the other values in this table, this requirement is expressed in international units (IU) per kilogram body weight.*
[d]*Nonhuman species.*
From National Research Council, 2015. Nutrient Requirements of Poultry. National Academy Press, Washington, DC; National Research Council, 2006. Nutrient Requirements of Dogs and Cats. National Academy Press, Washington, DC; National Research Council, 2008. Nutrient Requirements of Dairy Cattle, seventh ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2000. Nutrient Requirements of Beef Cattle, seventh ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2011. Nutrient Requirements of Fish and Shrimp. National Academy Press, Washington, DC; National Research Council, 1982. Nutrient Requirements of Mink and Foxes, second ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2006. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids and New World Camelids. National Academy Press, Washington, DC; National Research Council, 1995. Nutrient Requirements of Laboratory Animals, fourth ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2007. Nutrient Requirements of Horses, sixth ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2003. Nutrient Requirements of Nonhuman Primates, second ed. (rev.). National Academy Press, Washington, DC; National Research Council, 1977. Nutrient Requirements of Rabbits, second ed. (rev.). National Academy Press, Washington, DC; National Research Council, 2012. Nutrient Requirements of Swine, eleventh ed. (rev.). National Academy Press, Washington, DC.

most individuals by some **margin of safety**. This approach minimizes the probability of producing vitamin deficiencies in populations.

Some clinical conditions require the use of vitamin supplements at levels greater than those normally used to accommodate the usual margins of safety. These include specific vitamin deficiency disorders (e.g., xerophthalmia, rickets, and polyneuritis, encephalopathy related to alcohol abuse) and certain rare inherited metabolic defects (e.g., vitamin B_6-responsive cystathionase deficiency, vitamin B_{12}-responsive transcobalamin II deficiency, biotin-responsive biotinidase deficiency).[17] In such cases, vitamins are prescribed at doses that far exceed requirement levels.

Elevated doses of vitamins are also frequently prescribed by physicians or are taken as over-the-counter supplements by affected individuals in the treatment of certain other pathological states including neurological pains, psychosis, alopecia, anemia, asthenia, premenstrual tension, carpal tunnel syndrome, and prevention of the common cold. Although the efficacies of vitamin supplementation in most of these conditions remain untested in randomized, controlled trials, vitamin prophylaxis, and/or therapy for at least some conditions is perceived as effective by many in the medical community as well as in the general public. This view supports the widespread use of oral vitamin supplements at dosages greater than 50–100 times the RDAs.[18]

5. HYPERVITAMINOSES

Factors Affecting Vitamin Toxicity

Several factors can affect the toxicity of any vitamin. These include the route of exposure, the dose regimen (number of doses and intervals between doses), the general health of the subject, and potential effects of food and drugs. For example, parenteral routes of vitamin administration may increase the toxic potential of high vitamin doses, as the normal routes of controlled absorption and hepatic first-pass metabolism may be circumvented. Large single doses of the water-soluble vitamins are rarely toxic, as they are generally rapidly excreted, thus minimally affecting tissue reserves. However, repeated multiple doses of these compounds can produce adverse effects. In contrast, single large doses of the fat-soluble vitamins can produce large tissue stores that can steadily release toxic amounts of the vitamin thereafter. Some disease states, such as those involving malabsorption, can reduce the potential for vitamin toxicity; however, most increase that potential by compromising the subject's ability to metabolize and excrete the vitamin,[19] or by rendering the subject particularly susceptible to **hypervitaminosis**.[20] Foods and some drugs can reduce the absorption of certain vitamins, thus reducing their toxicities.

The signs of intoxication for each vitamin vary with the species affected and the timecourse of overexposure (Tables 5.8 and 5.9). Nevertheless, certain signs or syndromes are characteristic for each vitamin:

Hypervitaminosis A—The potential for vitamin A intoxication is greater than those for other hypervitaminoses, as its range of safe intakes is relatively small. For humans, acute exposures as low as 25 times the RDA are thought to be potentially intoxicating, although actual cases of hypervitaminosis A have been very rare[21] at chronic doses less than about 9000 µg of retinol equivalents per day. Hypervitaminosis A occurs when plasma retinol levels exceed 3 µmol/L (caused by increases in retinyl esters), which in humans can occur in response to single large doses (>660,000 IU for adults, >330,000 IU for children), or after doses >100,000 IU/day have been taken for several months.

Acute toxicity. Children with hypervitaminosis A develop transient (1–2 days) signs: nausea, vomiting, signs due to increased cerebrospinal fluid pressure (headache, vertigo, blurred, or double vision), and muscular incoordination. Studies have found that 3–9% of children given high, single therapeutic doses (200,000 IU) show transient nausea, vomiting, headache, and general irritability; a similar percentage of younger children may show fontanelle bulging, which subsides in 48–96 h.

Chronic toxicity. Chronic hypervitaminosis A occurs with recurrent exposures exceeding 12,500 IU (infants)–33,000 IU (adult). The early sign is commonly dry lips (cheilitis), which is often followed by dryness and fragility of the nasal mucosa, dry eyes, and conjunctivitis. Skin lesions include dryness, pruritis, erythema, scaling, peeling of the palms and soles, hair loss (alopecia), and nail fragility. Headache, nausea, and vomiting (signs of increased intracranial pressure) can also occur. Infants and young children can show painful periostitis. In animals, adverse effects have been reported at intakes

17. Other examples are given in Chapter 4.

18. For example, several studies have shown that athletes and their coaches generally believe that athletes require higher levels of vitamins than nonathletes. This attitude appears to affect their behavior, as athletes use vitamin (and mineral) supplements with greater frequencies than the general public. One study found that 84% of international Olympic competitors used vitamin supplements. Despite this widespread belief, it remains unclear whether any of the vitamins at levels of intake greater than RDAs can affect athletic performance.

19. For example, individuals with liver damage (e.g., alcoholic cirrhosis, viral hepatitis) have increased plasma levels of free (unbound) retinol and a higher incidence of adverse reactions to large doses of vitamin A.

20. For example, patients with nephrocalcinosis are particularly susceptible to hypervitaminosis D.

21. According to Bendich (1989. Am. J. Clin. Nutr. 49 (358)), fewer than 10 cases per year were reported in 1976–1987. Several of those occurred in individuals with concurrent hepatic damage due to drug exposure, viral hepatitis, or protein-energy malnutrition.

TABLE 5.8 Signs and Symptoms of Vitamin Toxicities in Humans

Vitamin	Children	Adults
Vitamin A	*Acute*: Anorexia, bulging fontanelles, lethargy, high intracranial fluid pressure, irritability, nausea, vomiting	*Acute*: Abdominal pain, anorexia, blurred vision, lethargy, headache, hypercalcemia, irritability, muscular weakness, nausea, vomiting, peripheral neuritis, skin desquamation
	Chronic: Alopecia, anorexia, bone pain, bulging fontanelles, cheilitis, craniotabes, hepatomegaly, hyperostosis, photophobia, premature epiphyseal closure, pruritus, skin desquamation, erythema	*Chronic*: Alopecia, anorexia, ataxia, bone pain, cheilitis, conjunctivitis, diarrhea, diplopia, dry mucous membranes, dysuria, edema, high CSF pressure, fever, headache, hepatomegaly, hyperostosis, insomnia, irritability, lethargy, menstrual abnormalities, muscular pain and weakness, nausea, vomiting, polydypsia, pruritus, skin desquamation, erythema, splenomegaly, weight loss
Vitamin D	Anorexia, diarrhea, hypercalcemia, irritability, lassitude, muscular weakness, neurological abnormalities, pain, polydypsia, polyuria, poor weight gain, renal impairment	Anorexia, bone demineralization, constipation, hypercalcemia, muscular weakness and pain, nausea, vomiting, polyuria, renal calculi
Vitamin E	No adverse effects reported	Mild gastrointestinal distress, some nausea, coagulopathies in patients receiving anticonvulsants
Vitamin K[a]	No adverse effects reported	No adverse effects reported
Vitamin C	No adverse effects reported	Gastrointestinal distress, diarrhea, oxaluria
Thiamin[b]	No adverse effects reported	Headache, muscular weakness, paralysis, cardiac arrhythmia, convulsions, allergic reactions
Riboflavin	No adverse effects reported	No adverse effects reported
Niacin	No adverse effects reported	Vessel dilation, itching, headache, anorexia, liver damage, jaundice, cardiac arrhythmia
Vitamin B_6	No adverse effects reported	Neuropathy, skin lesions
Pantothenic acid	No adverse effects reported	Diarrhea[c]
Biotin	No adverse effects reported	No adverse effects reported
Folate	No adverse effects reported	Allergic reactions[c]
Vitamin B_{12}	No adverse effects reported	Allergic reactions[c]

[a]*Adverse effects observed only for menadione; phylloquinone, and the menaquinones appear to have negligible toxicities.*
[b]*Adverse effects have been observed only when the vitamin was administered parenterally; none when it has been given orally.*
[c]*This sign has been observed in only a few cases.*

as low as 10 times the RDA; but intoxication typically follows chronic intakes of 100- to 1000-fold RDA levels. The most frequently observed signs are loss of appetite, loss of weight or reduced growth, skeletal malformations, spontaneous fractures, and internal hemorrhages. Most signs can be reversed by discontinuing excessive exposure to the vitamin. Ruminants appear to tolerate high intakes of vitamin A better than nonruminants, apparently due to destruction of the vitamin by the rumen microflora. That retinoids can be embryotoxic raises concerns about the safety of high-level vitamin A supplementation for pregnant animals and humans. High doses of retinol, all-*trans*-retinoic acid, or 13-*cis*-retinoic acid can disrupt cephalic neural crest cell activity, producing craniofacial, central nervous system, and cardiovascular and thymus malformations. Fetal malformations have been reported in cases of oral use of 20,000–25,000 IU/day all-*trans*-retinoic acid in treating *acne vulgaris*. Regular intakes exceeding 10,000 IU/day (preformed vitamin A) has been associated with increased risk of birth defects in a small cohort of women with very high vitamin A intakes (mean > 21,000 IU/day). Rare cases of premature closure of lower limb epiphyses have been reported in animals, e.g., "hyena disease" in calves.

The toxicities of carotenoids appear to be low. Regular intakes as great as 30 mg β-carotene per day are without side effects other than carotenodermia.

Hypervitaminosis D—Vitamin D_3 has been found safe for pregnant and lactating women and their children at oral doses of 100,000 IU/day; however, intakes as low as 50 times the RDA have been reported to be toxic to

TABLE 5.9 Signs of Vitamin Toxicities in Animals

Vitamin	Sign	Species
Vitamin A	Alopecia	Rat, mouse
	Anorexia	Cat, cattle, chicken, turkey
	Cartilage abnormalities	Rabbit
	Convulsions	Monkey
	Elevated heart rate	Cattle
	Fetal malformations	Hamster, monkey, mouse, rat
	Hepatomegaly	Rat
	Gingivitis	Cat
	Irritability	Cat
	Lethargy	Cat, monkey
	Reduced CSF pressure	Cattle, goat, pig
	Poor growth	Chicken, pig, turkey
	Skeletal abnormalities	Cat, cattle, chicken, dog, duck, mouse, pig, rabbit, rat, turkey, horse
Vitamin D[a]	Anorexia	Cattle, chicken, fox, pig, rat
	Bone abnormalities	Pig, sheep
	Cardiovascular calcinosis	Cattle, dog, fox, horse, monkey, mouse, pig, rat, sheep, rabbit
	Renal calcinosis	Cattle, chicken, dog, fox, horse, monkey, mouse, pig, rat, sheep, turkey
	Cardiac dysfunction	Cattle, pig
	Hypercalcemia	Cattle, chicken, dog, fox, horse, monkey, mouse, pig, rat, sheep, trout
	Hyperphosphatemia	Horse, pig
	Hypertension	Dog
	Myopathy	Fox, pig
	Poor growth, weight loss	Catfish, chicken, horse, mouse, pig, rat
	Lethality	Cattle
Vitamin E	Atherosclerotic lesions	Rabbit
	Bone demineralization	Chicken, rat
	Cardiomegaly	Rat
	Hepatomegaly	Chicken
	Hyperalbuminemia	Rat
	Hypertriglyceridemia	Rat
	Hypocholesterolemia	Rat
	Impaired muscular function	Chicken
	Increased hepatic vitamin A	Chicken, rat
	Increased prothrombin time	Chicken
	Reduced adrenal weight	Rat
	Poor growth	Chicken
	Increased hematocrit	Rat
	Reticulocytosis	Chicken
	Splenomegaly	Rat

TABLE 5.9 Signs of Vitamin Toxicities in Animals—cont'd

Vitamin	Sign	Species
Vitamin K[b,c]	Anemia[c]	Dog
	Renal failure[c]	Horse
	Lethality	Chicken,[c,d] mouse,[c,d] rat[c]
Vitamin C	Anemia	Mink
	Bone demineralization	Guinea pig
	Decreased circulating thyroid hormone	Rat
	Liver congestion	Guinea pig
	Oxaluria	Rat
Thiamin	Respiratory distress (i.p. dose)	Rat
	Cyanosis (i.p. dose)	Rat
	Epileptiform convulsions (i.p. dose)	Rat
Riboflavin	No adverse effects reported for oral doses lethality (parental dose)	Rat
Niacin	Impaired growth	Chicken (embryo)
	Developmental abnormalities	Chicken (embryo), mouse (fetus)
	Liver damage	Mouse
	Mucocutaneous lesions	Chicken
	Myocardial damage	Mouse
	Decreased weight gain	Chicken
	Lethality (i.p. dose)	Chicken (embryo), mouse[d]
Vitamin B_6	Anorexia	Dog
	Ataxia	Dog, rat
	Convulsions	Rat
	Lassitude	Dog
	Muscular weakness	Dog
	Neurologic impairment	Dog
	Vomiting	Dog
	Lethality	Mouse, rat
Pantothenic acid	No adverse effects reported for oral doses lethality (i.p. dose)	Rat
Biotin	No adverse effects reported for oral doses Irregular estrus (i.p. dose)	Rat
	Fetal resorption (i.p. dose)	Rat
Folate	No adverse effects reported for oral doses	
	Epileptiform convulsions (i.p. dose)	Rat
	Renal hypertrophy (i.p. dose)	Rat
Vitamin B_{12}	No adverse effects reported for oral doses Irregular estrus (i.p. dose)	Rat
	Fetal resorption (i.p. dose)	Rat
	Reduced fetal weights (i.p. dose)	Rat

[a]*Vitamin D_3 is much more toxic than vitamin D_2.*
[b]*Only menadione produces adverse effects; phylloquinone and the menaquinones have negligible toxicities.*
[c]*These effects observed after parenteral administration of the vitamin.*
[d]*Nicotinamide is more toxic than nicotinic acid.*

humans, particularly in children. Affected individuals show anorexia, vomiting, headache, drowsiness, diarrhea, and polyuria. There have been no documented cases of hypervitaminosis D due to excessive sunlight exposure. Excessive intakes of vitamin D increase circulating levels of 25-OH-D_3, which at high levels appears to bind VDR, thus, bypassing the regulation of the 25-OH-D_3-1-hydroxylase to induce transcriptional responses normally signaled only by 1,25-$(OH)_2$-D_3. Hypervitaminosis D is characterized by increases in both the enteric absorption and bone resorption of calcium. This produced hypercalcemia and, ultimately, calcinosis, i.e., deposition of calcium and phosphate in soft tissues (heart, kidney, and vascular and respiratory systems). Thus, the risk of hypervitaminosis D depends on concomitant intakes of calcium and phosphorus and is in conditions, such as chronic inflammation, in which the normal feedback regulation of the renal 25-OH-D_3-1-hydroxylase is compromised. Studies with animals indicate that vitamin D_3 is 10–20 times more toxic than vitamin D_2,[22] apparently because it is more readily metabolized than the latter to the 25-hydroxy metabolites.

Hypervitaminosis E—Vitamin E is one of the *least toxic* of the vitamins. Both animals and humans appear to be able to tolerate high levels of exposure. For humans, daily doses as high as 400 IU have been be considered harmless, and large oral doses as great as 3200 IU have not been found to have consistent ill effects. There have been isolated reports of headache, fatigue, nausea, double vision, muscular weakness, mild creatinuria, and gastrointestinal distress in humans consuming as much as 1000 IU per day. For animals, doses at least two orders of magnitude above nutritional requirements, e.g., to 1000–2000 IU/kg, are without untoward effects. Studies with animals indicate that excessive dosages of tocopherols exert most, if not all, of their adverse effects by antagonizing the utilization of the other fat-soluble vitamins: reducing hepatic vitamin A storage, impairing bone mineralization and producing coagulopathies. In each case, these signs could be corrected with supplements of the appropriate vitamin (A, D, and K, respectively). These effects appear to involve impaired absorption, and inhibition of retinyl ester hydrolase and vitamin K-dependent carboxylations.

Hypervitaminosis K—The toxic potentials of the naturally occurring forms of vitamin K are negligible. Phylloquinone exhibits no adverse effects when administered to animals in massive doses by any route. The menaquinones are similarly thought to have little, if any, toxicity. The synthetic vitamer menadione, when administered parenterally, can at high doses produce fatal anemia, hyperbilirubinemia, and severe jaundice. However, its toxic threshold appears to be at least three orders of magnitude greater than nutritionally required levels. At such doses, menadione appears to cause oxidative stress by reduction to the semiquinone radical, which, in the presence of O_2, is reoxidized to the quinone, resulting in the formation of the superoxide radical anion. Menadione can also react with free sulfhydryl groups; thus, high levels may deplete reduced glutathione (GSH) levels. The horse appears to be particularly vulnerable to menadione toxicity. Parenteral doses of 2–8 mg/kg have been found to be lethal in that species, whereas the parenteral LD_{50}[23] values for most other species are an order of magnitude greater than that.

Hypervitaminosis C—The only adverse effects of large doses of vitamin C that have been consistently observed in humans are gastrointestinal disturbances and diarrhea occurring at levels of intake nearly 20–80 times the RDAs. Concern has also been expressed that excess ascorbic acid may be prooxidative, may competitively inhibit the renal reabsorption of uric acid, may enhance the enteric destruction of vitamin B_{12}, may enhance the enteric absorption of nonheme iron (thus leading to iron overload), may produce mutagenic effects, and may increase ascorbate catabolism that would persist after returning to lower intakes of the vitamin. Present knowledge indicates that most, if not all, of these concerns are not warranted. That ascorbic acid can enhance the enteric absorption of dietary iron has led to concern that megadoses may lead to progressive iron accumulation in iron-replete individuals (iron storage disease). This hypothesis has not been supported by results of studies with animal models. Nevertheless, patients with hemochromatosis or other forms of excess iron accumulation should avoid taking vitamin C supplements with their meals.

Perhaps the greatest concern associated with high intakes of vitamin C concerns increased oxalate production. In humans, unlike other animals, oxalate is a major metabolite of ascorbic acid, accounting for 35–50% of the 35–40 mg of oxalate excreted in the urine each day.[24] The health concern is that high vitamin C intake may lead to increased oxalate production and, thus, to increased risk of urinary calculi.[25] Metabolic studies have indicated that

22. That is, vitamin D3 can produce effects comparable to those of vitamin D2 at doses representing only 5–10% of the latter.

23. The LD50 value is a useful parameter indicative of the degree of toxicity of a compound. It is defined as the lethal dose for 50% of a reference population and is calculated from experimental dose–survival data using the profit analysis.

24. The balance of urinary oxalate comes mainly from the degradation of glycine (about 40% of the total); but some also can come from the diet (5–10%).

25. There is some question as to whether oxalate may have been produced as an artifact of the analytical procedure.

the turnover of ascorbic acid is limited for which reason high intakes of vitamin C would not be expected to greatly affect oxalate production. Clinical studies have revealed slight oxaluria in patients given daily multiple-gram doses of vitamin C. It is not clear whether this effect is clinically significant, as its magnitude is low and within normal variation.[26] Nevertheless, prudence dictates the avoidance of doses greater than 1000 mg of vitamin C for individuals with a history of forming renal stones. Little information is available on vitamin C toxicity in animals, although acute LD_{50} (50% lethal dose) values for most species and routes of administration appear to be at least several grams per kilogram of body weight. Dietary vitamin C intakes 100–1000 times the allowance levels appear safe for most species.

Thiamin hypervitaminosis—The toxic potential of thiamin appears to be low, particularly when administered orally. Parenteral doses of the vitamin at 100–200 times the RDAs have been reported to cause intoxication in humans, characterized by headache, convulsions, muscular weakness, paralysis, cardiac arrhythmia, and allergic reactions. Most of the available information pertinent to its toxic potential is for thiamin hydrochloride. At very high doses (1000-fold levels required to prevent deficiency signs) that form can be fatal by suppressing the respiratory center. Such doses of the vitamin to animals produce curare-like signs, suggestive of blocked nerve transmission: restlessness, epileptiform convulsions, cyanosis, and dyspnea. Lower levels, ≤300 mg/day, are used therapeutically in humans without adverse reactions.

Riboflavin hypervitaminosis—High oral doses of riboflavin are very safe, probably owing to the relatively poor absorption of the vitamin at high levels. Oral riboflavin doses as great as 2–10 g/kg body weight produce no adverse effects in dogs and rats. The vitamin is somewhat more toxic when administered parenterally. The LD_{50} (50% lethal dose) values for the rat given riboflavin by the intraperitoneal, subcutaneous, and oral routes have been estimated to be 0.6, 5, and >10 g/kg, respectively. No adverse effects in humans have been reported.

Niacin hypervitaminosis—*acute toxicity*. In humans, small doses (10 mg) of nicotinic acid can cause flushing, although this effect is not associated with other seriously adverse reactions. At high dosages (two to four g/day), nicotinic acid can cause vasodilation, itching, nausea, vomiting, headaches, and, less frequently, skin lesions. These responses appears to be mediated by the niacin receptor, which is expressed by macrophages and bone marrow-derived cells of the skin. They can be minimized by using a slow-release formulation of nicotinic acid or by using a cyclooxygenase inhibitor (e.g., aspirin, indomethacin). High nicotinic acid doses have been reported to cause itching urticaria (hives), gastrointestinal discomfort (heartburn, nausea, vomiting, rarely diarrhea) in humans. Nicotinamide only rarely produces these reactions. Many patients have taken daily oral doses of 200–1000 mg for periods of years with only occasional side effects (skin rashes, hyperpigmentation, reduced glucose tolerance in diabetics, some liver dysfunction) at the higher dosages. Doses 50–100 times the RDAs are considered safe.

Chronic toxicity. The longer-term effects of high nicotinic acid doses include cases of insulin resistance, which may involve a rebound in lipolysis that results in increased free fatty acid levels. Transient hepatic dysfunction has also been reported. Chronic, high intakes of nicotinamide may deplete methyl groups (to excrete the vitamin), which would be exacerbated by low intakes of methyl donors, methionine and choline, and suboptimal status with respect to folate and/or vitamin B_{12}. Available information on the niacin tolerances of animals is scant but suggests that toxicity requires daily doses greater than 350–500 mg of nicotinic acid equivalents per kilogram body weight.

Hypervitaminosis B_6—The toxicity of vitamin B_6 appears to be relatively low, with intakes as great as 100 times the RDAs having been used safely by many people. Very high doses of the vitamin (several grams per day) have been shown to induce reversible sensory neuropathies marked by changes in gait and peripheral sensation. The primary target appears to be the peripheral nervous system; although massive doses of the vitamin have produced convulsions in rats, central nervous abnormalities have not been reported frequently in humans. Reports of individuals taking massive doses of the vitamin (>2 g/day) indicate that the earliest detectable signs are ataxia and loss of small motor control. Doses up to 750 mg/day for extended periods of time (years) have been found safe. The vitamin can increase the conversion of L-dopa to dopamine to interfere with the former drug in the management of Parkinson's disease in those not taking a decarboxylase inhibitor. Substantial information concerning the safety of large doses of vitamin B_6 in animals is available only for the dog and the rat. Doses less than 1000 times the allowance levels are safe for those species and, by inference, for other animal species.

Biotin hypervitaminosis—Biotin is generally regarded as nontoxic. Adverse effects of large doses of biotin have not been reported in humans or animals given the vitamin at doses as high as 200 mg orally or 20 mg intravenously. Limited data suggest that biotin is safe for most people at doses as great as 500 times the RDAs and for animals at probably more than 1000 times allowance levels. Animal

26. Forty percent of subjects given 2 g of ascorbic acid daily showed increases in urinary oxalate excretion by more than 10% (Chai, W., Liebman, M., Kynast-Gales, S., et al., 2004. Am. J. Kidney Dis. 44, 1060–1066).

studies have revealed few, if any, indications of toxicity, and it is probable that animals can tolerate the vitamin at doses at least an order of magnitude greater than nutritional levels.

Pantothenic acid hypervitaminosis—Pantothenic acid is generally regarded as nontoxic. A few reports indicate diarrhea occurring in humans consuming 10–20 g of the vitamin per day. Thus, pantothenic acid is thought to be safe for humans at doses at least 100 times the RDAs. No adverse reactions have been reported in any animal species following the ingestion of large doses of the vitamin. It has been estimated that animals can tolerate doses of pantothenic acid as great as at least 1000 times their respective nutritional requirements for the vitamin. Parenteral administration of very large amounts (e.g., 1 g per kg body weight) of the calcium salt has been shown to be lethal to rats.

Folate hypervitaminosis—Folate is generally regarded as nontoxic. Other than a few cases of apparent allergic reactions, the only purported adverse effect in humans (interference with the enteric absorption of zinc) is not supported with adequate data. Intakes of 400 mg of folate per day for several months have been tolerated without side effects in humans, indicating that levels at least as great as 2000 times the RDAs are safe. No adverse effects of high oral doses of folate have been reported in animals, although parenteral administration of pharmacologic amounts (e.g., 250 mg/kg, which is about 1000 times the dietary requirement) has produced epileptic responses and renal hypertrophy in rats. High-folate treatment has been found to exacerbate teratogenic effects of nutritional Zn deficiency.

Hypervitaminosis B_{12}—Vitamin B_{12} has no appreciable toxicity. No adverse reactions have been reported for humans or animals given high levels of the vitamin. Upper safe limits of vitamin B_{12} use are, therefore, highly speculative; it appears that doses at least as great as 1000 times the RDAs/allowances are safe for humans and animals.

6. SAFE INTAKES OF VITAMINS

The risks of adverse effects (toxicity) of the vitamins, like those of any other potentially toxic compounds, are functions of dose level. In general, the risk–dosage function is curvilinear, indicating a **hazard threshold** for vitamin dosage at some level greater than the requirement for that vitamin. Thus, a dosage increment exists between the level required to prevent deficiency and that sufficient to produce adverse effects. That increment, the **range of safe intake**, is bounded on the low-dosage side by the allowance, and on the high-dosage side by the upper safe limit, each of which is set on the basis of similar considerations of risk of adverse effects within the population (Fig. 5.4).

Quantifying Safe Intakes

There is no standard algorithm for quantifying the ranges of safe intakes of vitamins, but an approach developed for environmental substances that cause systemic toxicities has recently been employed for this purpose with nutritionally essential inorganic elements. This approach involves the imputation of an acceptable daily intake (ADI)[27] based on the application of a safety factor (SF)[28] to an experimentally determined highest **no observed adverse effect level** (**NOAEL**) of exposure to the substance. In the absence of sufficient data to ascertain an NOAEL, an experimentally determined **lowest observed adverse effect level** (**LOAEL**) is used:

$$\text{ADI} = \text{LOAEL} \div \text{SF}$$

An extension of this approach is to express the comparative safety of nutrients using a **safety index** (**SI**). This index is analogous to the therapeutic index (TI) used for drugs; it is the ratio of the minimum toxic dose and the RI derived from the RDA:

$$\text{SI} = \text{LOAEL} \div \text{RI}$$

This approach was used by Hathcock[29] to express quantitatively the safety limits of several vitamins for humans (Table 5.10).

The DRIs of the U.S. Food and Nutrition Board (1997–2001, 2010) addressed the safety of high doses of essential nutrients with the UL, defined as the highest level of daily intake that is likely to pose *no* risks of adverse health effects to almost all healthy individuals in each age–sex-specific demographic subgroup. In this context "adverse effect" is defined as any significant alteration in structure or function. It should be noted that the Food and Nutrition Board chose to use the term "tolerable intake" to avoid implying possible beneficial effects of intakes greater than the RDA.[30] The ULs are based on chronic intakes. They are derived through a multistep process:

1. Hazard identification—involving the systematic evaluation of all information pertaining to adverse effects of the nutrient;

27. The U.S. Environmental Protection Agency has replaced the ADI with the reference dose (RfD), a name the agency considers to be more value neutral, i.e., avoiding any implication that the exposure is completely safe or acceptable.

28. SF values are selected according to the quality and generalizability of the reported data in the case selected as the reference standard. Higher values, e.g., 100, may be used if animal data are extrapolated to humans; whereas lower values, e.g., 1 or 3, may be used if solid clinical data are available.

29. Hathcock, J., 1993. Nutr. Rev. 51, 278–285.

30. *See* Food and Nutrition Board, 1998. Dietary Reference Intakes: A Risk Assessment Model for Establishing Upper Intake Levels for Nutrients. National Academy Press, Washington, DC, 71 pp.

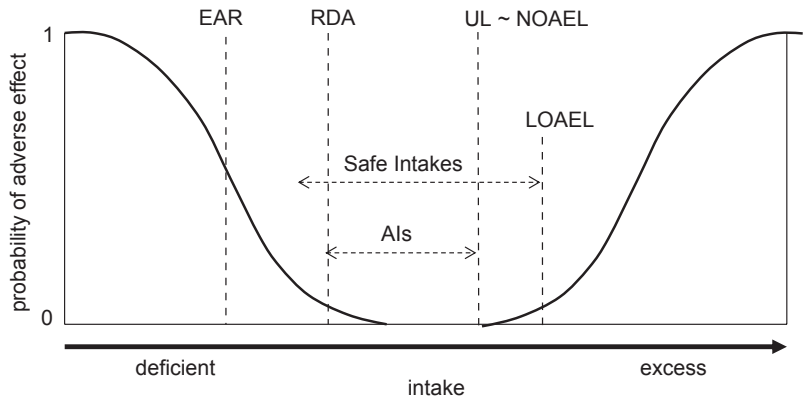

FIGURE 5.4 Vitamin safety follows a biphasic dose–response curve: just as very low intakes of vitamins can produce deficiency disorders, very high intakes can also have potential to produce adverse effects. The inflection points are the RDA and UL (which, in principle, should be comparable to the "no observed adverse effect level," NOAEL) and the "low observed adverse effect level", LOAEL, i.e., the upper end of the range of safe intakes.

TABLE 5.10 Use of a Safety Index to Quantitate the Toxic Potentials of Selected Vitamins for Humans

Parameter	Vitamin A	Vitamin D	Vitamin C	Niacin	Vitamin B_6
RDI (Recommended dietary intake)[a]	3300 IU	20 µg	60 mg	20 mg	2 mg
LOAEL (lowest observed adverse effect level)	25,000 IU	250 µg	2000 mg	500 mg	50 mg
Safety index (SI)	7.6	12.5	33	25	25

[a]*The greatest RDA for persons ≥4 years of age, excluding pregnant and lactating women.*
From Hathcock, J.N., 1993. J. Nutr. Rev. 51 (278); Hathcock, J.N., Shao, A., Vieth, R., et al., 2007. Am. J. Clin. Nutr. 85 (6).

2. Dose–response assessment—involving the determination of the relationship between level of nutrient intake and incidence/severity of adverse effects;
3. Intake assessment—involving the evaluation of the distribution of nutrient intakes in the general population; and
4. Risk characterization—involving the expression of conclusions from the previous steps in terms of the fraction of the exposed population having nutrients in excess of the estimated UL.

In practice, ULs are set at less than the respective LOAELs and no greater than the NOAELs (Fig. 5.4) from which they are derived, subject to uncertainty factors (UFs) used to characterize the level of uncertainty associated with extrapolating from observed data to the general population.[31] The ULs for the vitamins are presented in Table 5.9. Table 5.10 presents the authors' recommended upper safe vitamin intakes for animals.

Ranges of Safe Vitamin Intakes

The vitamins fall into four categories of relative toxicity at levels of exposure above typical allowances (Tables 5.11 and 5.12):

- **Greatest toxic potential**—vitamin A, vitamin D
- **Moderate toxic potential**—niacin
- **Low toxic potential**—vitamin E, vitamin C, thiamin, riboflavin, vitamin B_6
- **Negligible toxic potential**—vitamin K, pantothenic acid, biotin, folate, vitamin B_{12}.

Under circumstances of vitamin use at levels appreciably greater than the standard allowances (RDAs for humans or recommended use levels for animals), prudence dictates giving special consideration to those vitamins with greatest potentials for toxicity (those in the first two or three categories). In practice, it may only be necessary to consider the most potentially toxic vitamins of the first category (vitamins A and D).

31. Small UFs (close to one) are used in cases where little population variability is expected for the adverse effects, where extrapolation from primary data is not believed to under-predict the average human response, and where a LOAEL is available. Larger UFs (as high as 10) are used in cases where the expected variability is great, where extrapolation is necessary from primary animal data, and where a LOAEL is not available and a NOAEL value must be used.

TABLE 5.11 Food and Nutrition Board Tolerable Upper Intake Limits (ULs) for Vitamins

Age–Sex Group	Vitamin A (μg)[a]	Vitamin D (μg)	Vitamin E (mg)[b]	Vitamin K (μg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)[c]	Vitamin B_6 (μg)	Pantothenic Acid (μg)	Biotin (μg)	Folate (μg)[d]	Vitamin B_{12} (μg)
Infants													
0–11 months	600	25–38	–[e]	–[e]	–[e]	–[e]	–[e]		–[e]	–[e]	–[e]	–[e]	–[e]
Children													
1–3 years	600	63	200	–[e]	400	–[e]	–[e]	10	–[e]	–[e]	–[e]	300	–[e]
4–8 years	600	75	300	–[e]	650	–[e]	–[e]	15	–[e]	–[e]	–[e]	400	–[e]
Males													
9–13 years	1700	100	600	–[e]	1200	–[e]	–[e]	20	–[e]	–[e]	–[e]	600	–[e]
14–18 years	2800	100	800	–[e]	1800	–[e]	–[e]	30	–[e]	–[e]	–[e]	800	–[e]
19 + years	3000	100	1000	–[e]	2000	–[e]	–[e]	35	–[e]	–[e]	–[e]	1000	–[e]
Females													
9–13 years	1700	100	600	–[e]	1200	–[e]	–[e]	20	–[e]	–[e]	–[e]	600	–[e]
14–18 years	2800	100	800	–[e]	1800	–[e]	–[e]	30	–[e]	–[e]	–[e]	800	–[e]
>18 years	3000	100	1000	–[e]	2000	–[e]	–[e]	35	–[e]	–[e]	–[e]	1000	–[e]
Pregnancy													
≤18 years	2800	100	800	–[e]	1800	–[e]	–[e]	30	–[e]	–[e]	–[e]	800	–[e]
>18 + years	2800	100	1000	–[e]	2000	–[e]	–[e]	35	–[e]	–[e]	–[e]	1000	–[e]
Lactation													
≤18 years	2800	100	800	–[e]	1800	–[e]	–[e]	30	–[e]	–[e]	–[e]	800	–[e]
>18 + years	3000	100	1000	–[e]	2000	–[e]	–[e]	35	–[e]	–[e]	–[e]	1000	–[e]

[a] *Retinol equivalents.*
[b] *α-Tocopherol.*
[c] *Niacin equivalents.*
[d] *Folate equivalents.*
[e] *UL not established.*

From Food and Nutrition Board, 1997. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride. National Academy Press, Washington, DC, 432 pp.; Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.; Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, 506 pp.; Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC, 773 pp.; Food and Nutrition Board, 2010. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.

TABLE 5.12 Recommended Upper Safe Intakes of the Vitamins for Animals

Vitamin	Safe Intake (Multiple of Allowance Level[a])
High Toxic Potential	
Vitamin A	10[b]–30[c]
Vitamin D	10–20[d]
Moderate Toxic Potential	
Niacin[e]	50–100
Low Toxic Potential	
Vitamin E	100
Vitamin C	100–1000
Thiamin	500
Riboflavin	100–500
Vitamin B_6	100–1000
Negligible Toxic Potential	
Vitamin K[f]	1000
Pantothenic acid	1000
Biotin	1000
Folate	1000
Vitamin B_{12}	1000

[a]*From Committee on Animal Nutrition, 1987. Vitamin Tolerance in Animals. National Academy Press, Washington, DC.*
[b]*For nonruminant species.*
[c]*For ruminant species.*
[d]*Vitamin D_3 is more toxic than vitamin D_2.*
[e]*Nicotinamide is more toxic than nicotinic acid.*
[f]*Only menadione has significant (low) toxicity.*

7. STUDY QUESTIONS AND EXERCISES

1. Prepare a concept map illustrating the relationships of the concepts of minimal and optimal nutrient requirements and nutrient allowances to the concepts of physiological function and health.
2. What issues relate to the consideration of nutritional status in such areas as immune function or chronic and degenerative diseases in the development of dietary standards?
3. Which vitamins are most likely to present potential for hazards for humans?
4. Use specific examples to discuss the relationship of the toxic potential of vitamins to their absorption and metabolic disposition.
5. Prepare a concept map illustrating the relationships of the concepts of minimal and optimal nutrient requirements and nutrient allowances to the concepts of physiological function and health.
6. What considerations are necessary in applying the DRIs to individuals?

RECOMMENDED READING

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Part II

Considering the Individual Vitamins

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Chapter 6

Vitamin A

Chapter Outline

Anchoring Concepts

1. Vitamin A is the generic descriptor for compounds with the qualitative biological activity of **retinol**, i.e., retinoids and some (provitamin A) carotenoids.
2. Vitamin A-active substances are hydrophobic and, thus, are insoluble in aqueous environments (intestinal lumen, plasma, interstitial fluid, and cytosol). Accordingly, vitamin A-active substances are absorbed by micelle-dependent diffusion.
3. Vitamin A was discovered by its ability to prevent **xerophthalmia**.

Nobody was willing to accept that two cents worth of vitamin A was going to reduce childhood mortality by a third or half…A lot of people had spent their lives studying the complex amalgam of elements leading to childhood deaths, and here we were suggesting that we can cut right through this complex, causal web and give two cents worth of vitamin A and prevent those deaths. It didn't sit well.

Al Sommer[1]

1. Alfred Sommer (b. 1942) is professor and Dean Emeritus of the Bloomberg School of Public Health, Johns Hopkins University. In the 1970s, he conducted studies of the impacts of vitamin A deficiency on children in Indonesia. In reanalyzing those results some time later, he noted that the survival of children with vitamin A-deficiency blindness was much lower than those without blindness. He went on to demonstrate that vitamin A treatment, which was known to prevent blindness, also prevented deaths. His remarkable discovery shifted the paradigm for the role of vitamin A in nutrition and health.

LEARNING OBJECTIVES

1. To understand the nature of the various sources of vitamin A in foods.
2. To understand the means of vitamin A absorption from the small intestine.
3. To become familiar with the carriers involved in the extra- and intracellular transport of vitamin A.
4. To understand the metabolic conversions involved in the activation and degradation of vitamin A in its absorption, transport and storage, cellular function, and excretion.
5. To understand current knowledge of the biochemical mechanisms of action of vitamin A and their relationships to vitamin A deficiency diseases.
6. To understand the physiologic implications of high doses of vitamin A.

VOCABULARY

Abetalipoproteinemia
Acyl-CoA:retinol acyltransferase (ARAT)
Aldehyde dehydrogenase
Bleaching
α-Carotene
β-Carotene
β-Carotene 15,15′-oxygenase
γ-Carotene
Canthaxanthin
Carotenodermia
Carotenoid
cGMP phosphodiesterase

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00006-X

Chylomicron
β-Ionone nucleus
Conjunctival impression cytology
CRABP (cellular retinoic acid-binding protein)
CRABP(II) (cellular retinoic acid-binding protein type II)
CRALBP (cellular retinal-binding protein)
CRBP (cellular retinol-binding protein)
CRBP(II) (cellular retinol-binding protein type II)
β-Cryptoxanthin
3,4-Didehydroretinol
Glycoproteins
High-density lipoproteins (HDLs)
Holo-RBP4
Hyperkeratosis
International unit (IU)
Iodopsins
IRBP (interphotoreceptor retinol-binding protein)
Keratomalacia
Low-density lipoproteins (LDLs)
Lecithin–retinol acyltransferase (LRAT)
Lycopene
Measles blindness
Melanopsin
Metarhodopsin II
Modified relative dose–response (MRDR) test
Night blindness
Nyctalopia
Opsins
Pancreatic nonspecific lipase
Peroxisome-proliferator activation receptor (PPAR)
Protein–calorie malnutrition
Provitamin A
Relative dose–response (RDR) test
all-*trans*-Retinal
Retinal isomerase
Retinal oxidase
Retinal reductase
9-*cis*-Retinoic acid
11-*cis*-Retinoic acid
13-*cis*-Retinoic acid
all-*trans*-Retinoic acid
all-*trans*-Retinyl phosphate
apo-RBP4
Retinoic acid receptors (RARs)
Retinoic acid response elements (RAREs)
Retinoids
Retinoid X receptors (RXRs)
all-*trans*-Retinol
13-*cis*-Retinol
Retinol dehydrogenases
Retinol equivalents (RE)
Retinol phosphorylase
Retinyl ester hydrolase
Retinyl β-glucuronide
Retinyl acetate
Retinyl palmitate
Retinyl phosphate
Retinyl stearate
Rhodopsin
STRA6
Thyroid hormone (T_3)
Transducin
Transgenic
Transthyretin
Very low-density lipoproteins (VLDLs)
Xerophthalmia
Xerosis
Zeaxanthin

1. SIGNIFICANCE OF VITAMIN A

Vitamin A is a nutrient of global importance. More than 254 million people are estimated to have deficient serum retinol levels, i.e., <0.7 μM and/or related eye disease. This includes 69% of people in southeast Asia, 49% of people in Africa, and a more out of every four to five living in other regions. Vitamin A deficiency remains the single most important cause of childhood blindness in developing countries. In the mid-1990s, nearly 14 million preschool children (three-quarters from south Asia) were estimated to have clinical eye disease due to vitamin A deficiency. Within a decade that prevalence had declined; yet, more than 5 million children remained affected. If untreated, two-thirds of such children die within months of going blind, due to increased susceptibility to infectious diseases (e.g., measles, severe diarrhea, dysentery, and respiratory diseases) caused by the deficiency. Yet, vitamin A supplementation has been consistently effective[2] in reducing mortality in at-risk children; so much so that vitamin A intervention has been removed from the realm of research and into that of programming.[3]

Despite these gains, the prevalence of subclinical deficiency (serum retinol levels <0.7 μM) has increased. One-third of the world's preschool children appear to be growing up with insufficient vitamin A (Table 6.1). More than 19 million pregnant women in developing countries are vitamin A-deficient; one-third is affected by night blindness.

Subclinical vitamin A deficiency is also associated with increased child mortality in at least 122 countries in Africa, southern and Southeast Asia, and some parts of Latin America and the western Pacific. High rates of morbidity and mortality have long been associated with vitamin A deficiency; recent intervention trials have indicated that

2. 200,000 IU as retinyl palmitate every 6 months.
3. A meta-analysis of 17 clinical trials showed vitamin A supplementation to reduce mortality by an average of 24% (Imdad, A., Herzer, K., Mayo–Wilson, E., et al., 2010. Cochrane Database Syst. Rev. CD008524).

TABLE 6.1 Global Prevalence of Vitamin A Deficiency Among Preschool Children and Pregnant Women

Region	Children (0–5 years)		Pregnant Women	
	Night Blindness % (millions)	Low-Serum Retinol[a] % (millions)	Night Blindness (millions)	Low Serum Retinol[a] % (millions)
Africa	2.0 (2.55)	44.4 (56.4)	9.8 (3.02)	13.5 (4.18)
Americas	0.6 (0.36)	15.6 (8.68)	4.4 (0.50)	2.0 (0.23)
Southeast Asia	0.5 (1.01)	49.9 (91.5)	9.9 (3.84)	17.3 (6.69)
Europe	0.8 (0.24)	19.7 (5.81)	3.5 (0.22)	11.6 (0.72)
Eastern Mediterranean	1.2 (0.77)	20.4 (13.2)	7.2 (1.09)	16.1 (2.42)
Western Pacific (including China)	0.2 (0.26)	12.9 (14.3)	4.8 (1.09)	21.5 (4.90)
Global	0.9 (5.17)	33.3 (190)	7.8 (9.75)	15.3 (19.1)

[a]*Serum retinol <0.7 μM.*
After WHO Global Database on Vitamin A Deficiency, 2009. Global Prevalence of Vitamin A Deficiency in Populations at Risk 1995–2005, WHO, Geneva, 55 pp.

providing vitamin A can reduce child mortality by about 25% and birth-related, maternal mortality by 40%. Vitamin A deficiency in these areas does not necessarily imply insufficient national or regional supplies of food vitamin A, as vitamin A deficiency can also be caused by insufficient dietary intakes of protein, fats, and oils. Still, most studies indicate that children with histories of xerophthalmia consume fewer dark green leafy vegetables than their counterparts without such histories.

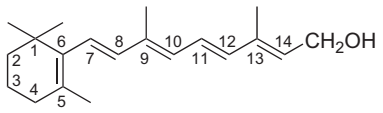
2. PROPERTIES OF VITAMIN A

Vitamin A is the generic descriptor for compounds with the qualitative biological activity of retinol. These compounds are formally derived from a monocyclic parent with five carbon–carbon double bonds and a functional group at the terminus of the acyclic portion. Due to their structural similarities to retinol, they are called **retinoids**. Those with vitamin A activity have the following features of chemical structure:

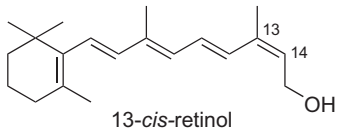
- a substituted β-ionone nucleus [4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one]; a side chain composed of three isoprenoid units joined head to tail at the 6-position of the β-ionone nucleus;
- a conjugated double-bond system among the side chain and 5,6-nucleus carbon atoms.

All three basic forms (retinol, retinal, and retinoic acid) can occur as two variants: with the β-ionone nucleus (vitamin A_1) or with the dehydrogenated β-ionone nucleus (vitamin A_2). Because the former is both quantitatively and qualitatively more important as a source of vitamin A activity, the term vitamin A typically refers to vitamin A_1.

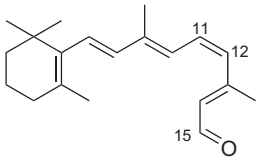
Chemical structures of the vitamin A group:



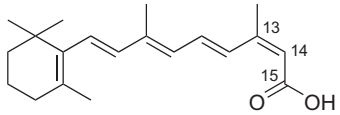
all-*trans*-retinol



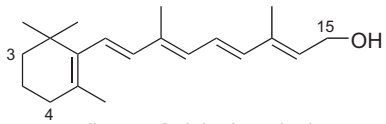
13-*cis*-retinol



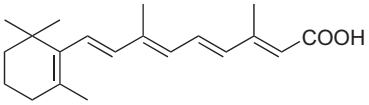
11-*cis*-retinal



13-*cis*-retinoic acid



all-*trans*-3-dehydroretinol
(sometimes called **vitamin A_2**)

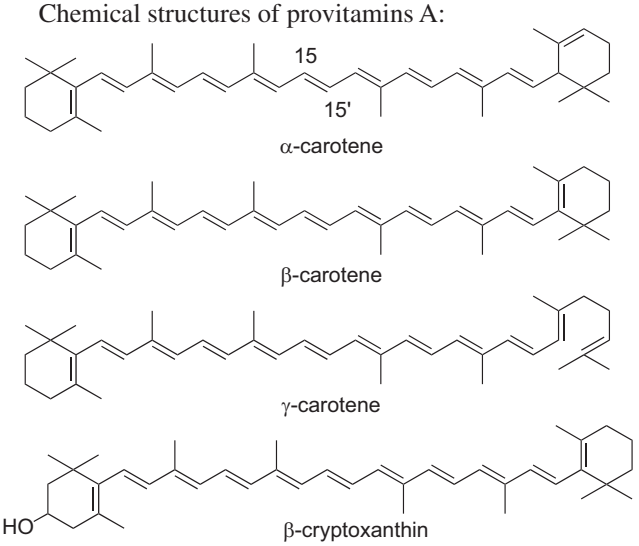


all-*trans*-retinoic acid

Vitamin A-active retinoids occur in three forms:
the alcohol… **retinol**
the aldehyde… **retinal** (also *retinaldehyde*)
the acid… **retinoic acid**.

Some compounds of the class of polyisoprenoid plant pigments called **carotenoids**, owing to their relation to the carotenes, yield retinoids metabolically and, thus, also have vitamin A activity. These **provitamin A** carotenoids include β-carotene, a tail-conjoined retinoid dimer.

Chemical structures of provitamins A:



Chemical Properties of Vitamin A

Of the 16 stereoisomers of vitamin A made possible by the four side chain double bonds, most of the potential *cis* isomers are sterically hindered; thus, only a few isomers are known. In solution, retinoids and carotenoids can undergo slow conversion by light, heat, and iodine through *cis–trans* isomerism of the side chain double bonds (e.g., in aqueous solution, all-*trans*-retinol spontaneously isomerizes to an equilibrium mixture containing one-third *cis* forms).

Contrary to what might be expected by their larger number of double bonds, carotenoids in both plants and animals occur almost exclusively in the all-*trans* form. These conjugated polyene systems absorb light, and, in the case of the carotenoids, appear to quench free radicals weakly. For the retinoids, the functional group at position 15 determines specific chemical reactivity. Thus, retinol can be oxidized to retinal and retinoic acid or esterified with organic acids; retinal can be oxidized to retinoic acid or reduced to retinol; and retinoic acid can be esterified with organic alcohols. Retinol and retinal each undergo color reactions with such reagents as antimony trichloride, trifluoroacetic acid, and trichloroacetic acids, which were formerly used as the basis of their chemical analyses by the Carr–Price reaction.[4]

The vitamers A are insoluble in water, but soluble in ethanol, and freely soluble in organic solvents including fats and oils. Most are crystallizable but have low melting points (e.g., retinol, 62–64°C; retinal, 65°C). Both retinoids and carotenoids have strong absorption spectra. Vitamin A and the provitamin A carotenoids are very sensitive to oxygen in air, especially in the presence of light and heat; therefore, isolation of these compounds requires the exclusion of air (e.g., sparging with an inert gas) and the presence of a protective antioxidant (e.g., α-tocopherol). The esterified retinoids and carotenoids in native plant matrices are fairly stable.

3. SOURCES OF VITAMIN A

Dietary Sources of Vitamin A

Vitamin A exists in natural products in many different forms. It exists as preformed **retinoids**, which are stored in animal tissues, and as provitamin A **carotenoids**, which are synthesized as pigments by many plants and are found in green, orange, and yellow plant tissues. In milk, meat, and eggs, vitamin A exists in several forms, mainly as long-chain fatty acid esters of **retinol**, the predominant one being **retinyl palmitate**.

Foods. Provitamin A carotenoids are present in both plant and animal food products; in animal products their occurrence results from dietary exposure. Carotenoid pigments are widespread among diverse animal species, with more than 500 different compounds estimated. About 60 of these have provitamin A activity, i.e., those that can be cleaved by animals to yield at least one molecule of retinol.[5] In practice, however, only five or six of these provitamins A are commonly encountered in foods.

Of the some 600 carotenoids in nature, only about 50 have provitamin A activity—those that can be cleaved metabolically to yield at least one molecule of retinol. A half-dozen of these are common in foods.

Therefore, actual vitamin A intakes depend on the patterns of consumption of vitamin A-bearing animal food products and provitamin A-bearing fruits and vegetables

4. Reaction of antimony trichloride with vitamin A in chloroform, which yields a quantifiable blue color.

5. These are synthesized by plants from isopentyl diphosphate and its isomer dimethylallyl diphosphate. The condensation of those precursors (in a 3:1 ratio, respectively) yields geranylgeranyl pyrophosphate two molecules of which are condensed to form phytoene, a colorless 40-carbon tetraterpenoid. Phytoene undergoes a series of desaturation and isomerization reactions to yield all-*trans*-lycopene, which is cyclized to generate β-carotene the hydroxylation which yields α-carotene and β-cryptoxanthin.

TABLE 6.2 Sources of Vitamin A in Foods

	Percentage Distribution of Vitamin A Activity		
Food	**Retinol**	**β-Carotene**	**Non-β-carotenoids**
Animal Foods			
Red meats	90	10	
Poultry meat	90	10	
Fish and shellfish	90	10	
Eggs	90	10	
Milk, milk products	70	30	
Fats and oils	90	10	
Plant Foods			
Maize, yellow		40	60
Legumes and seeds		50	50
Green vegetables		75	25
Yellow vegetables[a]		85	15
Pale sweet potatoes		50	50
Yellow fruits[b]		85	15
Other fruits		75	25
Red palm oil		65	35
Other vegetable oils		50	50

[a]*e.g., Carrots and deep-orange sweet potatoes.*
[b]*e.g., Apricots.*
After Leung, W., Flores, M., 1980. Food Composition Table for Use in Latin America. Institute of Nutrition of Central America and Panama, Guatemala City, Guatemala; and Interdepartmental Committee on Nutrition for National Defense, Washington, DC.

(Table 6.2), the relative contributions of which are influenced by food availability and personal food habits. Nursing infants consume both preformed vitamin A and provitamin A, especially if their mothers have adequate vitamin A intakes. Two-thirds of the vitamin A consumed by American omnivores come from carrots, organ meats, fortified breakfast cereals, cheese, margarine, tomatoes, and eggs. Sixty percent of the vitamin A consumed in the Netherlands comes from meats, fats, and oils. Half of the vitamin A consumed by Inuits comes from the livers of fish, seals, and caribou. Most of the vitamin A in the diets of vegetarians and of individuals in low-income countries comes from plant foods (red palm oil, dark/medium green leaves, yellow/orange fruits, and yellow maize).

Fortified foods. Certain foods are fortified with retinyl esters in many countries: milk, margarine, formula foods, and in some cases wheat flour. In the United States, this practice is regulated by the Food and Drug Administration. The use of agricultural technologies to enhance the micronutrient contents of staple foods—an effort referred to as **biofortification** (*see* Chapter 20, Sources of the Vitamins. V. Biofortification). This has involved the use of molecular biological techniques to produce a rice variety ("Golden Rice") containing appreciable amounts (>35 μg/g) of β-carotene, and traditional plant breeding to make substantial improvements in the β-carotene contents of several crops: high β-carotene carrot, orange-fleshed sweet potato, yellow cassava, and high-β-carotene maize.

Breast milk. Breast milk is the key source of vitamin A for the nursing infant. Retinoid and carotenoid contents of milk depend on the stage of lactation and the vitamin A status of the mother, the patterns of carotenoids in colostrum tend to reflect those in maternal low-density lipoproteins (LDLs), while patterns in mature milk (19 days) reflect those in maternal high-density lipoproteins (HDLs).[6] Breast milk from well nourished, vitamin A-adequate mothers typically drops from c.5–7 μM in colostrum, to c.3–5 μM in transitional milk, to 1.4–2.6 μM in mature milk. These levels are enough to meet the infant's immediate metabolic needs while also supporting the development of adequate

6. Schwiegert, F.J., Bathe, K., Chen, F., et al., 2004. Eur. J. Nutr. 43, 39–44.

vitamin A stores.[7] Such an infant will consume over the first 6 months of life, nearly 60 times as much vitamin A from breast milk (c.300 μmoles) than it accumulated throughout gestation. Vitamin A-deficient mothers, however, produce breast milk that is low in the vitamin; in vitamin A-deficient areas of the world, levels average c.1 μM (levels <1.05 μM/L are considered indicative of maternal vitamin A deficiency[8]). This level appears to be sufficient to meet an infant's immediate metabolic requirements, but higher levels (at least 1.75 μM) are required to support adequate vitamin A stores to protect against the development of xerophthalmia during weaning.

Microbiome. There is no evidence for biosynthesis of vitamin A by the gut microbiome; however, a recent study the possibility of producing a vitamin A-producing probiotic. Wassaf et al.[9] inserted plant genes coding for four key enzymes in the β-carotene biosynthetic pathway into an intestine-adapted mutant strain of *E. coli*. When fed to mice lacking the capacity to cleave β-carotene, the altered bacteria was increased the β-carotene contents of host plasma and liver.

Bioavailability

Although 1 mol of β-carotene can, in theory, be converted (by cleavage of the C–15═C–15′ bond) to 2 mol of retinol, the physiological efficiency of this process appears to be much less. Until recently, the efficiency of bioconversion of β-carotene to retinol was assumed to be about 50%, and that of intestinal absorption was assumed to be about 33%; thus, β-carotene was regarded as having one-sixth the vitamin A value of retinol. Accordingly, carotenoids that yield only 1 mol of retinol metabolically were regarded as having one-twelfth the vitamin A value of retinol. This logic was the basis for older dietary recommendations, which used equivalency ratios of 6:1 and 12:1 in setting retinol equivalency values for provitamin A carotenoids in supplements and foods, respectively.

Subsequent research has shown that the bioconversion of food carotenoids to vitamin A can vary considerably (10–90%) (Table 6.3). Isotope dilution studies have shown purified β-carotene in oils and nutritional supplements to be utilized at about half the efficiency of retinol, while β-carotene from plant foods is utilized with much lower efficiencies than previously thought. Retinol equivalency ratios from 3.8:1 to 28:1 have been reported for humans. Low bioconversion appears to be particularly true in resource-poor countries in which children rely almost entirely on the conversion of β-carotene from fruits and vegetables for their vitamin A.[10] In addition to vitamin A status, which inversely affects carotenoid conversion, several other factors can reduce conversion efficiency: low fat intakes, intestinal roundworms, recurrent diarrhea, tropical enteropathy, and other factors that affect the absorptive function of the intestinal epithelium and intestinal transit time. Accordingly, West and colleagues have suggested the use of ratios of 21:1 for mixed diets (12:1 for fruits and 26:1 for vegetables) in such contexts.[11] While the supporting data are sparse, in 2001 the IOM revised its estimates of the vitamin A biopotency of carotenoids to the figures presented in Table 6.4.

Expressing Vitamin A Activities

Because vitamin A exists in foods and supplements in many different forms of differing biopotencies, the reporting of vitamin A activity in foods requires some means of standardization. Three systems are used for this purpose: **retinol equivalents** (**RE**)[12] for food applications and **international units** (**IU**) for pharmaceutical applications.

For reporting food vitamin A activity—retinol equivalents (or retinol activity equivalents, RAE)[13]

1 RE = 1 μg all-*trans*-retinol.
= 2 μg all-*trans*-β-carotene in dietary supplements.
= 12 μg all-*trans*-β-carotene in foods.
= 24 (12–26) μg other provitamin A carotenoids (α-carotene, β-cryptoxanthin) in foods[3]

For pharmaceutical applications—international units.

1 IU[14] = 0.3 μg all-*trans*-retinol.
= 0.344 μg all-*trans*-retinyl acetate.
= 0.55 μg all-*trans*-retinyl palmitate.

7. The normal weight (c.3.2 kg) infant of a well-nourished, vitamin A-adequate, mother is born with hepatic vitamin A stores of c.5 μmoles.
8. Stoltzfus, R.J., Underwood, B.A., 1995. Bull. WHO 73, 703–711.
9. Wassaf, L., Wirawan, R., Chikindas, M., et al., 2014. J. Nutr. 144, 608–613.
10. de Pee, S., West, C.E., Muhilal, X., et al., 1995. Lancet 346, 75–81.
11. The data of van Lieshout, M., West, C.E., van Breeman, R.B., 2001. Am. J. Clin. Nutr. 77, 12–28 suggest a ratio of 2.6 based on stable isotope (or circulating retinol) dilution studies in more than a hundred Indonesian children. Other isotope dilution studies show the conversion of β-carotene to retinol by humans to be quite variable: from individual equivalency ratios of 2:1 to 12:1. (Wang, Z., Yin, S., Zhao, X. et al., 2004. Br. J. Nutr. 91:121–131; Haskell, M.J., Jamil, K.M., Hassan, F., et al., 2004. Am. J. Clin. Nutr. 80:705–714), and from 6:1 to 13:1. De Pee (de Pee, S, West, K.P., Permaesih, D., et al., 1998. Am. J. Clin. Nutr. 68, 1058–1067) suggested that in developing countries this conversion ratio may be as great as 21:1. This implies limits to the contributions of horticultural approaches (e.g., "home-gardening" programs) to solving problems of vitamin A deficiency.
12. These equivalencies were established by the Food and Nutrition Board in 2001. USDA National Nutrient Database for Standard Reference lists both IU and RE; FAO tables list μg of retinol and β-carotene. INCAP (Instituto de Nutrición de Centroamérica y Panamá) tables list vitamin A as μg retinol; those values are not the same as RE values, as a factor of 0.5 was used to convert β-carotene to RE.
13. Proposed in 2001 by the Institute of Medicine, US National Academy of Sciences.
14. Sometimes called USP Unit, as it was adopted by the United States Pharmacopeia.

TABLE 6.3 Apparent Uptake Without Conversion to Vitamin A of Major Food Provitamin A Carotenoids in Humans

	α-Carotene	β-Carotene	β-Cryptoxanthin
Estimated dietary intake (μM/day)	1.18 ± 0.12	6.79 ± 0.36	0.45 ± 0.04
Plasma concentration (μM)	0.10 ± 0.01	0.40 ± 0.04	0.16 ± 0.02
Relative utilization as vitamin A (vs. β-carotene)	0.60	1.00	0.14

After Pooled analysis of several studies; Burri, B.J., Chang, J.S.T., Neidlinger, T.R., 2011. Br. J. Nutr. 105, 212–219.

TABLE 6.4 Relative Biopotencies of Vitamin A and Related Compounds

Compound	Relative Biopotency[a]
All-*trans*-Retinol	100
All-*trans*-Retinal	100
cis-Retinol isomers	23–75
Retinyl esters	10–100
3-Dehydrovitamin A	30
β-Carotene	50
α-Carotene	26
γ-Carotene	21
β–Cryptoxanthin	28
Zeaxanthin	0

[a]*Most relative biopotencies were determined by liver storage bioassays with chicks and/or rats. In the case of 3-dehydrovitamin A, biopotency was assessed using liver storage by fish. In each case, the responses were standardized to that of all-*trans*-retinol.*

Foods Rich in Vitamin A

Several foods contain vitamin A activity (Table 6.5). It is estimated that carotene from vegetables contributes two-thirds of dietary vitamin A worldwide and more than 80% in developing countries. Other than green and yellow vegetables, few other foods are rich sources of vitamin A, those being liver, oily fishes, and vitamin A-fortified products such as margarine. It should be noted that, for vitamin A and other vitamins that are susceptible to breakdown during storage and cooking, values given in food composition tables are probably high estimates of amounts actually encountered in practical circumstances.

4. ABSORPTION OF VITAMIN A

Absorption of Retinoids

Most of the preformed vitamin A in the diet is in the form of **retinyl esters**, but only free retinoids appear to be taken up by the enterocyte. The absorption occurs in three steps:

1. **Hydrolysis of esters**. Retinyl esters are hydrolyzed in the stomach and lumen of the small intestine to yield retinol; this step is catalyzed by hydrolases produced by the gastric lining, pancreatic lipases situated on the mucosal brush border,[15] and esterases intrinsic to the mucosal brush border.
2. **Micellar solubilization**. The retinoids, being hydrophobic, depend on micellar solubilization for their dispersion in the aqueous environment of the small intestinal lumen. For this reason, they likely to be poorly utilized from low-fat diets. The absorption of retinol esters appears to be fairly high (75–100%); the process is appreciably less efficient at very high vitamin A doses.
3. **Mucosal uptake**
   a. **Lymphatic uptake**. Retinol is taken up by mucosal cells by a saturable process thought to involve the multidomain transmembrane protein **STRA6**,[16] and/or **retinoid-binding protein 2 (RBP2)**.
   b. **Nonlymphatic uptake**. Studies have shown that retinoids can also be absorbed via nonlymphatic pathways. Rats with ligated thoracic ducts retain the ability to accumulate vitamin A in their livers. That such animals fed retinyl esters show greater concentrations of retinol in their portal blood than in their aortic blood suggests that, in mammals, the portal system may be an important alternative route of vitamin A absorption when the normal lymphatic pathway is blocked. This phenomenon corresponds to the route of vitamin A absorption in birds, fishes, and reptiles, which, lacking lymphatic drainage of the intestine, rely strictly on portal absorption.

15. One of these activities appears to be the same enzyme that catalyzes the intralumenal hydrolysis of cholesteryl esters; it is a relatively nonspecific carboxylic ester hydrolase. It has been given various names in the literature, the most common being **pancreatic nonspecific lipase** and **cholesteryl esterase**.

16. This protein was named because its expression is stimulated by retinoic acid.

TABLE 6.5 Vitamin A Contents of Foods

Food	Vitamin A (IUg/100 g)
Grains	
Cornmeal	214
Oats	0
Rice	0
Wheat flour	9
Wheat bran	9
Vegetables	
Asparagus	1006
Beans, green	633
Broccoli	623
Cabbage	98
Carrots	16,700
Cauliflower	0
Kale	13,600
Peas, green	765
Potatoes	0
Tomatoes	830
Fruits	
Apples	54
Apricots	1925
Bananas	64
Grapes	100
Oranges	250
Pears	25
Pineapples	38
Meats	
Beef	0–37
Chicken	80–200
Duck	80–210
Pork	0–37
Trout	50
Salmon	55–195
Liver	
Beef liver	16,900
Pork liver	18,000
Dairy Products and Eggs	
Cheese	8–1240
Milk	160–200
Eggs	540
Other	
Human milk	210

After USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

Absorption of Provitamin A Carotenoids

The major sources of vitamin A activity for most populations are the provitamin A carotenoids. Their utilization involves three steps:

1. **Release from food matrices**. A major factor limiting the utilization of carotenoids from food sources is their release from physical food matrices. Carotenoids can occur in cytosolic crystalline complexes or in chromoplasts and chloroplasts, where they are associated with proteins, polysaccharides, fibers, and phenolic compounds. Their release from chromoplasts appears to occur more readily than from chloroplasts and is facilitated by the presence of lipid. Many carotenoid complexes are resistant to digestion without heat treatment; therefore, cooking tends to improve the bioavailability of provitamin A compounds in plant food.
2. **Micellar solubilization**. The enteric absorption of carotenoids depends on their solubilization in mixed lipid micelles the formation of which requires the consumption and digestion of lipids. Absorption, particularly of the less polar carotenoids, can be impaired by the presence of undigested lipids or sucrose polyesters in the intestinal lumen. Gastric acidity may also be an affector, as patients with pharmaceutically obliterated gastric acid production showed reduced blood responses to test doses of β-carotene.[17] Carotenoids (and likely retinoids) are not well absorbed from low-fat meals.[18] One study found that as little as 3–5 g fat per meal may be sufficient for optimal absorption of provitamin A carotenoids,[19] although higher amounts of fat are likely to be necessary to render vitamin A accessible from plant matrices, e.g., at least 10 g/day.
3. **Mucosal uptake**. Uptake of carotenoids from micelles has been thought to involve the diffusion directly through the plasma membranes of the enterocytes; however, the process may actually be carrier-mediated. Reboul has pointed out that careful studies have demonstrated saturable uptake, and that passive diffusion cannot explain either the high interindividual variability in carotenoid absorption observed in humans (5–65%), or the antagonism of carotenoid absorption by tocopherols and other carotenoids.[20] Mucosal uptake appears to be impaired by soluble dietary fiber and, likely, other factors that interfere with the contact of the micelle with the mucosal brush

17. This finding has implications for millions of people, as atrophic gastritis and hypochlorhydria are common, particularly among older people.
18. In fact, Brown and colleagues found the use of fat-free dressing to completely block the absorption of β-carotene from fresh vegetable salad. (Brown, M.J., Ferruzi, M.G., Nguyen, M.L., et al., 2004. Am. J. Clin. Nutr, 80, 396–403).
19. Roodenburg, A.J., Leenen, R., van het Hof, K.H., et al., 2000. Am. J. Clin. Nutr. 71, 1187–1193.
20. Raboul, E., 2013. Nutrients 5, 3563–3581.

border. It is likely that lipid transporters may facilitate carotenoid uptake.[21]

Provitamin A Carotenoid Metabolism Linked to Absorption

While β-carotene can cross the mucosal epithelial cell intact, most is metabolized within the cell. Carotenoid absorption typically results in the accumulation in enterocytes of more all-*trans*-β-carotene than 9-*cis*-β-carotene. This suggests enterocytic capacity for *cis–trans* isomerization, which is also indicated by the fact that humans given 9-*cis*-β-carotene show detectable levels of 9-*trans*-retinol in their plasma. The capacity for isomerization would serve to limit the distribution of 9-*cis*-retinoids to tissues and render both isomers of β-carotene capable of being metabolized to **retinal**, thus serving as effective provitamins A.

Fewer than 10% of naturally occurring carotenoids are provitamins A, those capable of yielding retinal upon hydrolysis. This metabolism is catalyzed by **β-carotene oxygenases (BCOs)**[22] (Fig. 6.1). Most of this bioconversion occurs via the central cleavage of the polyene moiety by a predominantly cytosolic enzyme, **β-carotene 15,15′-oxygenase (BCO1)**, found in the intestinal mucosa, liver, and corpus luteum. BCO1, sometimes also called **carotene cleavage enzyme**, cleaves β-carotene into two molecules of retinal. Several variants of BCO1 have been identified; these vary in specific activity by as much as 100%. It contains ferrous iron (Fe^{++}) linked to a histidinyl residue at the axis of a seven-bladed, β-propeller chain fold covered by a dome structure formed by six large loops in the protein. Upon binding within that structure, the three consecutive *trans* double bonds of the carotenoid are isomerized to a *cis-trans-cis* conformation, leading to the oxygen cleavage of the central *trans* bond. Accordingly, the activity of BCO1 can be affected by intakes of iron and factors affecting iron utilization (e.g., copper, fructose). Expression of BCO1 is repressed by the intestinal transcription factor ISX, which is induced by retinoic acid. This factor also appears to repress the expression of a receptor (scavenger receptor B type 1, SR-B1) thought to facilitate the intestinal absorption of lipids including β-carotene. By this mechanism, both the absorption of β-carotene, as well as its cleavage to produce retinal, are reduced under conditions of vitamin A adequacy.

However, the BCOs are not highly specific for β-carotene. Then can cleave other carotenoids; those have provitamin A activities to the extent that they can also yield retinal. Apocarotenals yield retinal; epoxy carotenoids are

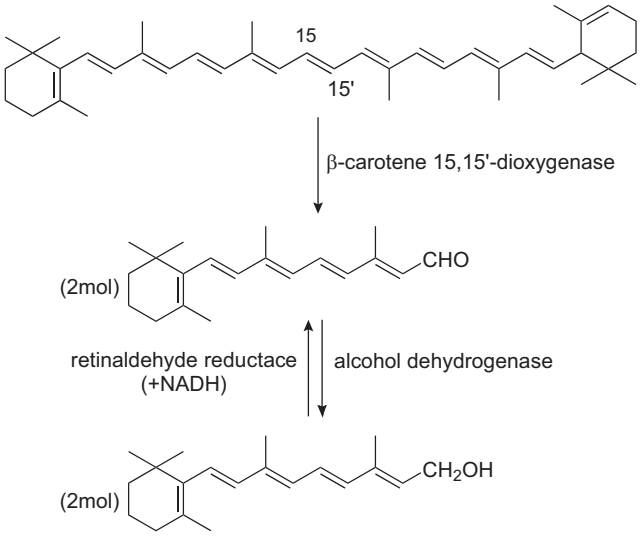


FIGURE 6.1 Bioconversion of provitamins A to retinal.

not metabolized. The reaction requires molecular oxygen, which reacts with the two central carbons (C-15 and C-15′), followed by cleavage of the C—C bond. It is inhibited by sulfhydryl group inhibitors and by chelators of ferrous iron (Fe^{++}). The enzyme has been found in a wide variety of animal species;[23] enzyme activities were found to be greatest in herbivores (e.g., guinea pig, rabbit), intermediate in omnivores (e.g., chicken, tortoise, fish), and absent in the only carnivore studied (cat). The enzyme activity is enhanced by the consumption of triglycerides,[24] suggesting that its regulation involves fatty acids. It is diminished by high intakes of β-carotene and protein deprivation, is induced by vitamin A deficiency, and can be inhibited by quercetin and other flavonols. The symmetric, central cleavage of β-carotene is highly variable between individuals (35–90%). In the bovine corpus luteum, which also contains a high amount of β-carotene, BCO1 activity has been shown to vary with the estrous cycle, showing a maximum on the day of ovulation. Studies with the rat indicate that the activity is stimulated by vitamin A deprivation and reduced by dietary protein restriction.

Low BCO1 activities are associated with the absorption of intact carotenoids; this phenomenon is responsible for the yellow-colored adipose tissue, caused by the deposition of absorbed carotenoids, in cattle. Thus, at low doses β-carotene is essentially quantitatively converted to vitamin A by rodents, pigs, and chicks; cats, in contrast, cannot perform the conversion, and therefore β-carotene cannot support their vitamin A needs.

The asymmetric cleavage of carotenoids also occurs by a second intestinal mucosal enzyme, β-carotene oxygenase

21. Candidates include two class B scavenger receptors, SR-BI and cluster determinant 36 (CD36); a cholesterol transporter, Neimann–Pick like C1 protein (NPV1L1); and a gut-specific transcription factor ISX, which may repress SR-BI expression in that organ.

22. More than 100 enzymes in this group are known. Two occur in animals: β-carotene-15,15'-oxygenase and β-carotene-9′,10′-oxygenase.

23. The β-carotene 15,15′-dioxygenase has also been identified in *Halobacterium halobium* and related halobacteria, which use retinal, coupled with an opsin-like protein, to form bacteriorhodopsin, an energy-generating, light-dependent proton pump.

24. Which also increase CRBP(II) levels.

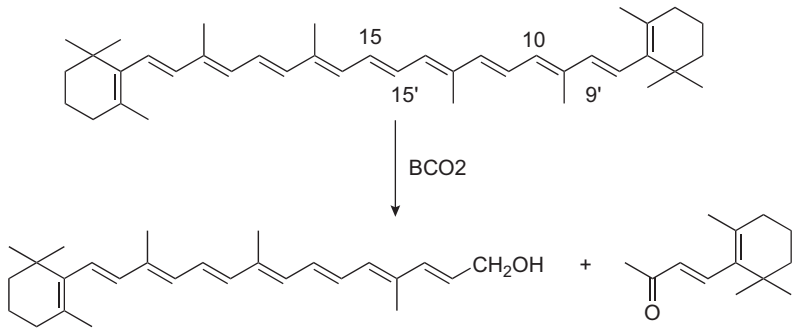


FIGURE 6.2 Asymmetric (or eccentric) cleavage of β-carotene by the β-carotene oxygenase 2 (BCO2) yields apo-10′-β-carotenal and β-ionone.

2 (BCO2), although this appears to be a quantitatively minor pathway. This enzyme cleaves the carotene-9′, 10′-bond to form apo-10′-β-carotenal (Fig 6.2), which can be chain-shortened directly to yield retinal or, first, oxidized to the corresponding apocarotenoic acids and, then, chain-shortened to yield **retinoic acid**.[25] It has been suggested that BCO2 functions in the metabolism of acylic, nonprovitamin A carotenoids such as lycopene, which accumulates when the enzyme is not expressed. Intestinal enzymes can cleave 9-*cis*-β-carotene (which comprises 8–20% of the β-carotene in fruits and vegetables but seems less well utilized than the all-*trans* isomer) to 9-*cis*-retinal which, in turn, appears to be oxidized to 9-*cis*-retinoic acid.

The turnover of carotenoids in the body occurs via first-order mechanisms that differ for individual carotenoids. For example, in humans the biological half-life of β-carotene has been determined to be 37 days, whereas those of other carotenoids vary from 26 days (lycopene) to 76 days (lutein).

Mucosal Metabolism of Retinol

Retinol formed either from the hydrolysis of dietary retinyl esters or from the reduction of retinal cleaved from β-carotene[26] is absorbed by facilitated diffusion via a specific transporter.[27] Retinal produced by the central cleavage of β-carotene is reduced in the intestinal mucosa to retinol (Fig. 6.1) by **retinaldehyde reductase**, which is also found in the liver and eye. The reduction requires a reduced pyridine nucleotide (NADH/NADPH) as a cofactor and has an apparent K_m of 20 mM. It can also be catalyzed by a **short-chain alcohol dehydrogenase/aldehyde reductase**, and there is some debate concerning whether the two activities reside on the same enzyme.

Retinol is quickly reesterified with long-chain fatty acids in the intestinal mucosa whereupon retinyl esters are transported to the liver (i.e., 80–90% of a retinol dose[28]). The composition of lymph retinyl esters is remarkably independent of the fatty acid composition of the most recent meal. **Retinyl palmitate** typically comprises about half of the total esters, with **retinyl stearate** comprising about a quarter and retinyl oleate/linoleate present in small amounts. Two pathways for the enzymatic reesterification of retinol have been identified in the microsomal fraction of the intestinal mucosa (Fig. 6.3): a low-affinity route involving uncomplexed retinol and catalyzed by **acyl-CoA:retinol acyltransferase** (**ARAT**); a high-affinity route involving retinol complexed with a specific binding protein, **cellular retinol-binding protein type II [CRBP(II)[29]]** and catalyzed by **lecithin–retinol acyltransferase** (**LRAT**). The expression of LRAT mRNA is induced by retinoic acid and downregulated by vitamin A depletion. It has been suggested that LRAT serves to esterify low doses of retinol, whereas ARAT serves to esterify excess retinol, when CRBP(II) becomes saturated. The identification of a retinoic acid-responsive element in the promoter region of the CRBP(II) gene suggests that the transcription of that gene may be positively regulated by retinoic acid, leading to increased CRBP(II) levels at high vitamin A doses. Experiments have shown that CRBP(II) expression is enhanced under conditions of stimulated absorption of fats, especially unsaturated fatty acids.

5. TRANSPORT OF VITAMIN A

Retinyl Esters Conveyed by Chylomicra in Lymph

Retinyl esters are secreted from the intestinal mucosal cells in the hydrophobic cores of chylomicron particles, by which

25. Studies of these processes are complicated by the inherent instability of carotenoids under aerobic conditions; many of the products thought to be produced enzymatically can also be produced by autoxidation.
26. It has estimated that humans convert 35–71% of absorbed β-carotene to retinyl esters.
27. This protein transports both all-*trans*-retinol and 3-dehydroretinol. Other retinoids appear to be taken up by enterocytes by passive diffusion.
28. Humans fed radiolabeled β-carotene absorbed some unchanged directly into the lymph, with only 60–70% of the label appearing in the retinyl ester fraction.
29. CRBP(II) is a 15.6 kDa protein that constitutes about 1% of the total soluble protein of the rat enterocyte.

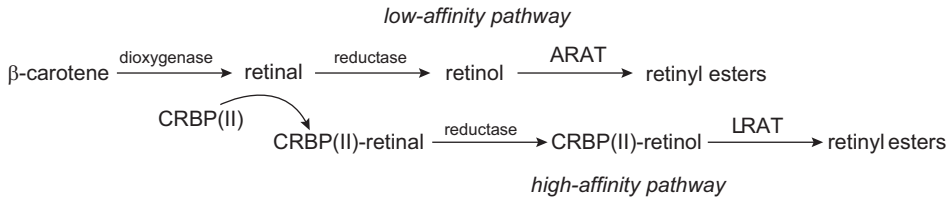


FIGURE 6.3 Intestinal metabolism of vitamin A.

TABLE 6.6 Distribution of Carotenoids in Human Lipoproteins

Carotenoid	VLDL (Very Low-Density Lipoprotein)	LDL (Low-Density Lipoprotein)	HDL (High-Density Lipoprotein)
Zeaxanthin/lutein (%)	16	31	53
Cryptoxanthin (%)	19	42	39
Lycopene (%)	10	73	17
α-Carotene (%)	16	58	26
β-Carotene (%)	11	67	22

After Reddy, P.P., Clevidance, B.A., Berlin, E., Taylor, P.R., Bieri, J.G., Smith, J.C., 1989. FASEB J. 3, A955.

absorbed vitamin A is transported to the liver through the lymphatic circulation, ultimately entering the plasma[30] compartment through the thoracic duct. Carnivorous species in general, and the dog in particular, typically show high plasma levels. Retinyl esters are almost quantitatively retained in the extrahepatic processing of chylomicra to their remnants; therefore, chylomicron remnants are richer in vitamin A than are chylomicra. Retinyl and cholesteryl esters can undergo exchange reactions between lipoproteins including chylomicra in rabbit and human plasma by virtue of a **cholesteryl ester transfer protein** peculiar to those species.[31] Although this kind of lipid transfer is probably physiologically important in those species, the demonstrable transfer involving chylomicra is unlikely to be a normal physiological process.

Transport of Provitamin A carotenoids. Carotenoids appear to be transported across the intestinal mucosa by a facilitated process similar to that of cholesterol. They are not metabolized in the epithelium but are transported from that organ by chylomicra via the lymphatic circulation to the liver, where they are transferred to lipoproteins. It is thought that strongly nonpolar species such as β-carotene and lycopene are dissolved in the chylomicron core; whereas species with polar functional groups may exist at least partially at the surface of the particle. Such differences in spatial distribution would be expected to affect transfer to lipoproteins during circulation and tissue uptake. Indeed, the distribution of carotenoids among the lipoprotein classes reflects their various physical characteristics, with the hydrocarbon carotenoids being transported primarily in LDLs and the more polar carotenoids being transported in a more evenly distributed manner among LDLs and HDLs (Table 6.6). It is thought that small amounts of the nonpolar carotenoids are transferred from chylomicron cores to HDLs during the lipolysis of the triglycerides carried by the former particles; however, because HDL transports only a small fraction of plasma β-carotene, the carrying capacity of the latter particles for hydrocarbon carotenoids would appear to be small. Therefore, it is thought that β-carotene is retained by the chylomicron remnants to be internalized by the liver for subsequent secretion in very low-density lipoproteins (VLDLs).

Abetalipoproteinemia. Absorption of vitamin A and other fat-soluble vitamins is a particular problem in patients with **abetalipoproteinemia**, a rare autosomal recessive disorder characterized by general lipid malabsorption, acanthosis (diffuse epidermal hyperplasia), and hypocholesterolemia. These patients lack apo B and consequently

30. On entering the plasma, chylomicra acquire apolipoproteins C and E from high-density lipoproteins (HDLs). Acquisition of one of these (apo C-II) activates lipoprotein lipase at the surface of extrahepatic capillary endothelia; that lipase hydrolyses the core triglycerides, causing them to shrink and transfer surface components (e.g., apo A-I, apo A-II, some phospholipid) to HDLs and fatty acids to serum albumin, and to lose apo A-IV and fatty acids to the plasma and other tissues. These processes leave a smaller particle, a **chylomicron remnant**, which is depleted of triglyceride but relatively enriched in cholesteryl esters, phospholipids, and proteins (including apo B and apo E). Chylomicron remnants are removed from the circulation almost entirely by the liver by a rapid, high-affinity receptor-mediated process stimulated by apo E.

31. This protein has not been found in several other mammalian species examined.

cannot synthesize any of the apo B-containing lipoproteins (i.e., LDLs, VLDLs, and chylomicra). Having no chylomicra, they show hypolipidemia and low plasma vitamin A levels. However, when given oral vitamin A supplementation, their plasma levels are normal. Although the basis of this response is not clear, it has been suggested that these patients can transport retinol from the absorptive cells via their remaining lipoprotein (HDLs), possibly by the portal circulation.

Vitamin: An Uptake by the Liver

Most of the recently absorbed vitamin A is taken up by the liver from chylomicron remnants, which hepatic parenchymal cells[32] remove from the circulation via a high-affinity receptor-mediated process stimulated by apo E.[33] Because this process is rapid and nearly quantitative, vitamin A (mostly as retinyl esters with smaller amounts of β-carotene) circulates in chylomicra only for a short time.[34] After being taken up by the liver retinyl esters are hydrolyzed to yield retinol in parenchymal cells from which it is transferred by a retinol-binding protein to stellate cells,[35] which also contain appreciable amounts of triglycerides, phospholipids, free fatty acids, and cholesterol. There it is re-esterified and stored in droplets (some that are membrane-bound and appear to be derived from lysosomes and other, larger ones not associated with membranes). It is likely that the reesterification of retinol proceeds by a reaction similar to that of the intestinal microsomal acyl CoA:retinol acyltransferase (ARAT). The liver thus serves as the primary storage depot for vitamin A, normally containing 50–80% of the total amount of the vitamin in the body.[36] Most of this (80–90%) is stored in stellate cells, which account for only about 2% of total liver volume. The balance stored in parenchymal cells. These are the only types of hepatocytes that contain retinyl ester hydrolase activities. Almost all (about 95%) of hepatic vitamin A occurs as long-chain retinyl esters, the predominant one being retinyl palmitate. Kinetic studies of vitamin A turnover indicate the presence, in both liver and extrahepatic tissues, of two effective pools (i.e., fast- and slow-turnover pools) of the vitamin. Of rat liver retinoids, 98% were in the slow-turnover pool (retinyl esters of stellate cells), with the balance corresponding to the retinyl esters of parenchymal cells.

In addition to retinol ester hydrolyases and ARAT, stellate cells contain two other retinoid-related proteins: **cellular retinoid-binding protein (CRBP)** and **cellular retinoic acid-binding protein** (**CRABP**). The storage or retinyl esters appears not to depend on the expression of CRBP, as **transgenic** mice that overexpressed CRBP in several organs have not shown elevated vitamin A stores in those organs. The metabolism of vitamin A by hepatic cytosolic retinal dehydrogenase increases with increasing hepatic retinyl ester stores.

Mobilization from the liver. Vitamin A is mobilized as retinol from the liver by hydrolysis of hepatic retinyl esters.[37] This mobilization accounts for about 55% of the retinol discharged to the plasma, the balance coming from recycling from extrahepatic tissues. The retinyl ester hydrolase involved in this process remains poorly characterized; it shows extreme variation between individuals.[38] The activity of this enzyme is known to be low in protein-deficient animals and has been found to be inhibited, at least in vitro, by vitamins E and K.[39]

Extracellular transport. The transport of mobilized retinol from the liver to peripheral tissues is thought to depend on a specific carrier, **retinol-binding protein** (**RBP4**).[40] Human RBP4 consists of a single polypeptide chain of 182 amino acid residues, with a molecular mass of 21 kDa. Like other RBPs,[41] it is classified as a member of the lipocalin family of lipid-binding proteins. These are composed of an eight-stranded, antiparallel β-sheet that is folded inward to form a hydrogen-bonded, β-barrel that comprises the ligand-binding domain the entrance of which is flanked by a single loop scaffold. Within this domain, a single molecule of all-*trans*-retinol is completely encapsulated, being stabilized by hydrophobic interactions of the β-ionone ring and the isoprenoid chain with the amino acids lining the interior of the barrel structure. This structure protects the vitamin from oxidation during transport. RBP4 is synthesized as a 24-kDa *pre-RBP4* by parenchymal cells, which also convert it to RBP4 by the cotranslational removal

32. The parenchymal cell is the predominant cell type of the liver, comprising more than 90% of organ volume.
33. Chylomicron remnants are recognized by high-affinity receptors for their apo E moiety.
34. Their remnants are degraded in hepatic parenchymal lysosomes.
35. Are also called **pericytes**, **fat-storing cells**, **interstitial cells**, **lipocytes**, **Ito cells,** or **vitamin A-storing cells**.
36. Mean hepatic stores have been reported in the range of 171–723 μg/g in children and 0–320 μg/g in adults (Panel on Micronutrients, Food and Nutrition Board [2002] Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Washington, DC: National Academy Press, p. 95.).
37. Retinol oxidation also produces some retinoic acid most of which in the plasma is bound to albumen.
38. In the rat, hepatic retinyl ester hydrolase activities can vary by 50-fold among individuals and by 60-fold among different sections of the same liver.
39. Each vitamin has been shown to act as a competitive inhibitor of the hydrolase. This effect may explain the apparently impaired hepatic vitamin A mobilization by animals fed very high levels of vitamin E.
40. That oral α-retinol, a structural isomer of retinol not bound by RBP4, could support the deposition of that isomer in the liver and milk in lactating sows. (Dever, J.T., Surles, R.L. and Davis, C.R., 2010. J. Nutr. 141, 42–47) suggests RBP4-independent transport of the vitamin.
41. Cellular retinal- and retinoic acid-binding proteins.

of a 3.5-kDa polypeptide.[42] This protein product (apo-RBP4) is secreted in a 1:1 complex with all-*trans*-retinol (holo-RBP4). Stellate cells also contain low amounts of RBP4; however, it is not clear whether they synthesize it or their apo-RBP4 derives from parenchymal cells to mobilize retinol to the circulation. According to the latter view, stellate cells may be important in the control of retinol storage and mobilization, a complex process that is thought to involve retinoid-regulated expression of CRBPs.

The secretion of holo-RBP4 from the liver is regulated in women by estrogen level,[43] and in all individuals by vitamin A status (i.e., liver vitamin A stores), protein, and zinc status; deficiencies of each markedly reduce RBP4 secretion and, thus, reduce circulating levels of retinol (Table 6.7). In cases of protein–energy malnutrition, RBP4 levels (and, thus, serum retinol levels) can be decreased by as much as 50%. Except in the postprandial state, virtually all plasma vitamin A is bound to RBP4. In the plasma, almost all RBP4 forms a 1:1 complex with **transthyretin**[44] (TTR, a tetrameric, 55-kDa protein that strongly binds four thyroxine molecules). The formation of the relatively large RBP4–TTR complex reduces the loss of RBP4 by glomerular filtration.[45] This effect may also involve **megalin**,[46] which has been shown to bind the RBP4–TTR complex. The kidney appears to be the only site of RBP4 catabolism, which normally turns over rapidly, in 11–16 h in humans.[47]

TABLE 6.7 Percentage Distribution of Vitamin A in Sera of Fasted Humans

Fraction	Retinol (%)	Retinyl Palmitate (%)
RBP4 (retinol-binding protein 4)	77	–
VLDL (very low-density lipoprotein)	6	71
LDL (low-density lipoprotein)	8	29
HDL (high-density lipoprotein)	9	–
Total	100	100[a]

[a]*This represents only about 5% of the total circulating vitamin A.*

Computer modeling studies indicate that more than half of hepatically released holo-RBP4 comes from apo-RBP4 recycled from RBP4–TTR complexes. Apo-RBP4 is not secreted from the liver. Vitamin A-deficient animals continue to synthesize apo-RBP4, but the absence of retinol inhibits its secretion (a small amount of denatured apo-RBP4 is always found in the plasma). Owing to this hepatic accumulation of apo-RBP4, vitamin A-deficient individuals may show a transient overshooting of normal plasma RBP4 levels on vitamin A realimentation.

Other factors can alter the synthesis of RBP4 to reduce the amount of the carrier available for binding retinol and secretion into the plasma. Dietary deficiencies of protein (e.g., **protein–energy malnutrition**) and/or zinc can reduce the hepatic synthesis of apo-RBP4. Because, RBP4 is a negative acute phase reactant, subclinical infections, or inflammation can also decrease circulating retinol levels. Thus, low-serum retinol levels in malnourished individuals may not be strictly indicative of a dietary vitamin A deficiency. Also, because vitamin A deprivation leads to reductions in plasma RBP4 only after the depletion of hepatic retinyl ester stores (i.e., reduced retinol availability), the use of plasma RBP4/retinol as a parameter of nutritional vitamin A status can yield false-negative results in cases of vitamin A deprivation of short duration.

A two-point mutation in the human RBP4 gene has been shown to result in markedly impaired circulating retinol levels; surprisingly, this is associated with no signs other than night blindness.[48] This suggests that other pathways are also important in supplying cells with retinol, presumably via retinyl esters and/or β-carotene, and/or with retinoic acid. Indeed, the genetic ablation of RBP4 did not impair the ability of β-carotene to prevent signs of vitamin A deficiency in the mouse, indicating that the provitamin, which is transported independently of RBP4, can serve as a tissue source of retinol.[49] That the systemic functions of vitamin A can be discharged by retinoic acid, which is ineffective in supporting vision, indicates that the metabolic role of RBP4 is to deliver retinol to the pigment epithelium as a direct requirement of visual function and to other cells as a precursor to retinoic acid. Retinoic acid is not transported by RBP4, but it is normally present in the plasma, albeit at very low concentrations (1–3 ng/mL), tightly bound to albumin.

42. Retinol-binding proteins isolated from several species (rat, chick, dog, rabbit, cow, monkey, human) have similar sizes and binding properties.
43. Seasonally breeding animals show threefold higher plasma RBP4 levels in the estrous compared with the anestrous phase. Women using oral contraceptive steroids frequently show elevated plasma RBP4 levels.
44. Formerly, *prealbumin*.
45. The half-life of *holo*-RBP4–transthyretin complex in human adult males has been found to be 11–16 hr; whereas, that of free RBP4 was only 3.5 h. Half-lives of both increase (i.e., turnover decreases) under conditions of severe protein–calorie malnutrition.
46. Megalin is a 600 kDa transmembrane protein in the LDL-receptor family.
47. For this reason, patients with chronic renal disease show markedly elevated plasma levels of both RBP4 (which show a half-life 10– to 15-fold normal) and retinol, although transthyretin levels remain normal. Turnover studies have indicated that some retinol recirculates to the liver, perhaps via transfer to lipoproteins.
48. Biesalski, H.K., Frank, J., Beck, S.C., et al., 1999. Am. J. Clin. Nutr. 69, 931–936.
49. Kim, Y., Wassef, S., Chang, S., et al., 2011. FASEB J. 25, 1641–1652.

It is presumed that the cellular uptake of retinoic acid from serum albumin is very efficient.

RPB4 is also expressed in adipose and other tissues, although liver is the predominant source of the protein in circulation. Appreciable amounts of vitamin A are stored in adipocytes (15–20% of total body store), more than half as retinyl esters. That visceral fat expresses more RBP4 than subcutaneous fat makes serum apo-RBP4 a candidate biomarker for visceral adiposity. Unlike other tissues, which take up retinol from RBP4, adipocytes take up retinyl esters from chylomicra. Studies with the rat have shown that the mobilization of vitamin A from adipocytes also differs from that process in other cells. A cAMP-sensitive, hormone-dependent lipase converts adipocyte retinyl esters to retinal in a manner analogous to the liberation of free fatty acids from adipocyte triglyceride depots.

RBP4 secreted from adipocytes has been found to be elevated in overweight/obese individuals, although much of this appears not to be bound to retinol.[50] That elevated apo-RBP4 levels may be related to the development of type 2 diabetes was suggested by the finding on increased gluconeogenic capacity on mice treated with recombinant RPB4. Studies with humans have found plasma RPB4 level to be positively correlated with body mass index (BMI, kg/m^2) (Fig 6.4), the degree of insulin resistance and impaired glucose tolerance in subjects with obesity or family histories of type 2 diabetes.[51] In contrast, plasma RBP4 levels have been found to be reduced by treatments that reduce insulin resistance, e.g., exercise training, gastric bypass surgery, and positively correlated with the expression of p85[52] in adipose tissue. These observations reflect increased apo-RBP4 secretion under conditions in which adipocytes downregulate the expression of GLUT4, the insulin-responsive transporter required for cellular uptake of glucose. This evidence suggests that adipocyte-derived RBP4 may acting as an adipokine.[53]

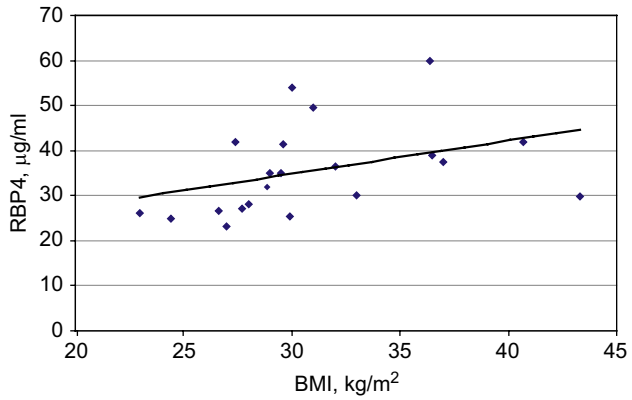


FIGURE 6.4 Positive association of plasma RBP4 level and body mass index (BMI). *Graham, T.E., Yang, Q., Blűher, M., et al., 2006. N. Eng. J. Med. 354, 2552–2563.*

Cellular Uptake of Retinol

Due to their hydrophobic character, the plasma membranes do not present a barrier to retinol uptake and, thus, retinol can enter target cells by nonspecific partitioning into the plasma membrane from holo-RBP4.[54] Nevertheless, most of the vitamin appears to enter cells through specific holo-RBP4-TTR–receptor-mediated mechanisms. The retinol ligand of holo-RBP4-TTR is taken into cells via binding of the complex to a specific cell surface receptor, a multidomain transmembrane protein **STRA6**.[55] STRA6 is expressed in all tissues *except* liver. It is upregulated by retinoic acid.[56] In extrahepatic tissues, STRA6 transfers vitamin A bidirectionally between extracellular and intracellular RBPs, net cellular accumulation being accomplished by trapping the vitamin within the cell through its esterification. The extracellular region of STRA6 binds tightly to the holo-RPB4; however, the presence of TTR inhibits that binding. Thus, it is thought that reductions in circulating TTR, as occur in the acute phase response, release holo-RBP4 for binding to STRA6 to facilitate cellular uptake of retinol. Transfer of retinol into the cell also depends on access to CRBP(I); in cells capable of esterifying retinol,[57] esterification constitutes a sink that drives cellular uptake of the vitamin. In extrahepatic tissues, this process appears to be regulated by all-*trans*-retinoic acid. STRA6 also has a signaling function; when bound to holo-RBP, it activates a Janus kinase (JAK[58]) by catalyzing the phosphorylation of a C-terminal tyrosine residue in that factor.

50. Yang, Q., Graham, T.E., Mody, N., et al., 2005. Nature 436, 356–362.
51. Graham, T.E., Yang, Q., Blűher, M., et al., 2006. New Eng. J. Med. 354, 2552; Broch, M., Vendrell, J., Ricart, W., et al., 2007. Diabetes Care 30, 1802; Chavez, A.O., Coletta, D.K., Kamath, S., et al., 2008. Am. J. Physiol. Endorcrinol. Metab. 296, E768; Chavez, A.O., Coletta, D.K., Kamath, S., et al., 2009. Am. J. Physiol. Endorcrinol. Metab. 296, E768; Kelly, K.R., Kashyap, S.R., O'Leary, V.B., et al., 2009. Obesity 18, 663.
52. p85 is a regulatory subunit of a protein involved in insulin signaling, phosphoinositol 3-kinase (PI3K). PI3K is activated by phosphorylation by insulin receptor substrate-1.
53. Adipokines are cell-signaling proteins (cytokines) secreted by adipocytes. They include several that may be involved in insulin resistance: C-reactive protein (CRP), leptin, adiponectin, resistin, and tumor necrosis factor α (TNFα).
54. Indeed, retinol has been shown to move spontaneously between the layers of artificial phospholipid bilayers.
55. Kelly, M., von Lintig, J., 2015. HepatoBiliary Surg. Nutr. 4, 229–242.
56. The STRA6 gene transcriptionally upregulated by the RARγ/RXRα heterodimer and repressed by RARα. Mutations in the gene produce diffferent developmental defects.
57. i.e., That express lecithin–retinol acyltransferase (LRAT).
58. Originally named "just another kinase," these tyrosine kinases transduce cytokine-mediated signals via the JAK/STAT pathway.

The resulting signaling cascade induces expression of STAT[59] target genes including PPARγ (peroxisome proliferator-activated receptor), which promotes lipid accumulation, and SOCS3,[60] which suppresses insulin signaling.

Another RBP receptor, RBPR2, has been identified. This receptor would appear to play an important role in retinol uptake in most tissues, as STRA6-null mice show reduced levels of vitamin A only in the eye. RBPR2 is a double-chain protein in humans, but a single chain in the mouse. Its primary amino acid structure shows only 18% homology with STRA6. It is thought that this receptor must be active in hepatic uptake of vitamin A. Expressed in liver, RBPR2 is thought to function in the hepatic uptake of vitamin A from chylomicrons, which is quantitatively greater than that of extrahepatic tissues with the exceptions of the mammary gland and bone marrow.

Release of retinol to the target cell increases in the negative charge of the resulting apo-RBP4; this reduces its affinity for TTR, which is subsequently lost. The residual apo-RBP4 can then be filtered by the kidney, where it is degraded. Thus, plasma apo-RBP4 levels are normally low but can be elevated (by about 50%) under conditions of acute renal failure. Studies have shown that apo-RBP4 can be recycled to the holo form; injections of apo-RBP4 into rats produced marked (70–164%) elevations in serum retinol levels. It is thought, therefore, that circulating apo-RBP4 may be a positive feedback signal from peripheral tissues for the hepatic release of retinol, the extent of which response is dependent on the size of hepatic vitamin A stores.

Intracellular Retinoid-Binding Proteins

On entry into the target cell, retinol combines with other binding proteins:

- cellular retinol-binding proteins, CRBP(I) and CRBP(II)
- cellular retinoic acid-binding proteins, CRABP(I) and CRABP(II)
- cellular retinal-binding protein, CRALBP
- interphotoreceptor retinol-binding protein, IRBP

The CRBPs and CRABPs have the same general tertiary structure as the **lipocalins**, a class of low-molecular weight proteins with multistranded β-sheets folded into a deep hydrophobic pocket suitable for binding hydrophobic ligands.[61] Each consists of some 135 amino acid residues and shows with pairwise sequence homologies of 40–74%. Each has been highly conserved (91–96% sequence homology among the human, rat, mouse, pig, and chick proteins). Their genes each contain four exons and three introns, the latter being positioned identically. The proximity of the genes for CRBPs (I) and (II) (only 3 centimorgans) suggests that this pair resulted from the duplication of the same ancestral gene. Their gene products show different tissue distributions: CRBP(I) is among the most abundant cytosolic proteins; it is expressed in most fetal and adult tissues, particularly those of the liver, kidney, lung, choroids plexus. and pigment epithelium; CRBP(II) is expressed only in mature enterocytes in the villi of the mucosal epithelium (especially jejunum) and in the fetal and neonatal liver.

CRALBP and IRBP are in the group of intracellular lipid-binding proteins that include the fatty acid-binding proteins. Like the lipocalins, they also have antiparallel, β-barrel structure that binds lipophilic ligands; however, they bind vitamin A in the reverse orientation from the CRBPs and CRABPs, i.e., with its polar group buried internally and the β-ionone ring close to the surface. The IRBPs can bind three retinol molecules (all other RBPs bind only a single ligand molecule) as well as two long-chain fatty acid molecules.

Tissue levels of the mRNAs for the CRBPs are influenced by vitamin A status. Both CRBP(I) protein and mRNA are reduced by deprivation of the vitamin; however, CRBP(II) protein and mRNA levels are increased by vitamin A deficiency. The CRBP gene appears to be inducible by retinoic acid and **retinoic acid response elements (RAREs)** have been identified in both the CRBP(I) and CRBP(II) promotors. Whether other transcriptional factors also bind to those elements remains to be learned, yet it appears that these genes are responsive to other hormones, including glucocorticoids and 1,25-dihydroxyvitamin D_3, which have been shown to have negative effects on CRBP(I) and CRBP(II), respectively.

Because CRBP(I) is present at high levels in cells that synthesize and secrete RBP4, it has been suggested that it may interact at specific sites to effect the transfer of retinol to RBP4 for release to the general circulation. The synthesis and/or the retinol-binding affinity of CRBP(I) may be affected by thyroid and growth hormones, both of which promote the cellular uptake of retinol.

The intracellular vitamin A-binding proteins appear to be important in the cellular uptake and the intracellular and transcellular transport of vitamin A metabolites. Both the CRBPs and CRABPs serve as carriers of their respective ligands from the cytoplasm into the nucleus, where they are transferred to the chromatin with release of the binding proteins possibly to return to the cytoplasm. The CRBPs appear to have more specialized transport functions in certain tissues. In the liver, their concentrations increase with increasing retinyl ester contents, suggesting that they may function in the transport of retinol from parenchymal cells into the stellate cells, which store retinyl esters. CRBP localization has been found in endothelial cells of the brain microvasculature, in cuboidal cells of the choroid plexus, in

59. Signal transducers and activators of transcription.
60. This gene encodes the protein suppressor of cytokine signaling 3.
61. Other lipocalins bind fatty acids, cholesterol, and biliverdin.

the Sertoli cells of the testis, and in the pigment epithelium of the retina. Because these tight-junctioned cells also have surface receptors for the plasma holo-RBP4–TTR complex, it is thought that their abundant CRBP(I) concentrations are involved in the transport of retinal across the blood–brain, blood–testis, and retinal blood–pigment epithelial barriers. Studies with mice have shown that CRBP(I) is necessary for the hepatic uptake of retinol: CRBP(I)-null individuals exhausted their hepatic retinyl ester stores even when they were fed vitamin A.

CRBP(II) appears to be restricted largely to the enterocytes of the small intestine (particularly, the jejunum). Its abundance in mature enterocytes, where it comprises 1% of the total soluble protein, as well as the absence of CRBP(I) in these cells, suggest that CRBP(II) is involved in enteric absorption of vitamin A, presumably by transporting it across the cell. Both CRBP(II), as well as a high-capacity esterase that esterifies CRBP(II)-bound retinol, have been identified in hepatic parenchymal cells of fetal and newborn rats. After birth, CRBP(II) appears to be replaced by CRBP(I), such that mature animals show none of the former binding protein in that organ. The presence of CRBP(II) in fetal liver corresponds to the increased concentration of retinyl palmitate in that organ at birth.

Some extracellular RBPs appear to serve similar transport functions. These include two low-molecular weight retinoic acid-binding proteins generally related to RBP4 that are secreted into the lumen of the epididymis where they are thought to participate in the delivery of all-*trans*-retinoic acid to sperm, which are rich in CRBP(I).[62] Other retinol-binding proteins are synthesized in the uterine endometrium and secreted into the uterus; these show some sequence homology with RBP4 but are slightly larger. They are thought to be involved in the transport of retinol to the fetus.

CRALBP is a 36 kDa protein that binds 11-*cis*-retinal and appears to be expressed only in pigment epithelial and Müller glial cells of the retina where it is thought to facilitate the intracellular transport of 11-cis-retinal. That it is essential for cone function has been demonstrated in mice: deletion of CRALBP results in severe (20-fold) reduction in cone dark adaptation.[63] This effect was appeared to be due to impaired chromophore recycling by Müller cells, as adenovirus-mediated restoration of CRALBP specifically to Müller cells restored visual sensitivity. Mutations in the CRALBP gene are known to cause forms of retinitis pigmentosa.

IRBP is thought to function in the transport of retinol between the pigment epithelium and photoreceptor cells where it is synthesized and binds to the extracellular matrix of cone outer segments. IRBP is a large (140-kDa) glycoprotein; it can bind 6 mol of long-chain fatty acid in addition to 2 mol of retinol. It has been suggested that its relatively low affinity for retinol, in comparison with the other retinol-binding proteins, facilitates rapid, high-volume transport of that ligand along a series of IRBPs. That IRBP is involved in the visual process, perhaps by delivering the chromophore, is indicated by the fact that its binding specificity shifts from mainly 11-*cis*-retinol to mostly all-*trans*-retinol as eyes become light adapted.[64] The two IRBP binding sites for retinoids have been found to be quite different. One is a strongly hydrophobic binding pocket; the other is a surface site that interacts with retinol via its polar head group. The protein shows higher affinities for all-*trans*-retinol and 11-*cis*-retinal than for other retinoids. Studies have shown that docosahexanoic acid (DHA) induces a rapid and specific release of 11-*cis*-retinal from the IRBP hydrophobic site; whereas, palmitic acid is without effect. This suggests that DHA may function in the targeting of 11-*cis*-retinal to photoreceptor cells, the DHA concentrations of which are much greater than those of pigment epithelial cells.

Retinol Recycling and Homeostasis

The majority of retinol that leaves the plasma appears to be recycled, as plasma turnover rates have been found to exceed utilization rates by more than an order of magnitude. Kinetic studies indicate that in adult rats a retinol molecule recycles via RBP4 12–13 times before its irreversible utilization;[65] in humans, number appears to be much smaller (e.g., 3). In the rat, newly released retinol has been found to circulate in the plasma for 1–3.5 h before leaving that compartment; it recycles in the plasma for a week or more. It is estimated that some 50% of plasma turnover in the rat is to the kidneys, 20% to the liver, and 30% to other tissues. It has been suggested that retinol leaves the plasma bound to RBP4. These parameters are quite different in the neonatal rat, indicating much faster turnover (turnover number 144) and much shorter transit time (0.4 h in plasma.) Although the source of RBP4 for retinol recycling is not established, it is worth noting that mRNA for RBP4 has been identified in many extrahepatic tissues, including kidney.

In healthy individuals, plasma retinol is maintained within a narrow range (40–50 mg/mL in adults; typically about half that in newborn infants) in spite of widely varying intakes of vitamin/provitamin A (Fig. 6.5).[66] This control appears to be effected by several factors: CRBP(II) expression in stellate cells, the esterification of retinol

62. The initial segment of the epididymis contains the greatest concentration of CRBP found in any tissue.

63. Saari, J.C., Nawrot, M., Kennedity, B.N., et al., 2001. Neuron 29, 739–748; Xue, Y., Shen, S., Jui, J., et al., 2015. J. Clin. Invest. 125, 727–738.

64. IRBP is also found in another photosensitive organ, the pineal gland.

65. Lin, L., Green, M.H., Ross, A.C., 2014. J. Nutr. 145, 403–410.

66. In contrast, plasma levels of all-*trans*-retinoic acid and 4-oxo-retinoic acid respond to the level of ingested vitamin A.

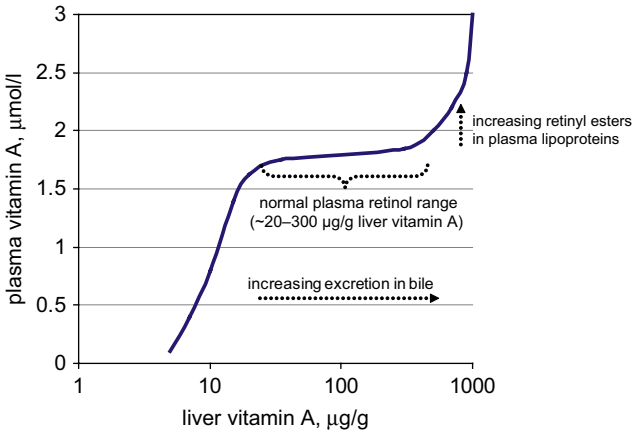


FIGURE 6.5 Regulation of plasma vitamin A levels. *After Olson, J.A., 1984. J. Nat. Cancer Inst. 73, 1439–1446.*

and hydrolyze retinyl esters, and retinol release to and/or removal from the plasma. Liver and kidneys play important roles in these various processes. Renal dysfunction has been shown to increase plasma retinol levels; this may involve a regulatory signal to the liver that alters the secretion of RBP4–retinol. Serum retinol levels can also be affected by nutritional status with respect to zinc, which is required for the hepatic synthesis of RBP4.

Plasma levels of carotenoids, in contrast, do not appear to be regulated but reflect intake of carotenoid-rich foods. Careful studies have revealed, however, cyclic changes of up to nearly 30% in the plasma β-carotene concentrations during the menstrual cycles of women. Whether these fluctuations are physiologically meaningful or whether they relate to fluctuations in plasma lipids is not clear.

Vitamin A in the Eye

Retinol is taken up by the retinal pigment epithelium in preference to uptake by nonocular tissues. However, this process, as in other tissues, involves transfer of retinol from RBP4 by the cellular receptor STRA6, which depends on the functional coupling of STAR6 and LRAT via CRBP. While pigment epithelial and Müller cells store the vitamin in esterified form in lipid droplets,[67] their accumulation appears to be less sensitive than other tissues to high doses of the vitamin. Epithelial reserves are mobilizable, and retinol is transported to rod cells by IRBP for discharge of the visual function of the vitamin. RBP4 is also expressed in the lacrimal glands; retinol appears to reach the cornea via holo-RBP4 secreted in tears. Both retinal pigment epithelial and Müller cells express CRALBP, which is thought to facilitate the intracellular transport of 11-*cis*-retinal and has been demonstrated to be essential for cone function. Retinal pigment epithelial cells also accumulate, by phagocytosis of photoreceptor outer segments, complex mixtures of bisretinoids as lipofuscin. This process appears to involve nonenzymatic reactions of retinal and is accelerated in some retinal disorders.[68]

67. Some investigators referred to these structures as "*retinosomes*".

Milk Retinol

Retinol is transferred from mother to infant through milk. Studies in animal models suggest that this retinol is drawn preferentially from recently consumed vitamin A, rather than from hepatic stores. The retinoid and carotenoid contents of milk depend on the stage of lactation and the vitamin A status of the mother, the patterns of carotenoids in colostrum tending to reflect those in maternal LDLs, while patterns in mature milk (19 days) reflecting those in maternal HDLs.[69] Retinol concentration of at least 1.75 µM is required to support adequate vitamin A stores to protect against the development of xerophthalmia during weaning. For this reason, vitamin A supplementation of mothers in vitamin A-deficient areas is regarded as a prudent public health strategy. A meta-analysis of randomized controlled trials showed that such measures have reduced infant mortality by 23% in children under 5 years of age in populations at risk to vitamin A deficiency.[70] The success of postpartum maternal supplementation depends on the prevailing breast feeding practices, with the simultaneous promotion of optimal practices (including the feeding of colostrum) being highly effective in improving infant vitamin A status. For example, the administration of 60,000 RE to a low-vitamin A mother can produce a 29% increase in the retinol contents of her breast milk over 6 months.

6. METABOLISM OF VITAMIN A

Metabolic Fates of Retinol

The metabolism of vitamin A (Fig. 6.6) centers around the transport form, retinol, and the various routes of conversion available to it: *esterification*, *conjugation*, *oxidation*, and *isomerization*.

Esterification. Retinol is esterified in the cells of the intestine and most other tissues via enzymes of the endoplasmic reticulum, which use acyl groups from either phosphatidylcholine (LRAT) or acylated coenzyme A (ARAT). These systems show marked specificities for saturated fatty acids, in particular, palmitic acid; thus, the most abundant product is retinyl palmitate.

68. e.g., Stargardt disease (also, fundus flavimaculatus), a recessive juvenile form of macular degeneration.

69. Schwiegert, F.J., Bathe, K., Chen, F., et al., 2004. Eur. J. Nutr. 43, 39–44.

70. Beaton, G.H., Mortorell, R., Aronson, K.J., et al., 1993. Nutrition Policy Discussion Paper No. 13, UN Administrative Committee of Coordination –Subcommittee on Nutrition, New York, 120 pp.

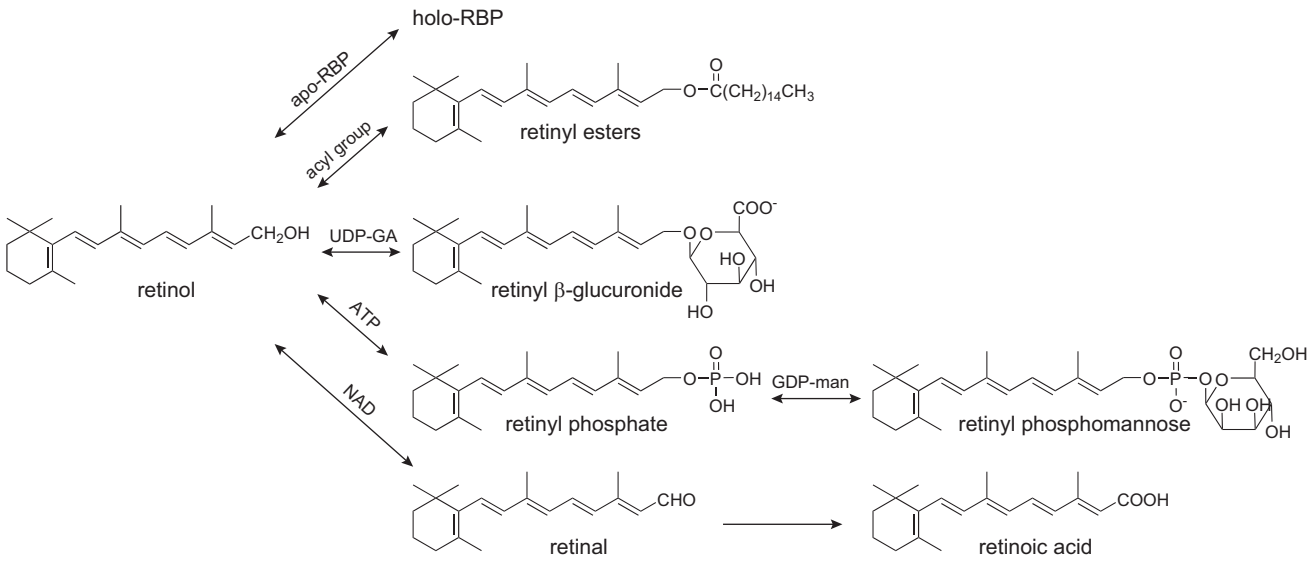


FIGURE 6.6 Metabolic fates of retinol.

Conjugation. Retinol may also be conjugated in either of two ways. The first entails the reaction catalyzed by retinol–UDP–glucuronidase, present in the liver and probably other tissues, which yields retinyl β-glucuronide, a metabolite that is excreted in the bile.[71] The second path of conjugation involves ATP-dependent phosphorylation to yield retinyl phosphate catalyzed by retinol phosphorylase. That product, in the presence of guanosine diphosphomannose (GDP-man), can be converted to the glycoside retinyl phosphomannose, which can transfer its sugar moiety to glycoprotein receptors. However, because only a small amount of retinol appears to undergo phosphorylation in vivo, the physiological significance of this pathway is not clear.

Oxidation. Retinol can also be reversibly oxidized to retinal by multiple NADH-/NADPH- and zinc-dependent retinol dehydrogenases. These cytosolic and microsomal activities are found in many tissues, the greatest being in the testis.[72] A short-chain aldehyde dehydrogenase has been described that can oxidize 9-*cis*- and 11-*cis*-retinol to the corresponding aldehyde. This activity has been identified in several tissues including the retinal pigment epithelium, liver, mammary gland, and kidney. That 9-*cis*-retinol can be converted to 9-cis-retinoic acid is evidenced by the finding of a 9-*cis*-retinol dehydrogenase. The enzyme in both humans and mice is inhibited by 13-*cis*-retinoic acid at levels similar to those found in human plasma, suggesting that 13-*cis*-retinoic acid may affect the regulation of retinoid metabolism.

Retinal can be irreversibly oxidized to retinoic acid by multiple retinal dehydrogenases (RALDHs), which are also aldehyde dehydrogenases. One of these, RALDH2, is critical for embryonic development; it is the first RALDH expressed in the mouse embryo and its genetic ablation produces malformations and death at midgestation.[73] It has been suggested that cytochrome *P*-450 monoxygenases may also produce retinoic acid from retinol through retinal. Because retinoic acid is the active ligand for the nuclear retinoid receptors, it is very likely that this metabolism is tightly regulated.[74] The rate of that reaction is several fold greater than retinol dehydrogenase that, plus the fact that the rate of reduction of retinal back to retinol is also relatively great, results in retinal being present at very low concentrations in tissues. Several retinoic acid isomers have been identified in the plasma of various species;[75] the number having physiological significance is presently unclear.

Isomerization. Interconversion of the most common all-*trans* and various *cis* vitamers occurs in the eye and is a key aspect of the visual function of vitamin A. That process involves light-induced isomerization of 11-*cis*-retinal to all-*trans*-retinal (Fig. 6.8), which alters the affinity of the visual pigment protein opsin for this ligand, leading

71. The former view of retinyl β-glucuronide as an excretory form has changed. About 30% of the amount excreted in the bile is reabsorbed and recycled in an enterohepatic circulation back to the liver, and retinyl β-glucuronide has been found to be produced in many extrahepatic tissues where it can support growth and tissue differentiation.

72. Male rats fed retinoic acid instead of retinol become aspermatogenic and experience testicular atrophy. It has been proposed that retinoic acid is required for spermatogenesis but cannot cross the blood–testis barrier; this is supported by the fact that the rat testis is also rich in CRABP.

73. Niederreither, K., Subbarayan, V., Dolle, P., et al., 1999. Nat. Genet. 21, 444–448.

74. Two microsomal proteins that catalyze this reaction have been isolated from rat liver; one cross-links with holo (but not apo)-CRBP in the presence of NADP.

75. In addition to all-*trans*-, 13-*cis*-, and 9-*cis*-retinoic acid, this number includes the 9,13-*dicis*-, 4-hydroxy-, 4-oxo-, 18-hydroxy-, 3,4-dihydroxy-, and 5,6-epoxy isomers, as well as such derivatives as retinotaurine.

to dissociation linked to nervous signaling and conversion back to the 11-*cis* vitamer by retinal isomerase (RPE65). That enzyme also catalyzes the isomerization of 11-*cis*-retinol to the all-*trans* form. The conversion of all-trans-retinoic acid to 9-cis-retinoic acid has also been demonstrated.

All-*trans*-retinoic acid is produced and catabolized by *unidirectional* processes. While retinol, retinyl esters, and retinal can be oxidized to retinoic acid, retinoic acid *cannot* be converted metabolically to any of the reduced vitamers.

Fates of Retinoic Acid

All-*trans*-retinoic acid is converted to forms that can be readily excreted (Fig. 6.7). It may be directly conjugated by glucuronidation in the intestine, liver, and possibly other tissues to retinyl β-glucuronide. Alternatively, it can be catabolized by several further oxidized products including the 4-hydroxy-, 4-oxo-, 4-dihydroxy-, 18-hyrodroxy-, 3-hyrdoxy-, and 5,6-epoxy metabolites. Several cytochrome *P*-450 enzymes have been implicated in this metabolism. These include different families: CYP2, CYP3, CYP4, and CYP26. Of these, three in the CYP26 family appear to be most important. These are monooxygenases that convert retinoic acid to the 4-hydroxy- (CYP26A), 4-oxo- (CYP26B), and 18-hydroxy- (CYP26C) metabolites. They are expressed early in development and their pattern of expression varies according to tissue and stage of development. The expression of CYP26A is known to upregulated by dietary vitamin A and retinoic acid and downregulated by vitamin A depletion; mice devoid of this isoform have neural tube defects and die shortly after birth.

The oxidative chain-cleavage metabolites are conjugated with glucuronic acid, taurine, or other polar molecules; these, being more polar, can readily be excreted. Glucuronides and retinotaurine comprise a significant portion of the retinoids excreted in the bile. That retinoyl-β-glucuronide has been found to show some vitamin A activity suggests that there may be some hydrolysis to yield retinoic acid.

Role of Retinoid-Binding Proteins in Vitamin A Metabolism

In addition to serving as reserves of retinoids, the CRBPs also serve to modulate vitamin A metabolism by holding the retinoids in ways that render them inaccessible to the oxidizing environment of the cell and by channeling the retinoids via protein–protein interactions among its enzymes (Fig. 6.8, Tables 6.8 and 6.9). Both CRBPs function in directing the metabolism to their bound retinoid ligands by shielding them from some enzymes that would use the free retinoid substrate and by making them accessible to other enzymes important in metabolism. For example, the esterification of retinol by LRAT occurs while the substrate is bound to CRBP(I) or CRBP(II). The abundance of CRBP(I) in the liver and its high affinity for retinol suggest that its presence directs the esterification of the retinoid ligand to the reaction catalyzed by LRAT, rather than that catalyzed by ARAT, which can use only free retinol. This also appears to be the case for CRBP(II), which can bind both retinol and retinal; only when retinal is bound to it can the reducing enzyme **retinal reductase** use that substrate. In addition, the binding of retinol to CRBP(II) greatly reduces the reverse reaction (oxidation to retinal). Thus, by facilitating retinal formation and inhibiting its loss, CRBP(II) seems to direct the retinoid to the appropriate enzymes, which sequentially convert

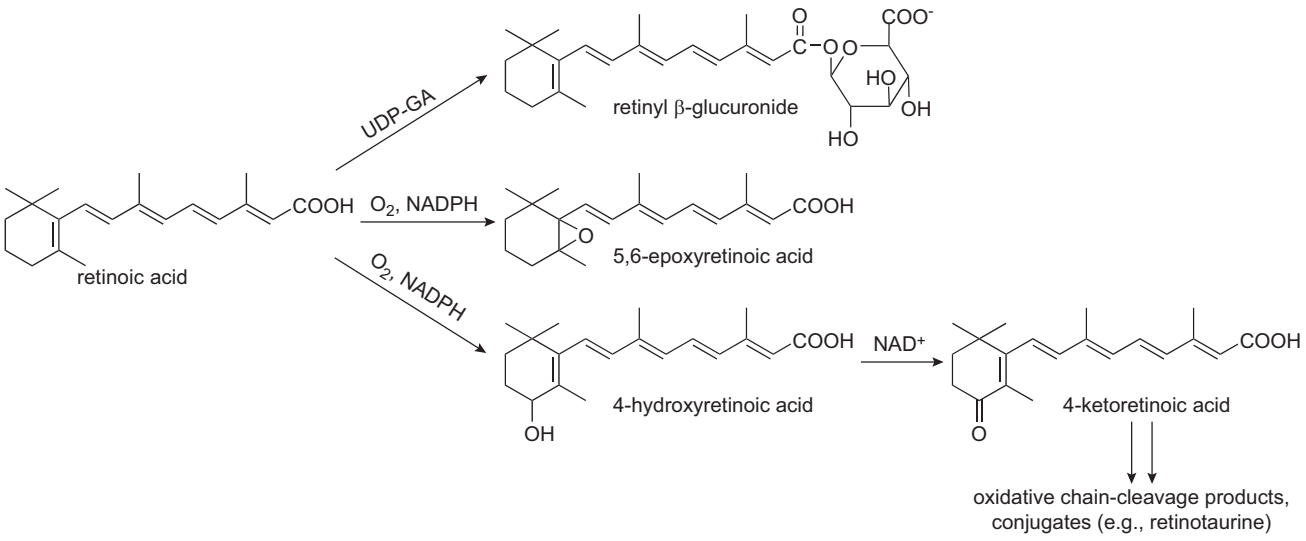


FIGURE 6.7 Catabolism of retinoic acid.

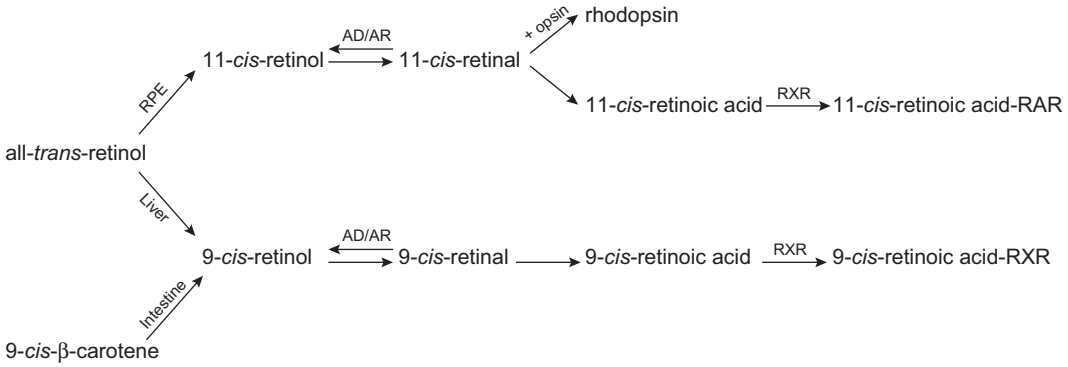


FIGURE 6.8 Roles of binding proteins in vitamin A metabolism.

TABLE 6.8 Vitamin A-Binding Proteins

Binding Protein	Abbreviation	MW (kDa)	Ligand	K_d (nM)	Tissues
Extracellular					
Retinol-BP	RBP4	21.2	All-*trans*-retinol	20	Plasma
Albumin	–	67	All-*trans*-retinoic acid		Plasma
Interphotoreceptor retinol-BP	IRBP	136	All-*trans*-/11-cis-retinol	50–100	Interphotoreceptor space
Intracellular					
Cellular retinol-BP, type I	CRBP(I)	15.7	All-*trans*-retinol	<10	Most *except* heart, adrenal, ileum
			All-*trans*-retinal	50	
Cellular retinol-BP, type II	CRBP(II)	15.6	All-*trans*-retinol(al)	90	Enterocytes, fetal and neonatal liver
Cellular retinal-BP	CRALBP	36	11-*cis*-retinol(al)	15	Retinal pigment epithelial and Müller cells
Cellular retinoic acid-BP, type I	CRABP(I)	15.5	All-*trans*-retinoic acid	0.1	Most *except* liver, jejunum, ileum
Cellular retinoic acid-BP, type II	CRABP(II)	15	All-*trans*-retinoic acid	0.1	Cytosol of embryonic limb bud
Epididymal retinoic acid-BP	–	18.5	All-*trans*-retinoic acid		lumen of epididymis
Uterine retinol-BP	–	22	All-*trans*-retinol		Uterus (sow)
Retinol pigment epithelium protein	RPE65	65	All-trans-retinyl esters		Retinal pigment epithelium
Cellular RBP4 receptor	STRA6	74	All-*trans*-retinol		Extraheptic tissues
Cellular RBP4 receptor 2	RBPR2		All-*trans*-retinol		Liver and other tissues
Nucleus					
Nuclear retinoic acid receptor-α	RARα	50	All-*trans*-retinoic acid		Most *except* adult liver
Nuclear retinoic acid receptor-β	RARβ	50	All-*trans*-retinoic acid		Most *except* adult liver
Nuclear retinoic acid receptor-γ	RARγ	50	All-*trans*-retinoic acid		Most *except* adult liver
Nuclear retinoid X receptor-γ	RXR	50	All-*trans*-retinoic acid		Most *except* adult liver
Rhodopsin	—	41	11-*cis*-retinal		Retina
Melanopsin	—		11-*cis*-retinal		Retina, brain, skin (*Xenopus* sp.)

TABLE 6.9 Apparent Metabolic Functions of the Intracellular Retinoid-Binding Proteins

Binding Protein	Function
CRBP(I) (cellular retinol-binding protein)	Directs retinol to LRAT (lecithin–retinol acyltransferase) and oxidative enzymes; regulates retinyl ester hydrolase
CRBP(II)	Directs retinol to LRAT and oxidative enzymes
CRABPs (I) and (II) (cellular retinoic acid-binding protein)	Regulates intracellular retinoic acid concentrations; direct retinoic acid to catabolizing enzymes
CRALBP (cellular retinal-binding protein)	Regulates enzymatic reactions of the visual cycle

retinal to the esterified form in which it is exported from the enterocytes. The preferential binding of 11-*cis*-retinal by the CRALBPs relative to CRBP(I) appears to be another example of direction of the ligand to its appropriate enzyme, i.e., a microsomal NAD-dependent retinal reductase in the pigment epithelium of the retina that uses only the carrier-bound substrate. In each of these cases, it is likely that the RBP–ligand complex interacts directly with the respective retinoid-metabolizing enzyme.

The CRABPs are noteworthy for their antagonistic effects on the trafficking of retinoic acid, which suggest their roles in regulating intracellular levels of the vitamer. Studies indicate that CRABP(I) facilitates its degradation by CYP26, while CRABP(II) facilitates its transfer to nuclear receptors. Mice in which CRABP genes had been deleted show normal phenotypes and normal susceptibility to the teratogenic effects of high doses of retinoic acid; yet, transgenic animals that overexpress CRABPs show significant pathology (cataracts and pancreatic endocrine tumors).

Excretion of Vitamin A

Vitamin A is excreted in various forms in both the urine and feces. Under normal physiological conditions, the efficiency of enteric absorption of vitamin A is high (80–95%), with 30–60% of the absorbed amount being deposited in esterified form in the liver. The balance of absorbed vitamin A is catabolized (mainly at C-4 of the ring and at C-15 at the end of the side chain[76]) and released in the bile or plasma, where it is removed by the kidney and excreted in the urine (i.e., short-chain, oxidized, conjugated products). About 30% of the biliary metabolites (i.e., retinoyl β-glucuronides) are reabsorbed from the intestine into the enterohepatic circulation back to the liver, but most are excreted in the feces with unabsorbed dietary vitamin A. In general, vitamin A metabolites with intact carbon chains are excreted in the feces, whereas the chain-shortened, acidic metabolites are excreted in the urine. The relative amounts of vitamin A metabolites in the urine and feces, thus, vary with vitamin A intake (i.e., at high intakes fecal excretion may be twice that of the urine) and the hepatic vitamin A reserve (i.e., when reserves are above the low normal level of 20 μg/g, both urinary and fecal excretion vary with the amount of vitamin A in the liver).

76. The chain-terminal carbon atoms (C-14, C-15) can be oxidized to CO_2; retinoic acid is oxidized to CO_2 to a somewhat greater extent than retinol.

7. METABOLIC FUNCTIONS OF VITAMIN A

Feeding provitamin A carotenoids retinyl esters, retinol, and retinal can support the maintenance of healthy epithelial cell differentiation, normal reproductive performance, and visual function. Each of these forms can be metabolized to retinol, retinal, or retinoic acid. But unlike retinol and retinal, retinoic acid cannot be reduced to retinal or retinol. Feeding retinoic acid can support only the systemic functions of vitamin A, e.g., epithelial cell differentiation. These observations and knowledge of retinoid metabolism led to the conclusion that whereas retinal discharges the visual functions, retinoic acid (and, specifically, **all-*trans*-retinoic acid**) must support the systemic functions of the vitamin.

Functional Forms of Vitamin A

Retinol	Transport, reproduction (mammals)
Retinyl esters	Storage
Retinal	Vision
Retinoic acid	Epithelial differentiation, immune function, gene transcription, reproduction

Vitamin A in Vision

The best elucidated function of vitamin A is in the visual process where, as 11-*cis*-retinal, it serves as the photosensitive

chromophore of the visual pigments of the ciliated photoreceptor cells of the retina, the rods and cones.[77] Rod cells contain the pigment **rhodopsin**, which has an absorption maximum at 498 nm. Cone cells contain one of three possible **iodopsins**: 64% are "red cones" (absorption maxima at 560–580 nm), 32% are "green cones" (absorption maxima at 530–540 nm), and 2% are "blue cones" (absorption maxima at 420–440 nm). In each case, photoreception is effected by the rapid, light-induced isomerization of 11-*cis*-retinal to the all-*trans* form. That product, present as a protonated Schiff base[78] of a specific lysyl residue of the protein (Fig. 6.9), produces a highly strained conformation, which results in the dissociation of the retinoid from the opsin complex. This process (**bleaching**) is a complex series of reactions, involving progression of the pigment through a series of unstable intermediates of differing conformations[79] and, ultimately, to *N*-retinylidene opsin, which dissociates to all-*trans*-retinal and opsin (Fig. 6.10).

The dissociation of all-*trans*-retinal and opsin is coupled to nervous stimulation of the vision centers of the brain. The bleaching of rhodopsin causes the closing of Na^+ channels in the rod outer segment, thus leading to hyperpolarization of the membrane. This change in membrane potential is transmitted as a nervous impulse along the optic neurons. This response appears to be stimulated by the reaction of an unstable "activated" form of rhodopsin, **metarhodopsin II**, which reacts with **transducin**, a membrane-bound

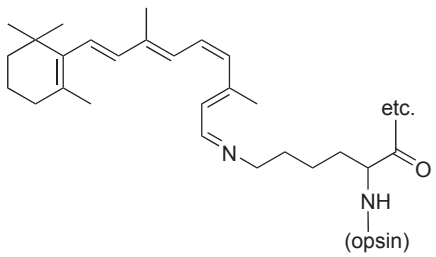


FIGURE 6.9 11-*cis*-Retinal binds to photopigment proteins via a lysyl linkage.

G protein of the rod outer segment disks. This results in the binding of the transducin α-subunit with **cGMP phosphodiesterase**, which activates the latter to catalyze the hydrolysis of cGMP to GMP. Because cGMP maintains Na^+ channels of the rod plasma membrane in the open state, the resulting decrease in its concentration causes a marked reduction in Na^+ influx. This results in hyperpolarization of the membrane and the generation of a nerve impulse through the synaptic terminal of the rod cell.

The visual process is a cyclic one, in that its constituents are regenerated. All-*trans*-retinal can be converted enzymatically in the dark back to the 11-*cis* form. After bleaching, all-*trans*-retinal is rapidly reduced to all-*trans*-retinol, in the rod outer segment. The latter is then transferred (presumably via IRBP) into the retinal pigment epithelial cells, where it is esterified (again, predominantly with palmitic acid) and stored in the bulk lipid of those cells. The regeneration of rhodopsin, which occurs in the dark-adapted eye, involves the simultaneous hydrolysis and isomerization of retinyl esters to yield 11-*cis*-retinol and then 11-*cis*-retinal, which is transferred into the rod outer segment via IRBP. Studies have revealed that the conversion of all-*trans*-retinyl esters to 11-*cis*-retinal in the retinal pigment epithelium is catalyzed by the microsomal membrane protein RPE65.[80] RPE65 is involved in the regeneration of rhodopsin in rods and of the opsins in cones. Its activity appears to be regulated through the addition/release of palmitic acid: palmitoylation of the protein converts the protein from a form soluble in the cytosol to a membrane-bound form, thus controlling its capacity to present its retinoid ligand to cytosolic isomerohydrolase. Nervous recovery is effected by the GTPase activity of the transducin α-subunit, which, by hydrolyzing GTP to GDP, causes the reassociation of transducin subunits and, hence, the loss of its activating effect on cGMP phosphodiesterase. Metarhodopsin II is also removed by phosphorylation to a form incapable of activating transducin and by dissociation to yield opsin and all-*trans*-retinal.

The visual cycle of cones differs from that of rods. Cones have 100-fold lower light sensitivity but 10-fold faster recovery rates compared to rods, suggesting that they may have access to a source of visual chromophore not available to rods. In cones, the oxidation of 11-*cis*-retinol to 11-*cis*-retinal is NADP-dependent, and the isomerization of all-*trans* to 11-*cis*-retinal occurs in a two-step process localized in Müller glial cells. In contrast, the oxidation step in rods is NAD-dependent and the isomerization is a one-step process localized in the retinal pigment epithelium.

Nonvisual opsins. Humans and other mammals have a subset of retinal ganglion cells, as well as cells in the

77. Rods are the most numerous (c.120 million) and are the very sensitive to light intensity, although not to color. Color vision is the provence of the 6–7 million cones, which are be concentrated in the **macula**, particularly in a small (0.3 mm diameter), rod-free area the **fovea centralis**, except for the "blue cones", which are distributed mostly outside of the fovea. While cones comprise only 5% of all photoreceptors in the human eye, they are more numerous in other species, e.g., comprising 60% of the photoreceptors in the chicken eye.

78. A Schiff base is a type of imine with the structure $R_2C{=}NR'$ often used in coordination complexes. It was described by the German chemist Hugo Schiff (1834–1915) who founded the Chemical Institute of the University of Florence.

79. The conformation of rhodopsin is changed to yield a transient photopigment, **bathorhodopsin**, which, in turn, is converted sequentially to **lumirhodopsin**, **metarhodopsin I**, and (by deprotonation) **metarhodopsin II**.

80. The protein was named for its apparent molecular mass under denaturing conditions, 65 kDa; further studies revealed that its true mass is 61 kDa. Mutations in RPE65 have been found to cause Leber's congenital amaurosis and other forms or autosomal recessive retinitis pigmentosa.

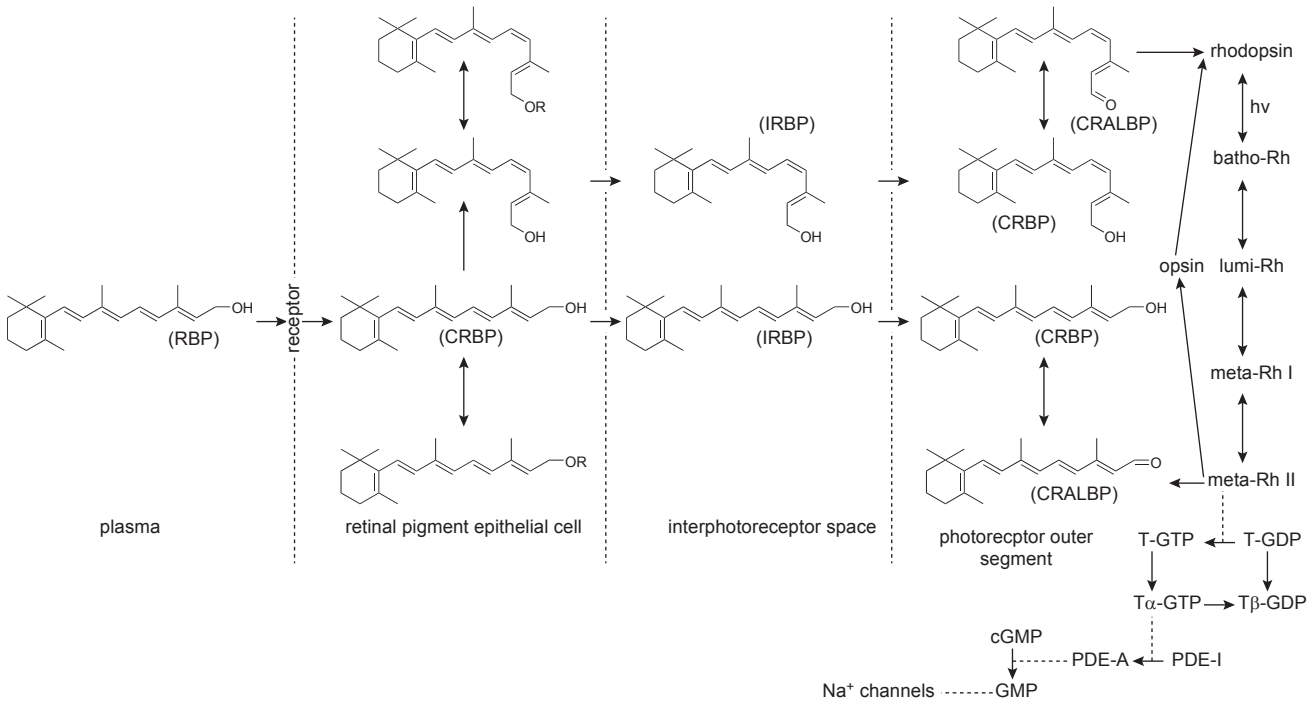


FIGURE 6.10 Vitamin A in the visual cycle. *PDE-A*, phosphodiesterase (active); *PDE-I*, phosphodiesterase (inactive); *Rh*, rhodopsin; *T*, transducin.

suprachiasmatic nucleus of the brain, that express the photopigment **melanopsin**. The light-induced isomerization of its ligand, 11-*trans*-retinal to the 11-cis-form suggests that melanopsin may function in circadian regulation. Other species express various nonvisual opsins (at least 17 have been described) in such tissues as the iris, skin, pineal, and deep brain, where they are thought to function in nonimage-forming photoreceptors that may be involved in photoisomerizations, detection of environmental light, and modulation of arousal states.

Systemic Functions of Vitamin A

Vitamin A supports systemic functions (e.g., corneal integrity, immunity, growth) in which vitamin A acts like a hormone. These functions appear to rely on retinoic acid; because the oxidation of retinal to retinoic acid is irreversible, retinoic acid can support only these systemic functions. Animals fed diets containing retinoic acid as the sole source of vitamin A grow normally and appear healthy in everyway except that they go blind.

Vitamin A-deficient animals die but not from lack of visual pigments. The extraretinal functions of vitamin A are of greater physiological importance than the visual function.

Epithelial differentiation. Chief among the systemic functions of vitamin A is its role in epithelial cell differentiation. Vitamin A-deficient individuals experience replacement of normal mucus-secreting cells by cells that produce keratin, particularly in the conjunctiva and cornea of the eye, the trachea, the skin, and other ectodermal tissues. Less severe effects occur in tissues of mesodermal or endodermal origin. The actions of retinoids in cell differentiation are analogous to those of the steroid hormones, i.e., they bind to the nuclear chromatin to signal transcriptional processes. In fact, studies have revealed that the differentiation of cultured cells can be stimulated by exposure to retinoids, and that abnormal mRNA species are produced by cells cultured in vitamin A-deficient media.[81] Further, retinoic acid has been found to stimulate, synergistically with **thyroid hormone (T_3)**[82], the production of growth hormone in cultured pituitary cells. Some studies with culture cell models have found all-*trans*-retinoic acid treatment to upregulate the expression of certain micro-RNAs,[83] suggesting epigenetic bases for some vitamin A actions.

Immune function. That vitamin A has an important role in immune function is indicated by the fact that vitamin

81. Epidermal keratinocytes cultured in a vitamin A-deficient medium made keratins of higher molecular weight than those made by vitamin A-treated controls. Different mRNA species encoded the different proteins produced under each condition.
82. Triiodothyronine.
83. miRNAs are a class of small (18–23 nucleotides), noncoding RNAs involved in posttranslational regulation of gene expression.

A-deficient animals and humans are typically more susceptible to infection than are individuals of adequate vitamin A nutriture.[84] They show changes in lymphoid organ mass, cell distribution, histology, and lymphocyte characteristics. Vitamin A deficiency leads to histopathological changes that provide environments conducive to secondary infection. This is supported by findings of excess bacteriuria among xerophthalmic compared to nonxerophthalmic, malnourished children in Bangladesh; and negative correlation of plasma retinol level and bacteria adherent to nasopharyngeal cells of children in India. Such outcomes involve impaired epithelial integrity (including mucosal immunity), antibody responses, and lymphocyte differentiation. Underlying these effects are roles of vitamin A in inducing heightened primary immune responses, enhancing memory responses, and accelerating the expansion of the mature B-lymphocyte pool. These functions appear to be discharged by retinoic acid acting at the nuclear level, i.e., by ligating RAR (retinoic acid receptor)–RXR (Retinoid X receptor) to alter the expression of genes affecting immune function. Retinoic acid has, therefore, been called a "*fourth signal*" of the antibody response.[85] In this way, retinoic acid induces changes that imprint antibody-secreting cells by permanently committing them. Studies have shown this function to be particularly important in maintenance of mucosal immunity, which depends on the retinoic acid-dependent modification of B cells by gut-associated dendritic cells within the lymphoid tissues of the small intestine. Both RAR and RXR receptors are constitutively expressed by both B and T cells. Vitamin A, which has long been known to prevent atrophy of the thymus, functions as retinoic acid in at least some subtypes of T cell, including T-helper lymphocytes, the antigen-presenting cells that stimulate B cells to produce immunoglobulin A (IgA). It is known that retinoic acid is a modulator of the differentiation of T cells from interleukin-17-secreting T-helper cells and toward $Foxp3^+$ T regulatory cells, and it may also play a role in determining the development of T cells toward CD^{+4} versus CD^{+8} status.

Reproduction. Studies with animals have demonstrated that vitamin A is required for normal reproduction. For example, rats maintained with retinoic acid grow well and appear healthy but lose reproductive ability, i.e., males show impaired spermatogenesis and females abort and resorb their fetuses. Injection of retinol into the testis restores spermatogenesis, indicating that vitamin A has a direct role in that organ. The chicken has been shown to require retinoic acid for spermatogenesis; all mammalian species examined to date require retinol or retinal. It has been proposed that these effects are secondary to lesions in cellular differentiation and/or hormonal sensitivity. Several researchers have found that vitamin A-deficient dairy cows show reduced corpus luteal production of progesterone and increased intervals between luteinizing hormone peak and ovulation. Some evidence indicates that hormonal parameters respond to oral treatment with β-carotene but *not* preformed vitamin A, suggesting the importance of retinol/retinal production in situ.

Retinoids play fundamental roles as differentiating agents in morphogenesis. Deprivation of vitamin A results in the Japanese quail results in the loss of normal specification of heart left–right asymmetry. That this effect is associated with the decreased expression of $RAR\beta_2$ in the presumptive cardiogenic mesoderm suggests that retinoids may direct the differentiation of mesoderm into the heart lineage. Studies of the regenerating amphibian limb have revealed profound effects or retinoids in providing positional information to enable cells to differentiate into the pattern of structures relevant to their appropriate spatial locations. On the basis of such observations the morphogenic role of vitamin A was proposed to involve concentration gradients of RARs/RXRs due to differential induction of the receptor by the retinoid, which established positional identity. However, it now appears that embryos have multiple areas with different responsiveness to retinoic acid caused by local differences in the production and binding of, and sensitivity to, retinoic acid. These differences vary among tissues during development, with retinoic acid acting primarily in a paracrine manner in pluripotent cells. In early development retinoic acid signals the posterior neuroectoderm, trunk mesoderm, and foregut endoderm in the organization of the trunk; in later development, it signals development of the other organs including the eye (Table 6.10).

The expression of many proteins results from retinoic acid-induced cell differentiation. Several are induced by activation through RXR/RAR binding: growth hormone[86] (in cultured pituitary cells), the protein laminin (in mouse embryo cells), the respiratory chain-uncoupling protein of brown adipose tissue (suggesting a role in heat production and energy balance), the vitamin K-dependent matrix Gla protein, and the RARs. This indicates autoregulation, i.e., retinoic acid induces its own receptor. In fact, the induction of RARs appears to be differentially selective among various tissues; retinoic acid has been found to induce mainly RARα in hemopoietic cells but RARβ in other tissues.

Bone metabolism. Vitamin A has an essential role in the normal metabolism of bone. Animal studies indicate that both low- and high-vitamin A intakes reduce bone mineral

84. In practice, it can be difficult to ascribe such effects simply to the lack of vitamin A, as deficient individuals generally also have protein–calorie malnutrition which, itself, leads to impaired immune function.

85. The others being signal 1—receptor-binding of antigen; signal 2—receptor-binding costimulatory/accessory factors; signal 3—binding of "*danger signals*", e.g., lipopolysaccharide, to Toll-like receptors.

86. That vitamin A may be required for the expression of growth hormone in humans is suggested by the correlation of plasma retinol and nocturnal growth hormone concentrations in short children.

TABLE 6.10 Genes Regulated by Retinoic Acid in Embryonic Development

Aspect of Development	Expression Induced	Expression Repressed
Hindbrain a.-p.[a] patterning	*Hoxa1, Hoxa3, Hoxb1, Hoxd4, vHnf1*	
Spinal cord motor neuron differentiation	*Pax6, Olig2*	
Early somite formation	*Cdx1*	*Fgf8*
Heart a.-p.[a] patterning		*Fgf8*
Forelimb differentiation		*Fgf8?*
Pancreas differentiation	*Pdx1*	
Lung differentiation	*Hoxa5*	*TGF-β1*
Anterior eye formation	*Pitx2*	
Kidney formation	*Ret*	
Meiosis induction	*Stra8*	

[a]*Anteroposterior.*
After Duester, G., 2008. Cell 134, 921–931.

TABLE 6.11 Prevalence of Vitamin A Deficiency Related to Hemoglobin Status in Indonesian Preschool Children

Hemoglobin (g/dL)	Prevalence of Vitamin A Deficiency (%)[a]
<11.0	54.2
11.1–12.0	43.3
>12.0	34.3

[a]*Based on conjunctival impression cytological assessment.*
After Lloyd-Puryear, M.A., Mahoney, J., Humphrey, F., et al., 1991. Nutr. Rev. 11, 1101–1110.

density.[87] Observational studies in humans, however, have yielded inconsistent results in this regard. Retinoids are thought to be involved in regulating the phenotypic expression of bone-mobilizing cells, osteoclasts, which are reduced in vitamin A deficiency. The consequently unchecked function of bone-forming cells, osteoblasts, would appear to result in excessive deposition of periosteal bone and a reduction in the degradation of glycosaminoglycans. It also appears that 9-*cis*-retinoic acid can serve as an affector of the vitamin D-induced renal calcification involving the vitamin K-dependent matrix γ-carboxyglutamic acid (GLA) protein.

Hematopoiesis. Chronic deprivation of vitamin A leads to anemia. Cross-sectional studies have shown low hemoglobin levels to be associated with the prevalence of xerophthalmia in children (Tables 6.11 and 6.12), and children with mild-to-moderate vitamin A deficiency or mild xerophthalmia have lower circulating hemoglobin levels than nondeficient children. In fact, serum retinol level explains 4–10% of the variation in hemoglobin level among preadolescent children in developing countries. The hematological response to vitamin A deficiency is biphasic, involving an initial fall in both hemoglobin and erythrocyte count due to apparently impaired hemoglobin synthesis, followed by the rise in both variables late in deficiency due to hemoconcentration resulting from dehydration secondary to reduced water intake and/or diarrhea.

The metabolic basis of the role of vitamin A in hematopoiesis appears to involve the mobilization and transport of iron from body stores as well as the enhancement of nonheme–iron bioavailability. The results of cross-sectional studies in developing countries have found serum iron to be positively correlated with serum retinol levels, and animal studies have shown vitamin A deprivation to cause decreases in both hematocrit and hemoglobin levels, which precede other disturbances in iron storage and absorption. Vitamin A deficiency reduces the activity of ceruloplasmin, a copper-dependent protein with ferroxidase activity, which is important in the enteric absorption of iron. This effect appears to occur as the results of a posttranscriptive disruption in the activity. In addition, the results of in vitro studies demonstrate that all-*trans*-retinol induces the differentiation and proliferation of pluripotent hemopoietic cells, which suggest that the anemia of vitamin A deficiency is initiated by impairments in erythropoesis and accelerated by subsequent impairments in iron metabolism. The presence of vitamin A or β-carotene has been found to increase the enteric absorption of iron from both inorganic and plant sources; this has been explained on the basis of the

87. See review: Henning, P., Conaway, H.H., Lerner, U.H., 2015. Front. Endocrinol. 6, 1–13.

TABLE 6.12 Efficacy of Vitamin A Supplementation on Increasing Hemoglobin Levels in Anemic Subjects

Country	Subject Age	Vitamin A Dosage (μg RE/d)	Follow-up	n	Hemoglobin (g/dL)	
					Baseline	Follow-up
Indonesia[a]	<6 years	0	5 months	240	11.4 ± 1.6	11.2 ± 1.5
		240	5 months	205	11.3 ± 1.6	12.3 ± 1.6*[b]
Guatemala[c]	1–8 years	0	2 months	20	10.4 ± 0.7	10.7 ± 0.6
		2400	2 months	25	10.3 ± 0.8	11.2 ± 0.8*
Indonesia[d]	17–35 years	0	2 months	62	10.4 ± 0.7	10.7 ± 0.6
		3000	2 months	63	10.3 ± 0.8	11.2 ± 0.8*

[a]*Mahilal, P.D., Idjradinata, Y.R., Muheerdiyantiningsih, K.D., 1988. Am. J. Clin. Nutr. 48, 1271–1276.*
[b]*Significantly different from baseline level, p < .05.*
[c]*Mejia, L.A., Chew, F., 1988. Am. J. Clin. Nutr. 48, 595–600.*
[d]*Suharno, D., West, C.E., Muhilal, K.D., et al., 1993. Lancet 342, 1325–1328.*

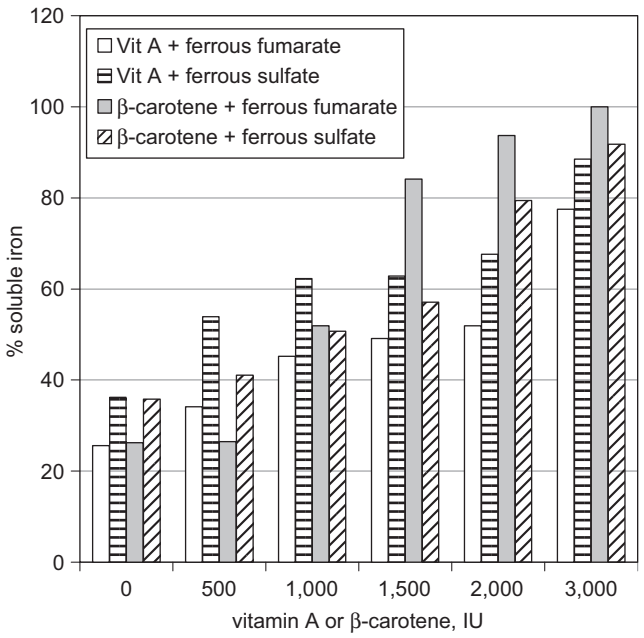


FIGURE 6.11 Effect of vitamin A and β-carotene on iron solubility (at pH 6). *After Garcia-Casal, M.N., Layrisse, Solano, L., et al., 1998. J. Nutr. 128, 646–650.*

TABLE 6.13 Effects of Vitamin A and β-Carotene on the Bioavailability of Nonheme Plant Iron for Humans

Iron Source	Vitamin A (μmol)	β-Carotene (μmol)	Iron Absorption (%)
Rice	0	0	2.1
	1.51	0	4.6[a]
	0	0.58	6.4[a]
	0	0.95	8.8[a]
Corn	0	0	3.0
	0.61	0	6.6[a]
	0	0.67	8.5[a]
	1	1.53	6.3[a]
Wheat	0	0	3.0
	0.66	0	5.5[a]
	0	0.85	8.3[a]
	0	2.06	8.4[a]

[a]$p < .05$, $n = 11–20$ *subjects.*
From Garcia-Casal, M.N., Layrisse, M., Solano, L., et al., 1998. J. Nutr. 128, 646–650.

formation of complexes with iron that are soluble in the intestinal lumen, thus, blocking the inhibitory effects of iron absorption of such antagonists as phytates and polyphenols (Fig. 6.11; Table 6.13). Clinical trials have shown intervention with both iron and vitamin A to be more effective on correcting anemia than intervention with iron alone.

Retinoids are involved in the differentiation of myeloid cells into neutrophils, which occurs in the bone marrow; this function appears to involve all-*trans*-retinoic acid, as RARα is the predominant retinoid receptor type found in hematopoietic cells. Vitamin A-deficient animals have been found to sequester retinol in their bone marrow;[88] this response may ensure adequate vitamin A for the growth and differentiation of myeloid cells, which would explain the observation that vitamin A-deficient individuals do not necessarily show neutropenia.[89]

88. Twining, S.S., Schulte, D.P., Wilson, P.M., et al., 1996. J. Nutr. 126, 1618–1626 found vitamin A deprivation to reduce the retinol contents of rat bone marrow by 75%.
89. Abnormally low circulating levels of neutrophils.

Skin health. Vitamin A has a role in the normal health of the skin. Its vitamers, as well as carotenoids, are typically found in greater concentrations in the subcutis than in the plasma (significant amounts are also found in the dermis and epidermis), indicating the uptake of retinol from plasma RBP4. Epithelial cell phenotypes are regulated by hormonal cycles and vitamin A intake; vitamin A deficiency impairs the terminal differentiation of human keratinocytes and causes the skin to be thick, dry, and scaly. It also results in obstruction and enlargement of the hair follicles.[90]

Vitamin A Regulation of Gene Transcription

Vitamin A discharges its systemic functions through the abilities of all-*trans*-retinoic acid and 9-*cis*-retinoic acid to regulate the expression of some 300 genes at specific target sites in the body. The hormone-like regulation of transcription by retinoids is receptor-mediated. Retinoic acid binds to two members of a highly conserved superfamily of proteins that act as nuclear receptors for steroid hormones including 1,25-$(OH)_2$-vitamin D_3 and thyroid hormone (T_3).[91] These nuclear receptors have similar ligand-binding and DNA-binding domains as well as substantial sequence homology. Retinoic acid is thought to interact with them in ways similar to their other ligands, with each receptor binding to regulatory elements upstream from the gene and acting as a ligand-activated transcription factor (Table 6.14). The **RAR**s function by attracting low-molecular weight coactivators, releasing corepressors, and forming obligate heterodimeric complexes with another retinoid receptor, the **RXR**. The RXRs form transcriptionally inactive homotetramers when not bound to ligand; these dissociate to form active homodimers upon retinoid binding. Because the tetramers can bind two DNA recognition sequences simultaneously, the retinoid-induced shift to the liganded dimer results in a change in DNA geometry. The involvement of RXRs with multiple binding partners, including PPARs, the vitamin D receptor (VDR), and farnesoid X receptors (FXR), makes them the master regulators of multiple pathways signaling transcription in response to lipophilic nutrients and hormones (Fig. 6.12). The regulation of gene expression also involves coactivators and corepressors that function through the modification of chromatin.

All isomers of retinoic acid bind the RARs; greatest affinities in vitro are shown by all-*trans*-retinoic acid (K_d = 1–5 nM). Three RAR subtypes have been identified (α, β, and γ). That only all-*trans*-retinoic acid functions as the endogenous ligand for RAR is suggested by the demonstration that growth arrest in mice lacking retinaldehyde dehydrogenase (incapable of producing all-*trans*-retinoic acid) can be rescued by the all-*trans* but not the 9-*cis* vitamer. The ligand-binding domains of the RARs are highly conserved (showing 75% identity in terms of amino acid residues). $RAR\alpha_1$ and $RAR\alpha_2$ share 7 of their 11 exons, suggesting that they arose from a common ancestral RAR gene. Different promoters, however, direct the expression of each in an unusual organization involving the 5′-untranslated regions of the genes divided among different axons. In the case of $RAR\alpha_1$, the 5′ region is encoded in three axons: two contain most of the untranslated region and the third encodes the remainder of that region plus the first 61 amino acids peculiar to $RAR\alpha_1$.

The RXRs show generally weak homology with the RARs, the highest degree of homology (61%) being in their DNA-binding domains. On the basis of homologies with an insect locus, it is thought that the RXRs may have evolved as the original retinoid-signaling system. Three RXR subtypes have been identified α, β, and γ); RXRα responds to somewhat higher retinoic acid levels than other RXR isoforms. Retainaldehyde has also been found to bind weakly RXR (and PPARγ) to inhibit activation (linked to inhibition of adipogenesis). Both RARs and RXRs are found in most tissues; greatest concentrations occur in adrenals, hippocampus, cerebellum, hypothalamus, and testis. Both

TABLE 6.14 Nuclear Retinoic Acid Receptors

Receptor	Isoforms[a]	Ligands
RARα (retinoic acid receptor)	$RAR\alpha_1$, $RAR\alpha_2$	All-*trans*-RA[b], 9-*cis*-RA[c], 13-*cis*-RA[d]
RARβ	$RAR\beta_1$, $RAR\beta_2$, RARβ[e]	All-*trans*-RA, 9-*cis*-RA[c], 13-*cis*-RA[d]
RARγ	$RAR\gamma_1$, $RAR\gamma_2$[c]	All-*trans*-RA, 9-*cis*-RA[c], 13-*cis*-RA[d]
RXRα (retinoid X receptor)		9-*cis*-RA
RXRβ		9-*cis*-RA
RXRγ		9-*cis*-RA
PPARβ/δ (peroxisome proliferator-activated receptor)		All-*trans*-RA[c]
PPARα		All-*trans*-RA[c]
PPARγ		All-*trans*-RA[c]

[a]*Isoforms differ only in their N-terminal regions.*
[b]*Retinoic acid.*
[c]*Binding shown only in vitro.*
[d]*Binding (weak) shown only in vitro.*
[e]*Identified in* Xenopus laevis.

90. That is, follicular hyperkeratosis, which can also be caused by deficiencies of niacin and vitamin A.
91. Triiodothyronine.

the RARs and RXRs can bind **9-*cis*-retinoic acid**[92] in vitro with high affinity ($K_d = 10$ nM); however, the physiological relevance of this vitamer is unclear as it has not been identified in vivo. RARs are abundant in the brain and pituitary gland (RARα distributed throughout; RXRα and RXRδ in striatal regions with dopaminergic neurons) where they function in regulating expression of the dopamine receptor.

All-*trans*-retinol can also bind **PPAR**s, PPARβ/δ having the greatest affinity. The shuttling of the retinoid to PPARβ/δ is accomplished by the fatty acid-binding protein 5 (FABP5), in contrast to CRABP (II), which delivers retinoids to other receptors. Therefore, the partitioning of retinoid between PPARβ/δ and the RARs and RXRs is a function of the relative amounts of FABP5 and CRABP (II) in cells. Cells in which CRABP (II) predominates express primarily through the RARs; cells with relatively high FABP5 levels express primarily through PPARβ/δ. In the presence of the coactivator SRC-1, the retinoid activation of PPARβ/δ led to the upregulation of expression of 3-phosphoinositide-dependent kinase 1 (PDK1), an activator of the antiapoptotic factor Akt1.[62] Activation of PPARδ, which is downregulated in adipose tissue in obese individuals, induces expression of genes affecting lipid and glucose homeostasis, including the insulin-signaling gene *PDK1*, resulting in improved insulin action. This mechanism is thought to underlie retinoic acid-induced weight loss in obesity-prone mice.[93]

Two types of high-affinity **RAREs** have been identified in the promoter regions of target genes near the transcription start: those that recognize the RXR homodimer and those that recognize the RXR–RAR heterodimer. The RXR–RAR heterodimer binds to RAREs, which consist of direct repeats of the consensus half-site sequence AGGTCA usually separated by five nucleosides. The RXR homodimer binds to cognate retinoid X response elements most of which are direct repeats of AGGTCA with only one nucleoside spacing. Gene expression is effected by activating each response element present in the promoter regions of responsive genes.[94] The RXRs can also form homotetramers as well as dimers with other members of the steroid/thyroid/retinoic acid family; heterodimerization in this system has usually been found to increase the efficiency of interactions with DNA and, thus, transcriptional activation. Further regulation is effected in this signaling system as the RXR–RAR heterodimer appears to repress the transcription-activating function of RXR–RXR.

In the absence of retinoic acid, the aporeceptor pair (RXR–RAR/RXR) binds to the RAREs of target genes, and RAR recruits corepressors that mediate negative transcriptional effects by recruiting histone deacetylase complexes, which modify histone proteins to induce changes in chromatin structure that reduce the accessibility of DNA to transcriptional factors. This process is reversed upon retinoic acid binding: a conformational change in the ligand-binding domain results in the release of the corepressor and the recruitment of coactivators of the AF-2 region of the receptor. Some cofactors interact directly to enhance transcriptional activation, while others can affect the acetylation histone proteins causing the conformational opening of chromatin and the activation of transcription of the target gene. Impairments in the process can lead to carcinogenesis.

The general picture is one of RXRs forming heterodimers with RARs that are activated by retinoids and with other receptors of the same superfamily, i.e., thyroid hormone receptors, the vitamin D_3 receptor, PPAR, and, probably, others yet unidentified (Fig. 6.12). The metabolite 9-*cis*-retinoic acid, which targets RXR, causes the formation of RXR homodimers that recognize certain RAREs. However, the same ligand inhibits the formation of heterodimers of RXR and the thyroid hormone receptor (TR), which reduces the expression of thyroid hormone-responsive genes.[95] In contrast, RXR-specific ligands do not appear to affect the formation of RAR-containing heterodimers. CRABP(II) facilitates interactions of RARα–RXRα heterodimers in forming a gene-bound receptor complex, delivering retinoic acid to its nuclear receptors and acting as a coactivator of the expression of retinoic acid-responsive genes. Retinoid responses appear to be restricted to a subset of retinoid-responsive genes through the action of the orphan COUP (chicken ovalbumin upstream promoter) receptors, which form homodimers that avidly bind several RAREs and repress both RAR–RXR and RXR–RXR activities. The RARs comprise a two-component signaling system for activating transcriptions in which retinoic acid can act in either a paracrine or autocrine manner, as both all-*trans*-retinoic acid and 9-*cis*-retinoic acid can be synthesized within the target cell (from retinol via retinal) or delivered to that cell from the circulation. In either case the ligand is transported intracellularly to the nucleus (via CRABP) and then to the appropriate receptor (RAR, RXR), which then can bind to its cognate RARE to regulate transcription. The RARs are part of a larger system of nuclear receptors (RARs, RXRs, PPARs, VDR, FXR, etc.) that integrates a range of metabolic signaling from retinoids, carotenoid metabolites, and fatty acids in

92. Yet to be identified in vivo, 9-*cis*-retinoic acid is known to be produced from 9-*cis*-retinol by 9-cis-retinol dehydrogenase with subsequent oxidation, by cleavage of 9-*cis*-β-carotene, and from the isomerization of all-*trans*-retinol in the lung.

93. Berry, D.C., Noy, N., 2009. Mol. Cell. Biol. 29, 3286.

94. The RXR–RAR response elements consist of polymorphic arrangements of the nucleotide sequence motif 5′-RG(G/T)TCA-3′. These gene elements are also responsive to thyroxine, suggesting that retinoic acid and thyroid hormone may control overlapping networks of genes.

95. This has been shown for the expression of uridine-5′-diphosphate-glucuronyl transferase, which is involved in the phase II metabolism of xenobiotic and endogenous substrates.

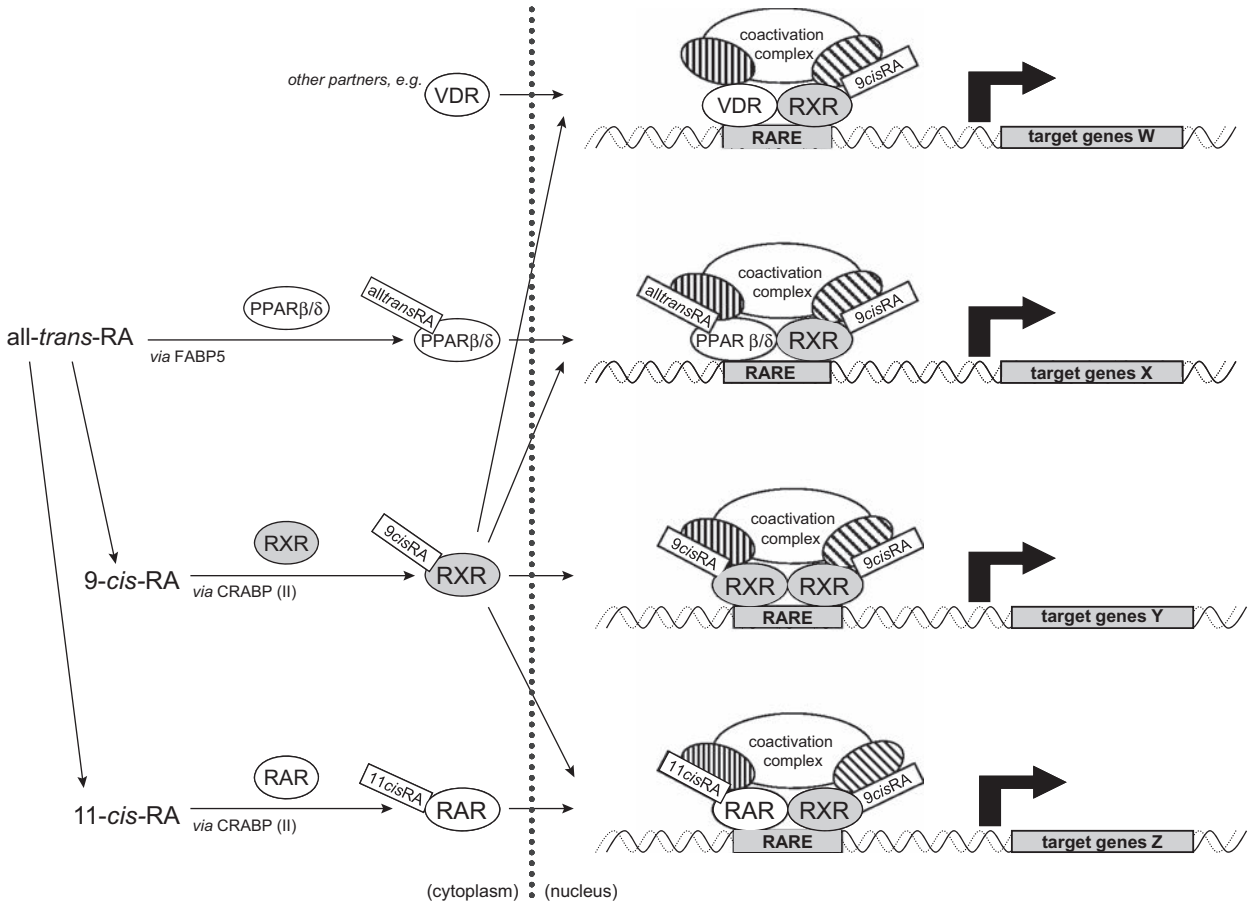


FIGURE 6.12 Participation of retinoid receptors and other nuclear receptors in integrating metabolic signaling in controlling expression of multiple genes. Retinoid activation of receptors that participate in complexes that bind to cognate DNA sequences to cause gene expression (*bold arrows*). The involvement of retinoid receptors (RARs and RXRs) with multiple binding partners allows retinoids to signal transcription of multiple pathways (*see text* for abbreviations.).

controlling the expression of multiple genes and affecting many tissues.

In this system, vitamin A and T_3 appear to play compensatory signaling roles. Studies with rats have shown that deprivation of either factor impairs thyroid signaling in the brain through reduced expression of RAR and TR, as well as a neuronal protein neurogranin.[96] Similarly, regulation of thyroid-stimulating hormone (TSH) has been found to be dependent on both the binding of both TR and RXR (which are activated by T_3 and 9-*cis*-retinoic acid, respectively) to the TSH gene.

Coenzyme Role for Vitamin A?

A coenzyme-like role has been proposed for vitamin A. This hypothesis holds that vitamin A can act as a sugar carrier in the synthesis of **glycoproteins**, which function on the surfaces of cells to effect intercellular adhesion, aggregation, and recognition. Indeed, retinol can be phosphorylated to yield retinyl phosphate, which can accept mannose from GDP-mannose (to form retinyl phosphomannose), which it then donates to a membrane-resident acceptor in the production of glycoproteins. Further, vitamin A-deficient animals appear to synthesize fewer glycoproteins and more abnormal ones (particularly in plasma, intestinal goblet cells, and corneal and trachea epithelial cells) than vitamin A-adequate animals. Still, the physiologic significance of this putative sugar carrier role of vitamin A is not clear. Retinoic acid, the vitamer that supports the systemic functions of the vitamin, cannot serve as a sugar carrier because it cannot be reduced to retinol. Neither can retinyl phosphate accept mannose. It has been proposed that retinoic acid may need to be hydroxylated to a phosphorylatable derivative that could serve as a sugar carrier.

8. BIOMARKERS OF VITAMIN A STATUS

Adequately nourished individuals have appreciable stores of vitamin A, which tend to mitigate against the effects

96. Neurogranin is a calmodulin-binding protein expressed in dendritic spines of the brain and participating in protein kinase C signaling.

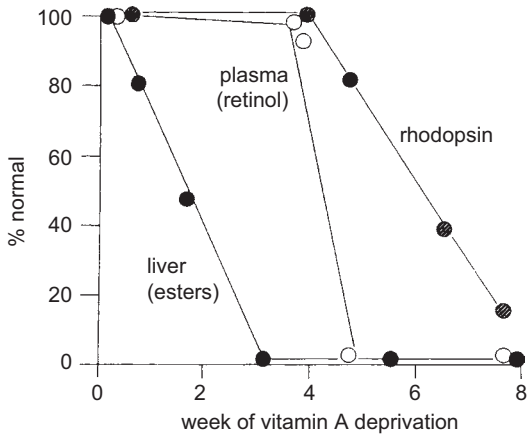


FIGURE 6.13 Hepatic vitamin A stores must be depleted before changes in retinol concentrations in circulation or photopigments occur.

of periods of low intakes of the vitamin. These stores are comprised of fast and slowly turning over pools (Fig. 6.13). As long as hepatic stores are sufficient to provide retinol, the plasma retinol level is minimally affected by vitamin A deprivation. Cellular functions of vitamin A can be expected to change only after plasma retinol–RBP4 concentrations have dropped below about 20 μg/g (0.07 μM). This phenomenon presents challenges in assessing vitamin A status, particularly at the margins of the normal range of plasma retinol concentrations.

Biochemical Assessment

The vitamers A can be accurately quantified in biological specimens using high-performance liquid–liquid partition chromatography (HPLC). Serum retinol is, therefore, a convenient parameter of vitamin A status. The homeostatic control of circulating retinol levels makes this parameter useful only in identifying subjects with chronically low-vitamin A intakes sufficient to exhaust their hepatic stores that support the synthesis of RBP4. Vitamin A-deficient subjects with still-appreciable liver stores will have serum retinol levels in the normal range. Under normal circumstances, 85% of plasma RBP4 exists in the holo-complex in with equimolar amounts of retinol; plasma levels of 0.70–1.05 μM indicate marginal vitamin A status and levels <0.70 μM (20 μg/dL) are considered indicative of vitamin A deficiency.

However, other factors can influence apo-RBP4 levels, affecting its value as a proxy for plasma retinol. Deficiencies of protein and zinc, hepatic steatosis, and injury can impair RBP synthesis in the liver. Hepatic release of apo-RBP to the circulation can be impaired under conditions of inflammation. Febrile illnesses and inflammation can reduce TTR binding to RBP4, allowing it to be lost by renal glomerular filtration. In contrast, obesity, in which RBP4 is released from adipocytes, and renal disease, in which RBP turnover can be less than one-10th of normal, can elevate plasma levels of RBP4, thus biasing assessments toward vitamin A adequacy. Such confounding effects may explain reports that one-fifth of night blind children have serum retinol greater than 0.70 μM and can show xerophthalmia and or conjunctival metaplasia, and that healthy adults depleted of vitamin A can show impaired dark adaptation at serum retinol levels of 20–30 μg/dL. Therefore, assessment of vitamin A status by RBP4 or plasma retinol should consider subject status with respect to general nutritional, inflammation, and general health; it is prudent to include such measures as BMI and, in plasma samples, albumin, C-reactive protein, and α_1-acid glycoprotein (AGP), which can suppress plasma retinol concentrations by as much as 24%.[97]

The concentrations of vitamin A in breast milk (i.e., primarily retinyl palmitate in milk fat) drop in vitamin A deficiency and can, therefore, be used to detect the deficiency in mothers.[98]

Dose–Response Tests

Because of the uncertainties of interpreting serum retinol values, tests have been devised to assess the mobilizable vitamin A capacity of the liver based on serum responses to oral doses of a retinoid.

- Relative **dose–response (RDR) test**. This test assesses hepatic capacity to mobilize vitamin A based on serum responses to oral doses of retinyl ester (retinyl acetate or retinyl palmitate). The test requires two samples of blood: a fasting sample drawn immediately before the administration of the test dose; another sample drawn 5 h later. Retinol is determined in each sample, and the percentage response over baseline is calculated.
- **Modified relative dose–response (MRDR) test**. This test assesses hepatic capacity to mobilize vitamin A based on serum responses to oral doses of **3,4-didehyd roretinol**.[99] It requires only a single blood sample taken 4–6 h after oral administration of the retinoid; both retinol and 3,4-didehydroretinol are determined in the sample, and the response is taken as the molar ratio of the two analytes.

Both tests assume that the appearance of retinoid in the plasma is a function of the amount also entering from endogenous hepatic stores. Therefore, an RDR value of

97. Thurnham, D.J., Northrop-Clewes, C.A., Knowles, J., 2015. J. Nutr. 145, 1137S–1143S.
98. Breast milk retinyl palmitate concentrations typically fall in the range of 1.75–2.45 μM; levels ≤1.05 μM (i.e., ≤8 μg/g milk fat) indicate vitamin A deficiency.
99. Also called **dehydroretinol** and **vitamin A_2**.

≥20% or an MRDR value of 20–30% is taken as indicative of inadequate hepatic storage (<0.07 μmol/g) of the vitamin; children with such low stores are almost certain to be vitamin A deficient. Determination of serum retinoids is accomplished using HPLC; smaller blood samples can be employed by using RPB4 as the endpoint, as that protein determined in very small volumes by enzyme-linked immunoassay. There is, however, a caveat: the use of RBP4 can yield high rates of false positives, particularly in subjects with high BMIs.[100] Such observations are presumably due to the presence in serum of adipose-derived apo-RPB4, which reduces the retinol:RBP4 ratio. Nevertheless, the RDR and MRDR tests remain the instruments of choice as categorical indicators of vitamin A status. While neither give quantitative measures of hepatic vitamin A stores, each can indicate changes in vitamin A status from low to adequate. The MRDR test is particularly useful in assessing impacts of interventions in which serum retinol is not expected to change, e.g., in subjects of marginal status. It has shown that preterm infants generally have low vitamin A stores.

Palmar Scanning

Noninvasive assessment of carotenoid status has been accomplished by scanning the palmar surface of the hand using resonance Raman spectroscopy. This procedure has been shown to be useful in monitoring subject responses to changes in the intake of carotenoid-rich foods;[101] however, as currently developed, it is not specific for provitamin A carotenoids (or vitamin A status).

9. VITAMIN A DEFICIENCY

Like most nutritional deficiencies in human populations, vitamin A deficiency is an outcome of a bio-eco-social system that fails to provide sources of the vitamin in ways that are at once accessible and utilizable. The complexity of this system has been captured in the WHO conceptual model (Fig. 6.14). In humans, vitamin A deficiency is typically associated with malnutrition, particularly protein–energy malnutrition. These conditions have common origins in grossly unbalanced diets and poor hygiene. Accordingly, malnourished children are likely to be deficient in vitamin A and other essential nutrients. Protein deficiency also impairs the synthesis of apo-RBP4, CRBP, and other retinol binding proteins, affecting vitamin A transport and cellular utilization. The storage of vitamin A mitigates the effects of periodic low dietary intakes of the vitamin. Recommended daily allowances for vitamin A (Table 6.15) are set to insure that body stores are sufficient to support adequate plasma retinol–RBP4 concentrations. When they are not, clinical signs of vitamin A deficiency are manifest.

Primary vitamin A deficiency can occur among children and adults who consume diets composed of few

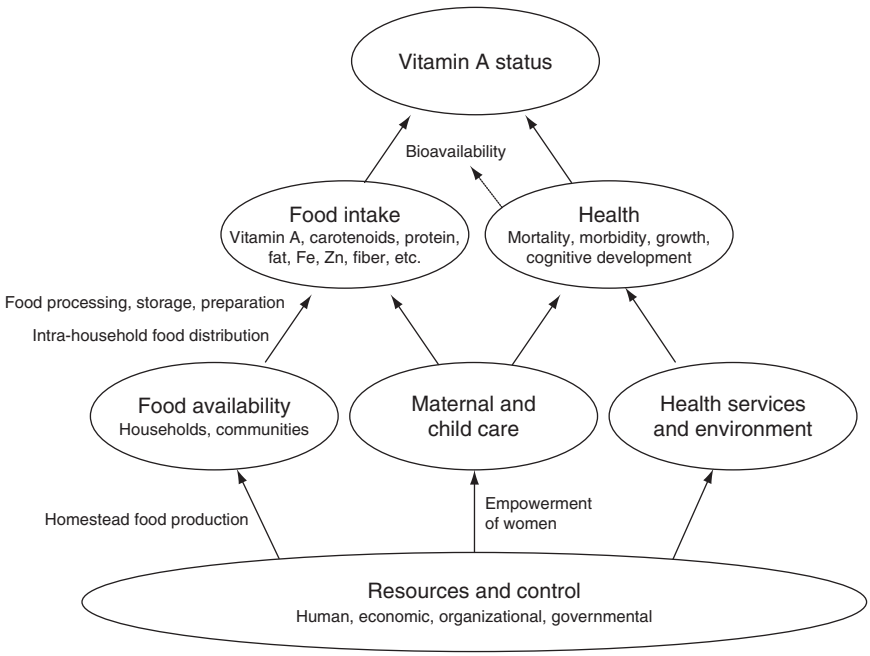


FIGURE 6.14 WHO conceptual framework of causes of vitamin A deficiency in human populations. *After UN Food Nutr. Bull. vol. 19, 1998.*

100. Fujita, M., Brindle, E., Rocha, A., et al., 2009. Am. J. Clin. Nutr. 90, 217–224.

101. Jahns, L., Johnson, L.K., Mayne, S.T., et al., 2014. Am. J. Clin. Nutr. 100, 930–937.

TABLE 6.15 Recommended Daily Allowances for Vitamin A

Age–Sex	US RDA[a] (μg RE/d)	Age–Sex	FAO/WHO RNI[b] (μg RE/d)
0–6 months	[400][c]	0–6 months	375
7–11 months	[500][c]	7–11 months	400
1–3 years	300	1–3 years	400
4–8 years	400	4–6 years	450
7–13 years	600	4–9 years	500
14–>70 years, females	700	10–18 years	600
Males	900	19–65 years, females	500
Pregnancy, ≤18 years	750	Males	600
≥19 years	770	>65 years	600
Lactation, ≤18 years	1200	Pregnancy	800
≥19 years	1300	Lactation	850

[a]*Recommended Daily Allowances; Food and Nutrition Board (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc, National Academy Press, Washington, DC, 773 pp.*
[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*
[c]*RDA has not been set; average intake (AI) is listed.*

servings of yellow and green vegetables and fruits and liver. For infants and young children, early weaning can increase the risk of primary deficiency. For livestock, it can occur with unsupplemented diets containing low amounts of yellow maize (corn) and corn gluten meal.

Secondary vitamin A deficiency can occur in several ways. One involves chronically impaired enteric absorption of lipids, such as in diseases affecting the exocrine pancreas (e.g., pancreatitis, cystic fibrosis, selenium deficiency) or bile production and release (e.g., biliary atresia, some mycotoxicoses in livestock), or due to the consumption of diets containing very low amounts of fat.[102] Chronic exposure to oxidants can also induce vitamin A depletion; an example is benzo(α)pyrene in cigarette smoke. Nutritional deficiencies of zinc can also impair the absorption, transport, and metabolism of vitamin A, as zinc is essential for the hepatic synthesis or RBP4 and the oxidation of retinol to retinal, which is catalyzed by a zinc-dependent retinol dehydrogenase. Malnourished populations, which typically have low intakes of several essential nutrients including vitamin A and zinc, are at risk to vitamin A deficiency. A prevalence of 25% or more of individuals with plasma retinol levels <0.70 μM (<20 μg/dL) is indicative of population-wide inadequacy with respect to the vitamin.

102. There are few data upon which to base estimates of the minimum amount of dietary fat needed to support the absorption of vitamin A and the other fat-soluble vitamins; in the absence of empirical data, the estimate of 5 g/day is frequently used.

Who is at risk of vitamin A deficiency?

- Infants and children whose mothers were vitamin A deficient during lactation
- Preterm infants whose in utero vitamin A uptake was interrupted
- Children and adults with poor diets and/or malabsorption conditions
- Animals fed unsupplemented grain-based diets free of provitamin A containing feedstuffs.

General Deficiency Signs

Insufficient intakes of vitamin A lead to a sequence of physiological events (Fig. 6.15) that, ultimately, are manifest in several clinical signs (Tables 6.16 and 6.17) and are classified accordingly (Table 6.18) for the purposes of evaluating risk and treatment efficacy (e.g., Table 6.19). Only two signs are unequivocal indicators of vitamin A deficiency; both are ocular lesions:

- **Nyctalopia** is impaired dark adaptation of the retina (night blindness); it can take a year to develop after the initiation of a vitamin A-deficient diet. In individuals of marginal vitamin A status, it can be brought on in a few days by febrile illness (e.g., measles). In either case, it quickly responds to vitamin A treatment.
- **Xerophthalmia** involves permanent morphological changes of the anterior segment of the eye that are not correctable without scarring. These epithelial changes involve dysfunction in the maintenance of normal

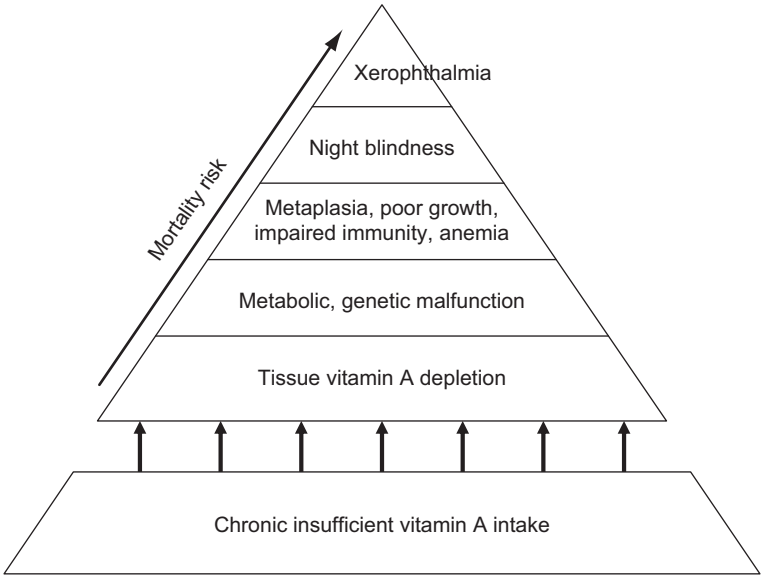


FIGURE 6.15 Progression of vitamin A deficiency. *After West, K.P., 2002. J. Nutr. 132, 2857S–2866S.*

TABLE 6.16 Signs of Vitamin A Deficiency

Organ System	Sign
General	Loss of appetite, retarded growth, drying and keratinization of membranes, infection, death
Dermatologic	Rough scaly skin, rough hair/feathers
Muscular	Weakness
Skeletal	Periosteal overgrowth, restriction of cranial cavity and spinal cord, narrowed foramina
Vital organs	Nephritis
Nervous system	Increased cerebrospinal fluid pressure, ataxia, constricted optic nerve at foramina
Reproductive	Aspermatogenesis, vaginal cornification, fetal death and resorption
Ocular	Nyctalopia, xerophthalmia, keratomalacia, constriction of optic nerve

TABLE 6.17 Stages of Xerophthalmia

Stage	Signs
1. Xerosis	Dryness of conjunctiva Bitot's spots on the conjunctiva near the cornea; ultimately extending to the cornea
2. Keratomalacia	Softening of cornea Ultimate involvement of iris/lens Secondary infection

TABLE 6.18 Clinical Classification of Eye Lesions Caused by Vitamin A Deficiency in Humans

Site Affected	Clinical Sign	Designation
Retina	Night blindness *also* nyctalopia	XN
	Fundus specs	XF
Conjunctiva	Xerosis	X1A
	Bitot's spots	X1B
Cornea	Xerosis	X2
	Ulceration/keratomalacia <1/3 of surface	X3A
	Ulceration/keratomalacia = 1/3 of surface	X3B
	Scar	XS

From WHO.

TABLE 6.19 Relationship of Serum Retinol Level and Clinical Vitamin A Deficiency in Indonesian Preschool Children

Clinical Status		n	Case Frequency, by Serum Retinol Level		
			Deficient	Low	Adequate
XN	X1B		<10 µg/dL	10–20 µg/dL	>20 µg/dL
+	–	174	27%	55%	18%
–	+	51	31%	57%	12%
+	+	79	38%	53%	9%
–	–	252	8%	37%	55%

After Sommer, A., Hussaini, G., Muhilal, L., et al., 1980. Am. J. Clin. Nutr. 33, 887–891.

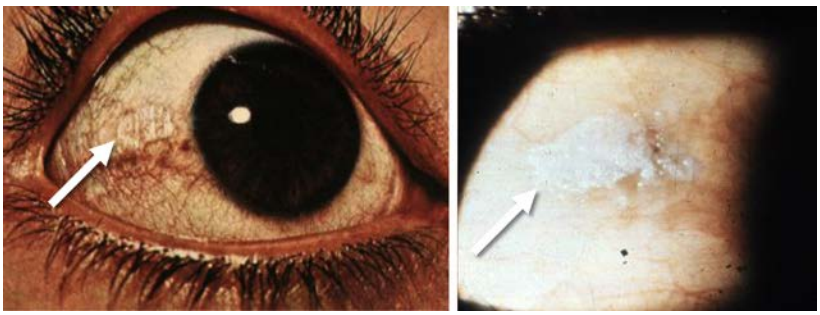


FIGURE 6.16 Bitot's spots on corneas of vitamin A-deficient children. *Courtesy of McLaren, D.S., American University of Beirut.*

epithelial architecture, particularly of the mucous membranes, resulting in keratinizing metaplasia. In the conjunctiva, goblet cells are lost and keratinized material accumulates on the surface over a thickened layer of flattened cells and a prominent granular cell layer. These changes ultimately lead to frank corneal necrosis with dissolution of stromal collagen and loss of keratocytes, i.e., the condition called **keratomalacia**.[103] While xerophthalmia can occur at any age, greatest prevalence is seen in pregnant women and children,[104] particularly those born to vitamin A-deficient mothers thus having low vitamin A stores. Early intervention is necessary to interrupt these progressive lesions in early stages before permanent blindness occurs (Table 6.10).

Deficiency Signs in Humans

Nyctalopia. This is the first functional sign of vitamin A deficiency that can be detected in humans. Dark adaptation is measured by the minimal intensity at which a subject can detect flashes of a white light while the subject fixes on a red light in a darkened environment. Dark adaptation is characterized by an initial decline in the luminance threshold due to cone adaptation, followed at the "rod-cone break" by a second luminance threshold decline. Vitamin A-deficient subjects show longer times to the "rod-cone break," which can occur before symptomatic night blindness. Dark adaptation can also be assessed by the pupillary response to a graduated light stimulus or by electroretinography. Examination by slit lamp can reveal fundus[105] specs, disrupted rod outer segments (with the possible involvement of similarly disrupted cones), and signs suggestive of visual field alterations. The technical expertise and subject burden has meant that these methods have not been widely used.

Ocular lesions. These include conjunctival xerosis with/without **Bitot's spots**[106] (Fig. 6.16), corneal xerosis, ulceration, and/or keratomalacia (Fig. 6.17), and corneal scars can be diagnosed by direct examination of the eye. Morphological changes in epithelial cells blotted from the conjunctival surface can be detected by histologic examination, a procedure called **conjunctival impression**

103. Keratomalacia can also occur in viral infections (e.g., measles) with general malnutrition.
104. Children face heavy demands for vitamin A: rapid growth; morbidity to gastroenteritis, measles, chickpox, pertussis, respiratory infections.
105. The fundus of the eye is the interior surface opposite the lens; it includes the retina, optic disc, macula and fovea, and posterior pole.
106. Described in the 1860's the eponymous Bitot's spots are patches of xerotic conjunctiva with keratin debris and *bacillus* growth.

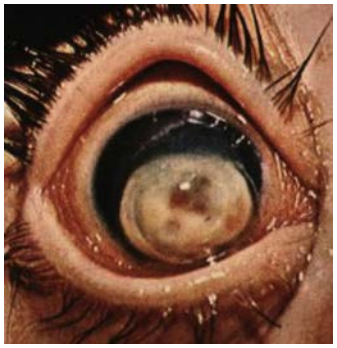


FIGURE 6.17 Keratomalacia in a vitamin A-deficient child. *Courtesy of McLaren, D.S., American University of Beirut.*

cytology.[107] The presence of enlarged, flattened epithelial cells, and few or no goblet cells is indicative of vitamin A deficiency. With progressing vitamin A deficiency, the conjunctival surface takes on a dry, corrugated, irregular surface, ultimately developing an overlay of white, foamy, or "cheesy" material consisting of desquamated keratin and a heavy bacterial growth.[108] Bitot's spots, the sine *qua non* of conjunctival xerosis, are almost always bilateral oval or triangular structures first appearing temporal to the limbus and comprising a thickened, superficial layer of flattened cells usually with a keratinizing surface, a prominent granular cell layer, acanthotic thickening with a disorganized basal cell layer, but with no goblet cells.

The earliest corneal signs of vitamin A deficiency are fine, fluorescein-positive, superficial punctuate keratopathy that usually begin in the inferior aspect of the cornea, particularly infranasally. These lesions can be seen using the slit lamp microscope. Studies have revealed punctuate keratopathy in 60–75% of patients with nyctalopia or vitamin A-responsive conjunctival xerosis. With progressive vitamin A deficiency, the lesions become more numerous and concentrated, involving larger portions of the corneal surface. By the time most of the corneal surface is involved, the lesions are generally apparent by hand-light examination as a haziness and diminished wettability on the corneal surface. At that point the condition is called **corneal xerosis**. In addition to punctuate keratopathy, corneas affected at this level also show stromal edema, again mostly in the inferior aspect. If untreated, the condition progresses to the point of corneal ulceration, frequently characterized by the presence of a single (in a minority of cases, by two to three) sharply defined ulcer varying in depth, usually one-fourth to one-half of corneal thickness, but sometimes deep enough to effect stromal loss. This can lead to deep stromal necrosis characterized by gray-yellow, edematous and cystic lesions varying in size from 2 mm in diameter to covering most of the cornea.

Impaired disease resistance. Vitamin A-deficient individuals are typically more susceptible to infection than are individuals of adequate vitamin A nutriture.[109] They show changes in lymphoid organ mass, cell distribution, histology, and lymphocyte characteristics. Accordingly, low-vitamin A status is frequently associated with increased morbidity and mortality.[110] Such findings were reported in a year-long study of school-age children in Colombia, which found that every 10 μg/dL decrement in plasma retinol was associated with 18% more days of diarrhea and vomiting, 10% more days of coughing and fever, and 6% more doctor visits.[111] A longitudinal study of preschoolers in Indonesia revealed that the overall mortality rate in children with xerophthalmia was 4–5 times that of children with no ocular lesions. Many studies have found positive associations between mild xerophthalmia and risks of diarrhea, respiratory infection, and measles among children. Vitamin A deficiency can reduce child survival by 30% or more,[112] and child mortality increases with increasing severity of the eye disease such that affected children die at nine times the rate of normal children.

Subclinical vitamin A deficiency can also affect resistance to infection. It has been suggested that foci of vitamin A-deficient cells in otherwise normal tissue may provide penetrable sites for bacteria and viruses, thus promoting infection. This hypothesis is consistent with the results of studies showing hypovitaminosis A coupled to secondary coliform infection in cattle, and more frequent bacteriuria among xerophthalmic compared to nonxerophthalmic, malnourished children. That vitamin A-deficient mice can survive enteric coliform infection without eliminating

107. Impression cytology is simple, noninvasive, and useful means of studying the conjunctiva. It involves filter-paper blotting of the anesthetized conjunctiva, followed by fixing, staining, and microscopically examining the lifted cells. Normal conjunctival cells appear in sheets of small, uniform, nonkeratinized epithelial cells with abundant mucin-secreting goblet cells. The procedure facilitates the correct identification of 82–93% of cases of xerophthalmia and 70–90% of unaffected; sensitivity declines and specificity increases when serum retinol or retinol relative dose–response cutoffs are also used in the definition of a case. The use of impression cytology yields estimates of vitamin A deficiency that are 5–10 times the rates of diagnosed xerophthalmia by direct visual examination. Somer suggested that vitamin A deficiency should be considered a public health problem when the prevalence of abnormal impression cytology reaches 20% in either women or children.

108. Most commonly, the "xerosis *Bacillus*," *Corynebacterium xerosis*, aerobic, Gram- and catalase-positive, nonspore-forming rod-shaped bacterium.

109. In practice, it can be difficult to ascribe such effects simply to the lack of vitamin A, as deficient individuals generally also have protein–calorie malnutrition, which, itself, leads to impaired immune function.

110. Although it was not until 1983 that this was noticed. (Sommer, A., Tarwojito, I., Hussaini, G., 1983. Lancet 2, 585–588).

111. Thornton, K., Mora-Plazas, M., Marin, C., et al., 2024. J. Nutr. 144, 496–503.

112. See meta-analyses: Fawzi, W.W., Chalmers, T.C., Herrara, M.G., et al., 1993. Med. Asso. 269, 898–903; Glasziou, P., Mackerras, D., 1993. Br. Med. J. 306, 366–370.

the infection suggests that hypovitaminosis A may create asymptomatic reservoirs for such enteric infections.[113]

Thus, vitamin A deficiency can induce and exacerbate inflammation and lead to histopathological changes that provide environments conducive to secondary infection in loci obstructed by keratinizing debris.[114] Such outcomes involve impairments in epithelial integrity (including mucosal immunity), antibody responses, and lymphocyte differentiation. Vitamin A deficiency impairs adaptive immunity and the development of both B cells and T-helper (Th) cells. Antibody responses, particularly those directed by Th2 cells, are reduced; at the same time chronic inflammatory responses, directed by Th1 cells, are increased, including increased production of proinflammatory cytokines. Children with xerophthalmia were found to have low $CD4^+$:$CD8^+$ ratios as well as other abnormalities in T cell subsets (Table 6.20); these signs reversed upon vitamin A supplementation.

TABLE 6.20 T Cell Abnormalities in Children

Measure	Without Xerophthalmia	With Xerophthalmia
CD4/CD8	1.11 ± 0.04	0.99 ± 0.05
% CD4/CD45RA (naive)	34.9 ± 1.7	29.9 ± 2.1[a]
% CD4/CD45RO (memory)	18.0 ± 1.1	17.4 ± 1.2
% CD8/CD45RA	37.3 ± 1.7	41.6 ± 2.1
% CD8/CD45RO	7.6 ± 0.6	10.2 ± 0.9[a]
Plasma retinol (μM)	0.84 ± 0.06	0.57 ± 0.04[a]

[a] $p < .05$.
After Semba, R.D., Muhilal, X., Ward, B.J., et al., 1993. Lancet 341, 5–8.

Measles. Measles infection[115] can potentiate subclinical vitamin A deficiency to cause xerophthalmia. "**Measles blindness**" is the leading cause of blindness among children in low-income countries, with as many as 60,000 cases each year. Systematic reviews of clinical intervention trials have confirmed that vitamin A supplementation can reduce mortality in children with measles.[116] The WHO recommends high-dose vitamin A treatment on two consecutive days for measles prophylaxis.

Malaria. Many subjects with malaria[117] are suboptimally nourished with respect to vitamin A and other micronutrients. Low-serum retinol levels are commonly found in patients with malaria. Such findings do not imply causality, as malaria is known to be associated with other factors capable of impairing vitamin A status (reduced food intake, helminth infections). The most direct evidence of a causal linkage comes from studies in Papua New Guinea, Burkina Faso, and Ghana demonstrated vitamin A treatment of apparently nondeficient children to produce 30% reductions in the prevalences of malaria-specific mortality or malarial fever.[118] Such results are supported by findings that the in vivo growth of *Plasmodium falciparum* can be inhibited by retinol, and that retinoic acid treatment of human monocytes can increase phagocytosis of *Plasmodium*-parasitized erythrocytes. It is possible that vitamin A deficiency and infections such as malaria are mutually potentiating, the deficiency compromising resistance to the parasite, and the parasitic infection impairing the utilization of the vitamin.

Deficiency Signs in Animals

Animals manifest signs of vitamin A deficiency similar to those of humans, the most notable being nyctalopia and xerophthalmia. Other signs are also of consequence in livestock species, i.e., those involving other systems:

- eyes—excessive lacrimation in cattle and horses
- integument—rough hair coat in cattle; ruffled feathers in poultry; shortened, weakened wool fibers in sheep
- nerves and muscles—seizures in cattle and sheep; unsteady gait in poultry and swine; weakness in horses
- bone—periosteal overgrowth in cattle
- reproduction—reduced conception rates and increased fetal deaths in cattle, swine and poultry; depressed egg production in hens
- disease resistance—increased risk for pinkeye in calves and mastitis in dairy cows
- growth—swine, poultry
- others—and impaired heat tolerance in cattle.

Treatment of Vitamin A Deficiency

Because vitamin A is stored in appreciable amounts in the liver, it can be administered in relatively large, infrequent doses with efficacy. In cases of clear or suspected

113. McDaniel, K., Restori, K.H., Dods, J.W., et al., 2015. Infec. Immun. 83, 2984–2991.
114. Such an example is *Bitot's spots*, which are patches of xerotic conjunctiva with keratin debris and *bacillus* growth. In Indonesia, the presence of Bitot's spots is associated with intestinal worms, such that they are referred to as "*worm feces*."
115. Measles affects an estimated 30 million children each year, causing at least a million deaths.
116. D'Souza, R.M., D'Souza, R., 2004. Cochrane Database Syst. Rev. CD001479; Sudfeld, C.R., Navar, A.M., Halsey, N.A., 2010. Int. J. Epidemiol. 39, 1148S–1155S.
117. Malaria affects populations that are often malnourished, particularly children. In Africa alone, children experience more than 100 million malarial episodes each year, killing some 80,000.
118. Shankar, A.H., Genton, B., Semba, R.D., et al., 1999. Lancet 354, 203–209; Zeba, A.N., Sorgho, H., Rouamba, N., et al., 2008. Nutr. J. 7, 7–14; Owusu-Agyei, S., Newton, S., Mahama, E., et al., 2013. Nutr. J. 12, 131–140.

TABLE 6.21 WHO Recommended Treatment Schedule for Vitamin A Deficiency

Case/Situation	Time	Vitamin A Dose, RE[a], by Age			
		Any Age	<6 months	6–12 months	1 year
Subjects with xerophthalmia	Day 1		15,000	30,000	60,000
	Day 2		15,000	30,000	60,000
	2–4 weeks		15,000	30,000	60,000
Women reproductive age, night blind or Bitot's spots	Weekly, 3 months	10,000 or 25,000			
Women reproductive age with corneal lesions	Day 1	200,000			
	Day 2	200,000			
	Day 14	200,000			
Subjects with measles	Day 1		15,000	30,000	60,000
	Day 2		15,000	30,000	60,000
Subjects with severe protein–energy malnutrition[b]	Day 1		15,000	30,000	60,000
	Every 4–6 months[c]	60,000			
Asymptomatic high-risk subjects	Once		15,000	30,000	60,000[d]
	Every 4–6 months	60,000			

[a]*Oil solution administered orally.*
[b]*<-3 Z scores for weight:height.*
[c]*Until signs of protein–energy malnutrition subside.*
[d]*Including postpartum mothers.*
After WHO, 1997. Vitamin A supplementation: a guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia. second ed. WHO, Geneva.

xerophthalmia, particularly in communities in which the deficiency is prevalent, vitamin A is administered orally in large doses, followed by an additional dose the next day and one-third a few weeks later (Table 6.20). Oral administration of water miscible or oil solutions of the vitamin is as effective as water-miscible preparations administered parenterally. Water-miscible preparations are much more effective than oil solutions when administered parentally, i.e., by intramuscular injection. Topical administration on the skin is ineffective.

High-dose treatment. Vitamin A can be administered safely in relatively high, infrequent oral doses to treat deficient individuals. The WHO dosing schedule (Table 6.21) has been demonstrated safe and effective, the only side effects being transient headache, nausea or vomiting, and diarrhea. Still, high-dose treatment is not recommended for pregnant women due to the risk of teratogenic effects on the fetus (see, *Vitamin A Toxicity*, later in this chapter). Periodic high doses of vitamin A are widely used to prevent deficiency in children living in high risk areas; in those circumstances, its efficacy in preventing xerophthalmia appears very high, i.e., 90%.[119]

Low-dose treatment. Retinyl palmitate in daily doses equal to the RDA, or weekly doses 7 times RDA, are very well absorbed and effective in supporting adequate serum retinol levels. These low-dose regimens greatly reduce the risk of side effects and appear to be particularly effective in reducing the incidence and duration of acute respiratory infections.

Responses to treatment. Night blindness due to vitamin A deficiency responds within hours to days upon the administration of vitamin A (Fig. 6.16), although full recovery of visual function may take weeks and the fading of retinal lesions may take up to 3 months. Active Bitot's spots and the accompanying xerosis responds rapidly to vitamin A treatment; in most cases lesions regress in days and disappear in 2–3 weeks. Punctate keratopathy of the cornea also responds rapidly to vitamin A, improving within a week in response to a large oral dose (Fig. 6.18).

Correction of vitamin A deficiency in children reduces morbidity rates, particularly from diarrhea and measles (Table 6.22). Meta-analyses of community-based, vitamin A intervention studies indicate an average 23% (range: 6–52%)[120] reduction in preschool mortality (Table 6.23).

119. Solon, F.S., Klemm, R.D., Sanchez, L., et al., 2000. Am. J. Clin. Nutr. 72, 738–744.

120. Beaton, G.H., Martorell, R., L'Abbe, et al., 1992. Report to CIDA, University of Toronto; Sommer, A., West, Jr., K.P., Olson, J.A., Ross, C.A., 1996. Vitamin A Deficiency: Health, Survival, and Vision. Oxford University Press, New York, p. 33.

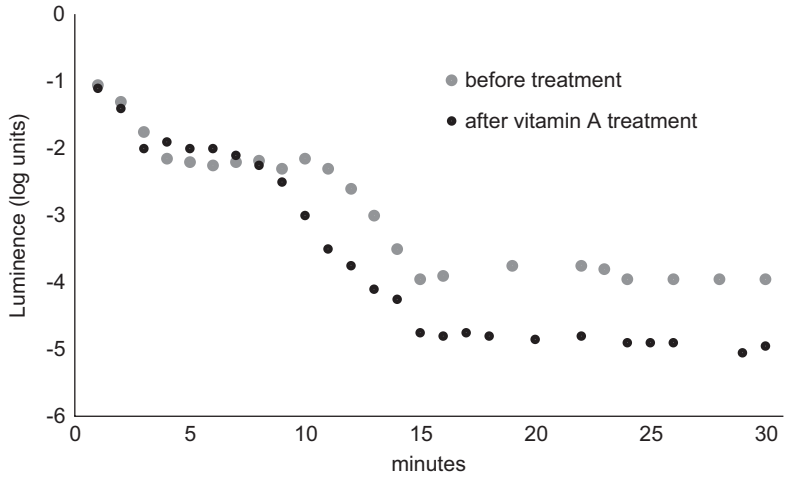


FIGURE 6.18 Dark adaptation in a vitamin A-deficient individual before and after vitamin A treatment. *After Russell, R.M., Multack, R., Smith, V., et al., 1973. Lancet 2, 1161.*

TABLE 6.22 Effects of Vitamin A on Morbidity of Children With Measles in South Africa

	Hospital Morbidity			6-Month Morbidity	
Outcome	**Placebo**	**Vitamin A**	**Outcome**	**Placebo**	**Vitamin A**
Clinical pneumonia (days)	5.7 ± 0.8	3.8 ± 0.4[b]	Weight gain (kg)	2.37 ± 0.24	2.89 ± 0.23[b]
Diarrhea duration (days)	4.5 ± 0.4	3.2 ± 0.7	Diarrheal episodes	6	3
Fever duration (days)	4.2 ± 0.5	3.5 ± 0.3	Respiratory infections	8	3[a]
Clinical recovery (<8 days) (%)	65	96	Pneumonia episodes	3	0[a]
Integrated morbidity score	1.37 ± 0.40	0.24 ± 0.15[b]	Integrated morbidity score	4.12 ± 1.13	0.60 ± 0.22[b]

[a] $p < .05$.
[b] *Based on incidence/severity of diarrhea, upper respiratory infections, pneumonia, and laryngotracheobronchitis.*
After Coutsoudis, A., Broughton, M., Coovadia, H.M., 1991. Am. J. Clin. Nutr. 54, 890–895.

TABLE 6.23 Effects of Vitamin A Supplementation on Child Mortality

		Deaths/Total		
Trial	**Months**	**Control**	**Vitamin A**	**Odds Ratio (95% CL)**
Sarlahi, Nepal	12	210/14,143	152/14,487	0.70 (0.57–0.87)
Northern Sudan	18	117/14,294	123/14,446	1.04 (0.81–1.34)
Tamil Nadu, India	12	80/7655	37/7764	0.45 (0.31–0.67)
Aceh, Indonesia	12	130/12,209	101/12,991	0.73 (0.56–0.95)
Hyderabad, India	12	41/8084	39/7691	1.00 (0.64–1.55)
Jumla, Nepal	5	167/3,4111	138/3786	0.73 (0.58–0.93)
Java, Indonesia	12	250/5445	186/5775	0.69 (0.57–0.84)
Bombay, India	48	32/1644	7/1784	0.20 (0.09–0.45)

After Fawzi, W.W., Chalmers, T.C., Merrara, M.G., et al., 1993. J. Am. Med. Assoc. 269, 898–903.

Vitamin A supplementation of children with active, severe, complicated measles has been shown to reduce in-hospital mortality by at least 50%. In other populations, vitamin A treatment has been shown to reduce the symptoms of diarrhea nearly as much (Table 6.24), as well as the symptoms of pneumonia and other infections substantially. Although attendant reductions in morbidity would be expected, studies have shown those effects to be variable. That not all interventions with vitamin A have reduced mortality rates of vitamin A-deficient children is not surprising, as other factors (e.g., time of initiation of breast feeding, poverty, poor sanitation, inadequate diets) clearly contribute to the diminished survival of vitamin A-deficient children. Because enteric pathogens induce unique immune responses not all of which may be comparably affected by the differential action of vitamin A on innate and adaptive immune responses, the efficacy of vitamin A supplementation is likely to depend on the dominant pathogens present in particular communities.

Of the newborn vitamin A supplementation trials conducted to date, vitamin A has been found to reduce mortality in trials with subjects of low-vitamin A status and/or late initiation of breast feeding; whereas, benefits have not been observed in trials with subjects of relatively good vitamin A status and/or early initiation of breast feeding.[121] It is likely that excess mortality occurs not only among xerophthalmic preschoolers but also among those who are mildly to marginally deficient in vitamin A but have not developed corneal lesions. In fact, a large portion of the deaths averted by vitamin A supplementation may be in this low-vitamin A group. Sommer and colleagues have estimated that the improvement of serum retinol levels in mildly deficient, asymptomatic children (with serum retinol levels of 18–20 μg/dL) to serum levels of 30 μg/dL would be expected to reduce mortality by 30–50%. A recent meta-analysis showed a 62% reduction in the risk of measles in response to a two-dose regimen of vitamin A administered with measles vaccination; however, other recent negative findings for vitamin A administered to children at the time of vaccination[122] raise the question of whether vitamin A may benefit mostly children who are not vaccinated, which is often the case in poor countries.

Public health programs. The successes of vitamin A supplementation trials have led to the widespread use of vitamin A capsules: UNICEF estimates that each year more than a half-billion capsules are distributed to some 200 million children in 100 countries. The efficacy of the large-dose, medical approach for reducing child morbidity has been questioned.[123] A meta-analysis of nine randomized controlled trials found that vitamin A supplementation had no consistent effect on the incidence of diarrhea, and may slightly increase the risk of respiratory tract infection,[124] and a very large trial in Ghana found vitamin A supplementation of women without effect on pregnancy-related mortality.[125] West and colleagues[126] noted that, in fact, maternal vitamin A supplementation has reduced maternal mortality in areas of prevalent gestational night blindness and the risk of maternal mortality is very high. Maternal interventions also offer the potential to produce health benefits, such as improved respiratory function, that become apparent in preadolescent children.

TABLE 6.24 Effects of Vitamin A Supplementation on Child Cause-Specific Mortality

Study Country	Relative Risk[a] of Death, by Disease		
	Measles	Diarrhea	Respiratory Disease
Indonesia[b]	0.58	0.48	0.67
Nepal[c]	0.24	0.61	1.00
Nepal[d]	0.67	0.65	0.95
Ghana[e]	0.82	0.66	1.00

[a]*Ratio of deaths occurring in the vitamin A-treated group to those occurring in the untreated control group.*
[b]*Rahmathullah, L., Underwood, B.A., Thulasiraj, R.D., et al., 1990. N. Eng. J. Med. 323, 929–935.*
[c]*Reanalysis of data of West, Jr., K.P., Pokhrel, R.P., Katz, J., et al., 1991. Lancet 338, 67–71 cited in Sommer, A., West, Jr., K.P., Olson, J.A., et al., 1996. Vitamin A Deficiency: Health, Survival, and Vision. Oxford University Press, New York, p. 41.*
[d]*Daulaire, N.M.P, Starbuck, E.S., Houston, R.M., et al., 1992. Br. Med. J. 304, 207–210.*
[e]*Ghana VAST Team, 1993. Lancet 342, 7–12.*

10. VITAMIN A IN HEALTH AND DISEASE

Infections

Active infection appears to alter the utilization or, at least, the distribution of vitamin A among tissues. Plasma retinol concentrations drop during malarial attacks, chickenpox, diarrhea, measles, and respiratory disease. Ocular signs of xerophthalmia following measles outbreaks are associated

121. Thurman, D., 2010. Newborn vitamin A dosing and neonatal mortality. Sight Life 1, 19–26.
122. Sudfeld, C.R., Navar, A.M., Halsey, N.A., 2010. Int. J. Epidemiol. 39, 148–155; Benn, C.S., Aaby, P., Nielsen, J., et al., 2009. Am. J. Clin. Nutr. 90, 626–639; Benn, C.S., Rodrigues, A., Yazdanbakhsh, M., et al., 2009. Vaccine 27, 2891–2898.
123. Latham, M., 2010. J. World Pub. Health Nutr. Assoc. 1, 12–24.
124. Grotto, I., Mimouni, M., Gdalevich, M., et al., 2003. J. Pediatrics 142, 297–304.
125. In 1999, West and colleagues. (West, K.P., Katz, J., Khatry, S.K., et al., 1999. Br. Med. J. 318, 570–575) reported vitamin A supplementation to reduce pregnancy-related mortality by 44%, although the apparent effects seemed unrelated to infection/immunity; however, in 2010 Kirkwood and colleagues (Kirkwood, B., Humphrey, J., Moulton, L., et al., 2010. Lancet 376, 1643–1644) found no such protection in an intervention trial with more than 200,000 women in Ghana.
126. West, K.P, Christian, P, Katz, J., et al., 2010. Lancet 376, 873–874.

with declines in plasma retinol levels, depending on the severity and duration of infection, and can be as great as 50%. Episodes of acute infection have been found to be associated with substantive (e.g., eightfold) increases in the urinary excretion of retinol and RBP4. That such insults to vitamin A status can be of clinical significance is indicated by the fact that vitamin A treatment can greatly reduce morbidity and mortality rates in measles and respiratory diseases. Stimulation of immunity and resistance to infection are thought to underlie the observed effects of vitamin A supplements in reducing risks of mortality and morbidity from some forms of diarrhea, measles, HIV infection, and malaria in children. Night blind women have a fivefold increased risk of dying from infections, and low doses of vitamin A have been found to reduce peri- and postpartum mortality in women (Table 6.25), presumably due to reduction in the severity of infections. Indeed, vitamin A supplementation has been found to reduce the incidence of uncomplicated malaria by more than 30%.

HIV. Low-serum retinol levels have been found to be more common among HIV+ than in noninfected individuals and to be highly predictive of vaginal HIV-1 DNA shedding. Whether this is cause or effect is not clear, as serum retinol is known to decrease in the acute-phase response to infection. Intervention studies have found vitamin A supplements effective in improving maternal vitamin A status. Clinical trials with HIV+ children have found vitamin A supplementation to reduce mortality; however, those with HIV+ mothers have not shown benefits in reducing the progress of disease, mother-to-child transmission of the virus, or the prevalence of infection in infants (Table 6.26). That vitamin A may affect other viral infections has been suggested by clinical findings of reduced viremia in some vitamin A-treated subjects with hepatitis C.

TABLE 6.25 Efficacy of Low-Dose Vitamin A Supplements in Reducing Mortality Related to Pregnancy in Nepal

Parameter	Placebo	Vitamin A	β-Carotene
Serum Levels, Midpregnancy[a] (μmol/L)			
Retinol	1.02 ± 0.35	1.30 ± 0.33	1.14 ± 0.39
β-Carotene	0.14 ± 0.12	0.15 ± 0.14	0.20 ± 0.17
Mortality, deaths/100,000 pregnancies (RR, 95% C.L.)			
During pregnancy	235 (1.0)	142 (0.60, 0.26–1.38)	111 (0.47, 0.18–1.20)
0–6 weeks postpartum	359 (1.0)	232 (0.65, 0.34–1.25)	222 (0.62, 0.31–1.23)
7–12 weeks postpartum	110 (1.0)	52 (0.47, 0.13–1.76)	28 (0.25, 0.04–1.42)

[a]*The 3.5 yr. trial involved some 44,646 women who had more than 22,000 pregnancies, 7200–7700 in each treatment group. Vitamin A (retinol) or β-carotene were given in weekly dosages; posthoc analyses revealed that only half of subjects took 80% of the intended doses (7000 IU), suggesting that the study underestimated the potential impact of vitamin A supplementation.*
After West, Jr., K.P., Katz, J., Kharty, S.K., et al., 1999. Br. Med. J. 318, 570–574.

Skin Health

Owing to their similarities to changes observed in vitamin A-deficient animals, certain dermatologic disorders of keratinization (e.g., ichthyosis,[127] Darier's disease,[128] pityriasis rubra pilaris[129]) have been treated with large doses of retinol. Clinical success of such treatments generally has been variable; high doses of the vitamin may be required for efficacy. Greater therapeutic efficacy has been achieved with all-*trans*-retinoic acid, 13-*cis*-retinoic acid, and an ethyl ester of all-*trans*-retinoic acid. The most successful of these has been 13-*cis*-retinoic acid, known generically as **tretinoin**, which is used for the treatment of **acne vulgaris**.[130] The vitamer decreases sebum production, inhibits the development of blackheads, reduces bacterial numbers in both the ducts and surface, and reduces inflammation by inhibiting the chemotactic responses of monocytes and neutrophils. It is also used to treat cystic acne, rosacea, gram-negative folliculitis, pyoderma faciale, hidradenitis suppurativa, and skin cancers.

Retinoids have also been found to produce rapid reductions in the incidence of new nonmelanoma skin cancers in high-risk patients. Therefore, it has been suggested that they may produce regressions of prediagnostic malignant and/or premalignant lesions. Indeed, regressions of cutaneous metastases of malignant melanoma and cutaneous T cell lymphoma have been reported in response to retinoid therapy. Topical treatment with all-*trans*-retinoic acid has been found to protect against photoaging signs by stimulating collagen synthesis and accumulation[131] in the upper papillary dermis, and downregulating the induction by UV light of metalloproteinase expression, thereby increasing collagen replacement. The action of retinoids in psoriasis

127. The term refers to a group of skin disorders, many with genetic components, characterized by varying degrees of dry, thickened, scaling/flaking skin.

128. An autosomally dominant dermatosis characterized by greasy hyperkeratotic papules in seborrheic regions, mucous, and nail changes.

129. Also, "*Devergie's disease*," a chronic disorder of unknown cause characterized by red-orange, scaling plaques, and keratotic follicular papules with itching and severe flaking.

130. Commonly called "*acne*," this chronic skin disease involves the blockade of hair follicles with dead skin cells and skin oils, often involving proliferation of the common skin bacterium *Propionibacterium acnes*. It is most common among teenagers and is thought to have a genetic component.

131. In particular collagen I, which comprises some 85% of dermal collagen.

TABLE 6.26 Vitamin A Supplementation Has Failed to Reduce Mother-To-Child HIV Transmission

Trial	Intervention Agent	RR[a] to Being HIV+, by Age			
		Birth	6 weeks	12 weeks	2 years
Malawi[b]	Retinol	–	0.96	–	0.84
Tanzania[c]	Retinol + β-carotene	1.60	1.22	–	1.38[d]
South Africa[e]	Retinol + β-carotene	0.85	–	0.91	–

[a]*Ratio of % HIV = children in treatment group to % HIV+ in control group.*
[b]*Kunwenda, N., Motti, P.G., Thaha, T.E., et al., 2002. Clin. Infect. Dis. 35, 618–624.*
[c]*Fawzi, W.W., Msamanga, G.I., Hunter, D., et al., 2002. AIDS 16, 1935–1944.*
[d]$p < .05$.
[e]*Coutsoudis, A., Pillay, K., Spooner, E., et al., 1999. AIDS 13, 1517–1524.*

appears to involve thinning of the stratum corneum, reduced keratinocyte proliferation, and reduced inflammation.

The therapeutic value of retinoids is limited by their dose-limiting side effects. Some retinoids, e.g., 13-*cis*-retinoic acid, can be teratogenic, limiting their use especially for women of child-bearing age. Therefore, alternative approaches have been of interest: use of synthetic, mono- and poly-aromatic retinoids; use of inhibitors (imidazoles and triazoles) of the cytochrome *P*-450-dependent 4-hydroxylation of all-*trans*-retinoic acid to sustain its intracellular concentrations. Clinical evaluation of the retinoic acid metabolism inhibitor liarozole,[132] has shown it to be comparably effective as 13-*cis*-retinoic acid in treating psoriasis and ichthyosis; when used topically the azole compound was more effective.

Obesity–Diabetes

Overweight/obese individuals show elevated plasma apo-RBP4 levels (Fig. 6.4).[133] These observations reflect increased apo-RBP4 secretion under conditions in which adipocytes downregulate the expression of GLUT4, the insulin-responsive transporter required for cellular uptake of glucose. This was confirmed by the finding that genetic ablation of adipose-specific GLUT4 increased RBP4 expression in mice. Because downregulation of GLUT4 occurs under conditions of food deprivation, obesity-stimulated secretion of apo-RPB4 may be a signal for restricting glucose uptake by peripheral tissues. Studies in mice have shown that overexpression of RPB4 or treatment with recombinant RPB4 increased expression of the gluconeogenic enzymes in liver and impaired insulin signaling in muscle. This is consistent with findings that obese humans have been found to have elevated plasma levels of RBP4, but normal levels of retinol (Table 6.27), and that prolonged elevation of RBP is associated with increased risk of type 2 diabetes (Table 6.28). Thus, adipocyte-derived apo-RBP4 may act as an adipokine to signal reductions in insulin sensitivity, thus promoting the development of type 2 diabetes. That signaling appears to depend on STRA6; when bound to holo-RBP, activates a JAK/STAT signaling cascade that induces expression of SOCS3, which suppresses insulin signaling. STRA6-null mice do not experience RBP-induced suppression of insulin signaling.[134] It has been suggested that lowering RBP4 should be considered in the prevention/treatment of type 2 diabetes. To that end, fenretinide,[135] a synthetic retinoid that increases urinary excretion of RBP4, has been found to normalize serum RBP4 levels and improve insulin sensitivity and glucose tolerance in obese mice.

Adipose tissue is responsive to vitamin A. Adipose tissue can store vitamin A as retinyl esters, and adipocyte differentiation is regulated by RARs, RXRs, and PPARs. Under conditions of vitamin A deficiency, adipocytes increase their storage of triglycerides and express high levels of RADH1 and mobilize those stores to make retinal and retinoic acid. Vitamin A has been found to be protective against the obesogenic effects of high-fat diets in the mouse. Signaling through RARγ, retinoic acid promotes the maintenance of the preadipocyte phenotype; in mature adipocytes, it upregulates lipid oxidation and energy expenditure. It is not clear, however, whether adipose vitamin A levels are related to obesity, as studies with normal animal models have yielded mixed results. Cattle show declines in plasma retinol at the fattening stage of their growth; but vitamin A deprivation has been found effective in increasing fattening, i.e., muscle marbling, only in animals with a novel single nucleotide polymorphism in aldehyde dehydrogenase I.[136] Still, it has been

132. 6-[(3-chlorophenyl)-imidazol-1-ylmethyl]-1*H*-benzimidazole.
133. Yang, Q., Graham, T.E., Mody, N., et al., 2005. Nature 436, 356–362.
134. Berry, D.C., Jacobs, H., Marwarha, G., et al., 2013. J. Biol. Chem. 34, 24528–24539.
135. 4-Hydroxy(phenyl)retinamide
136. Ward, A.K., McKinnon, J.J., Hendrick, S., et al., 2015. J. Anim. Sci. 90, 2476–2483.

TABLE 6.27 Parameters of Vitamin A Status in Obese and Nonobese Adults

Parameter	Obese	Nonobese
N	76	41
BMI (kg/m^2)	34.3 ± 4.0[a]	24.8 ± 3.2
Plasma retinol (μM)	2.23 ± 0.57	2.23 ± 0.58
Plasma RBP (μM)	3.13 ± 0.88[a]	2.67 ± 1.02

[a]$p < .05$.
After Mills, J.P., Furr, H.C., Tanumihardjo, S.A., 2008. Exp. Biol. Med. 233, 1255–1261.

TABLE 6.28 Association of Plasma RBP4 Level and Risk of Type 2 Diabetes in Adults

RBP4 (μg/mL)	Cases/Total	Relative Risk
(Quartile)	N	
<31.3	101/522	1.00
31.3–<38.1	116/525	1.16 (0.86, 1.58)
38.1–<46.1	125/522	1.18 (0.87, 1.61)
≥46.1	165/523	1.75 (1.30, 2.37)
P, trend		<0.001

After Sun, L., Qi, Q., Zong, G., et al., 2014. J. Nutr. 144, 722–728.

suggested that alterations in retinoid metabolism may affect the regulatory activities of PPARγ. For example, studies have found high-fat diets to be more obesogenic for mice lacking BCO1, CRBP(I), or CRBP(III), compared to wild-type mice.

Adipose tissue is also a major storage site of carotenoids, which partition into the bulk lipid droplets of adipocytes. Carotenoid concentrations tend to be inversely related to percentage body fat, due to the fact that the caloric excesses that drive adiposity tend to be unrelated to the intake of carotenoid-rich foods (fruits, vegetables). Therefore, body fat would appear to be a determinant of the tissue distribution of carotenoids including those with provitamin A potential.

Drug Metabolism

That the level of vitamin A intake has been found to affect negatively the genotoxic effect of several chemical carcinogens suggests that the vitamin may play a role in the cytochrome *P*-450-related enzyme system. Indeed, several studies have shown that vitamin A deficiency can reduce hepatic cytochrome *P*-450 contents and related enzyme activities, and vitamin A supplementation has been shown to increase the activities of cytochrome *P*-450 isozymes.[137]

Antioxidant Protection

It has been suggested that actions of vitamin A in supporting the health of the skin and immune systems may involve effects on systems that provide protection against the adverse effects of prooxidants.[138] Yet, it is unlikely that vitamin A, itself, is physiologically significant in this regard, as retinol and retinal cannot quench singlet oxygen ($^1O_2$) and have only weak capacities to scavenge free radicals. It can, however, affect tissue levels of other antioxidants; animal studies have shown that deprivation of vitamin A leads to marked increases in the concentrations of α-tocopherol in the liver and plasma, whereas high intakes of retinyl esters can enhance the bioavailability of selenium, an essential constituent of several glutathione-dependent peroxidases.

Several carotenoids, on the other hand, have been shown to have direct antioxidant activities. These include β-carotene, lycopene, and some oxycarotenoids (zeaxanthin, lutein), which can quench $^1O_2$ or free radicals in the lipid membranes into which they partition (Table 6.29). These antioxidant activities are due to their extended systems of conjugated double bonds, which are thought to delocalize the unpaired electron of a free-radical reactant.[139] At low (physiologic) partial pressures of oxygen, carotenoids can also participate in the reduction of free radicals; xanthophyll carotenoids (lutein, lycopene, and **β-cryptoxanthin**) are more effective than β-carotene and more efficient than α-tocopherol in vitro. Despite these differences, the carotenoids tend to be less plentiful in tissues, for which reason

137. These include CYP3A in rats, rabbits, and guinea pigs, and CYP2A in hamsters.

138. Because aerobic systems rely on O_2 as the terminal electron acceptor for respiration, they must also protect themselves against the deleterious effects of highly reactive O_2 metabolites that can be formed either metabolically or through the action of such physical agents as UV light or ionizing radiation. These reactive oxygen species (ROS) include singlet oxygen ($^1O_2$), superoxide (O_2^-), hydroxyl radical (OH·), and nitric oxide (NO). ROS can react directly or indirectly with polyunsaturated membrane phospholipids (to form scission products), protein thiol groups (to form disulfide bridges), nonprotein thiols (to form disulfides), and DNA (to cause base changes) to alter cellular function. They can also react with polyunsaturated fatty acid components of circulating lipoprotein complexes; such oxidative changes in low-density lipoproteins (LDLs) appear to be important in the development of atherosclerotic lesions. The systems that protect against these oxidative reactions include several reductants (e.g., tocopherols and carotenoids in membranes and lipoprotein complexes; glutathione, ascorbic acid, urate, and bilirubin in the soluble phases of cells) and antioxidant enzymes (e.g., superoxide dismutases, glutathione peroxidases, catalase).

139. This mechanism differs from that of the tocopherols, which donate a hydrogen atom to the lipid-free radical to produce a semistable lipid peroxide; the tocopherols in turn become semiquinone radicals. (The antioxidant function of the tocopherols is discussed in Chapter 8.)

TABLE 6.29 Antioxidant Abilities of Carotenoid and Other Antioxidants

Compound	ROO·Reduction[a]	$^1O_2$ Quenching[a]
Lycopene	–	9×10^9
β-Carotene	1.5×10^9	5×10^9
α-Tocopherol	5×10^8	8×10^7
L-Ascorbate	2×10^8	1×10^7

[a]*Bimolecular rate constants* ($M^{-1}s^{-1}$).
After Sies, H., Stahl, W., 1995. Am. J. Clin. Nutr. 62 (Suppl.), 1315S–1321S.

their contributions to physiologic antioxidant protection are likely to be less important than those of the tocopherols except, perhaps, in cases of high carotenoid intake. Cooperative antioxidant interactions between α-tocopherol and β-carotene have been observed in model systems and it is likely that in vivo carotenoids may serve to protect tocopherols.

The interactions of carotenoids with radicals result in the production of oxidation products of the former and in the bleaching of these pigments;[140] the decomposed product cannot be regenerated metabolically, thus, destroying its provitamin A potential. In ultraviolet (UV)-irradiated skin, lycopene is more susceptible to bleaching than is β-carotene, suggesting that it may be more important in antioxidant protection of dermal tissues. Supplementation with β-carotene has been found to improve antioxidant status in vivo. These results include the following: reduced pentane[141] breath output in smokers; reduced plasma concentrations of malonyldialdehyde[142] in cystic fibrosis; reduced lipid peroxidation products (TBARS) in mice; reduced lethality to cultured cells of prooxidant drugs; and reduced acetaminophen toxicity[143] in mice. These and other nonprovitamin A properties of carotenoids are discussed in greater detail in Chapter 19.

Cardiovascular Health

Epidemiologic investigations have repeatedly found inverse relationships between the level of consumption of provitamin A-containing fruits and vegetables and risks of cardiovascular disease. Indeed, plasma retinol levels have been found to be related inversely to the risk of ischemic stroke, and low-plasma β-carotene concentrations are associated with increased risk of myocardial infarction.[144] Such findings have provided the bases for hypothetical actions of vitamin A or, more often, provitamin A carotenoids in chronic disease prevention. Unfortunately, many of these hypotheses have not withstood experimental challenge. Well-designed, randomized, double-blind, clinical intervention trials have found supplements of β-carotene[145] or a combination of β-carotene and/or α-tocopherol[146] to be ineffective in reducing risk of either cardiovascular disease or angina pectoris. In fact, one found an increase in the incidence of angina associated with β-carotene use.

Anticartumorigenesis

Because vitamin A deficiency characteristically results in a failure of differentiation of epithelial cells without impairing proliferation (i.e., the keratinizing of epithelia), it has been reasonable to question the possible role of vitamin A in the etiology of epithelial cell tumors, i.e., carcinomas. The squamous metaplastic changes seen in vitamin A deficiency are morphologically similar to precancerous lesions induced experimentally, and both show downregulation of CRBP(I) expression. Indeed, patients with oral leukoplakia, a precancerous condition of the buccal mucosa, have been found to have lower serum retinol levels than healthy controls, and treatment with retinol has been found to reduce the development of new lesions and to cause remissions in the lesions of some patients. It has been proposed that retinoic acid, which in high doses can inhibit the conversion of papillomas (benign lesions) to carcinomas, can upregulate RARs, which can, in turn, complex with protooncogenes such as *c*-fos to prevent malignant transformation.

Studies with animal tumor models have found vitamin A deficiency to enhance susceptibility to chemical carcinogenesis and large doses of vitamin A (i.e., supranutritional but not toxic) to inhibit carcinogenesis in some models

140. This is seen in the loss of pigmentation from the shanks of poultry, which poultry keepers used historically to indicate reproductive status of hens. Immature pullets deposit carotenoids in dermis; whereas, actively laying hens deposit the pigments in the lipids of the developing oocyte. The bleaching of the skin, therefore, is a positive sign of good laying condition.
141. *n*-Pentane (C_5H_{12}) is a scission product of the peroxidative degradation of ω-6 fatty acids.
142. Malonyldialdehyde is also a peroxidative scission product of polyunsaturated fatty acids. It can be detected by reaction with 3-thiobarbituric acid and is the predominant, but not only, reactant in biological specimens. Due to the lack of specificity, results of this analysis are typically expressed as thiobarbituric-reactive substances (TBARS).
143. The microsomal metabolism of acetaminophen, like that of other prooxidant drugs metabolized by cytochrome *P*-450-related enzymes, produces $O_2^{\cdot-}$. Antioxidant status has been shown to affect its acute toxicity in animal models.
144. The Physicians Health Study, a prospective study of 22,071 male American physicians (Hak, A.E., Stampfer, M.J., Campos, H., et al., 2003. Circulation 108, 802–807).
145. The Dartmouth Skin Cancer Study involved 1188 male and 532 female Americans treated with 50 mg/day β-carotene for more than 4 years. (Greenberg, E.R., Baron, J.A., Karagas, M.R., et al., 1996. JAMA 275, 699–703.).
146. The Alpha Tocopherol and Beta Carotene (ATBC) Cancer Prevention Trial involved 29,133 Finish male smokers treated with β-carotene and α-tocopherol for nearly 5 years. (Törnwall, M.E., Virtamo, J., Korhonen, P.A., et al., 2004. Eur. Heart J. 25, 1171–1178).

TABLE 6.30 Inhibition by β-Carotene of Chemical Carcinogenesis in Rats: Reduced Hepatic γ-Glutamyltranspeptidase-Positive Foci

Treatment[a]	Foci (Number/cm²)	Focal Area (% Total Area)
Control	37.1 ± 9.7	1.267 ± 1.121
Retinyl acetate (10 mg/kg/2 days)	34.8 ± 9.6	0.911 ± 0.901
β-Carotene (70 mg/kg/2 days)	20.1 ± 12.5[b]	0.308 ± 0.208

[a]*Rats were also treated with diethylnitrosamine/2-acetylaminofluorene.*
[b]$p < .05$.
After Moreno, F.S., Wu, T.S., Penteado, M.V.C., et al., 1995. Int. J. Vit. Nutr. Res. 65, 87–94.

(Table 6.30). Retinoids appear to suppress carcinogenesis and tumor growth by increasing expression of tumor suppressors such as the retinoid signaling protein TRIM16,[147] and inducing apoptosis (programmed cell death) and/or terminal differentiation. The proapoptotic effects appear to be mediated by RARs the target genes of which include players in the intrinsic (caspase cascade initiated by cell stress, DNA damage, or deprivation of growth factor) and the extrinsic (triggered by activation of death receptor-associated caspases) pathways of apoptosis.

Studies have demonstrated the efficacy of retinoic acid in inhibiting the growth of several types of cancer cells and tumors that do not express the RARβ gene even in the presence of physiological levels of vitamin A. The mechanism of silencing of RARβ gene expression is thought to involve hypermethylation of the gene due to loss of heterozygosity of chromosome 3p24, the locus of RARβ, and/or impaired expression of other factors involved in RARβ expression.[148] Loss of RAR function under vitamin A-adequate conditions is associated with different cancers, the best studied of which is acute promyelocytic leukemia (APL[149]). This cancer has been found to result from a nonrandom chromosomal translocation or deletion that leads to the production of a fusion of RARα gene on chromosome 17 to the promyelocytic (PML) gene on chromosome 15. When expressed, the fusion product represses translation and initiates leukemogenesis. This appears to occur through that action of the PML–RAR protein, which is a transcriptional activator of retinoic acid target genes. Studies with one specific target, the tumor suppressor gene $RAR\beta_2$, have revealed that its promoter contains a high-affinity RARE near the transcription start site, but is inactivated by methylation.[150] It appears that the PML–RAR fusion protein can form a complex with histone deacetylase, which, in turn, becomes oncogenic by recruiting DNA methyltransferases to the promoters of $RAR\beta_2$ locking them in a stably silenced chromatin state by hypermethylation. Most (80%) APL patients, however, respond to treatment with very high doses of all-*trans*-retinoic acid, resulting in complete remission in more than half of cases. This effect appears to involve retinoic acid causing the dissociation of the PML–RAR protein–histone deacetylase complex, which converts the fusion protein into a transcriptional activator resulting in leukemia cell differentiation. Studies with breast cancer cells[151] suggest that retinoic acid can also cause histone acetylation (due to the release of histone deactylase) of the $RAR\beta_2$ gene resulting in the inhibition of cell growth. Thus, in sensitive cells retinoic acid can reactivate $RAR\beta_2$ gene expression through epigenetic means; such reactivation has been shown to suppress malignancy in lung cancer cells.

While the use of retinoic acid, which is rapidly metabolized and eliminated from the body, avoids the problem of chronic hypervitaminosis, its substantial toxicity makes it unsuitable for regular clinical use. Therefore, more than 1500 retinoids have been synthesized and tested for potential anticarcinogenicity.[152] Several[153] have been found to effectively inhibit experimentally induced tumors in several organs of animals[154] and have yielded hopeful results in clinical trials. The consensus, however, is that although retinoids currently available can delay tumorigenesis, they cannot do so at doses that are not, themselves, hazardous.

Epidemiological investigations of vitamin A intake and human cancer have produced mostly negative results, depending on study design. A recent meta-analysis of 15 prospective studies found prostate cancer risk to be positively associated with plasma retinol level.[155] Findings of significant, inverse associations of intakes/plasma level and cancer risk have come mostly from retrospective

147. Tripartite motif protein 16.
148. Possibilities include the orphan receptors nurr77 and COUP-TF, both of which are overexpressed in retinoic acid-resistant cells.
149. APL is characterized by a blockade in myeloid differentiation, resulting in the accumulation in the bone marrow of abnormal promyelocytes and in a coagulopathy involving disseminated intravascular coagulation and fibrinolysis.
150. DiCroce, L, Raker V.A., Corsaro, M., et al., 2002. Science 295, 1079–1082.
151. Sirchia, S.M., Ren, M., Pili, et al., 2002. Cancer Res. 62, 2455–2461.
152. These compounds are formal derivatives of retinal with differing modifications of the isoprenoid side chain (including modification of the polar end and cyclization of the polyene structure), or the cyclic head group (including replacement with other ring systems).
153. For example, 13-*cis*-retinoic acid, *N*-ethylretinamide, *N*-(2-hydroxyethyl)-retinamide, *N*-(4-hydroxyphenyl)-retinamide, etretinate, *N*-(pivaloyloxyphenyl)-retinamide, *N*-(2,3-dihydroxypropyl)-retinamide.
154. Several studies have shown that retinoids can inhibit the initiation and promotion of mammary tumorigenesis induced in rodents by dimethylbenz(α)anthracene or *N*-methyl-*N*-nitrosourea, as well as the induction of **ornithine decarboxylase**, an enzyme the induction of which appears to be essential in the development of neoplasia.
155. Key, T.J., Appleby, P.N., Travis, R.C., et al., 2015. Am. J. Clin. Nutr. 102, 1142–1157.

TABLE 6.31 Results of a Meta-Analysis of Results of 57 Epidemiological Studies of β-Carotene Status and Human Cancer Risk

Design	Pooled Estimate of Risk
Cohort	1.013 (0.884, 1.16)[a]
Nested case–control	0.977 (0.864, 1.105)
Case–control	0.729 (0.640, 0.831)

[a]*Mean (95% confidence limits).*
After Musa-Velosa, K., Card, J.W., Wong, A.W., et al., 2009. Nutr. Rev. 67, 527–545.

TABLE 6.32 Increased Mortality Among Male Smokers Taking β-Carotene

	Mortality Rate (per 10,000 Person-Years)	
Causes of Death	No β-Carotene	β-Carotene
Lung cancer	30.8	35.6
Other cancers	32.0	33.1
Ischemic heart disease	68.9	77.1
Hemorrhagic stroke	6.0	7.0
Ischemic stroke	6.5	8.0
Other cardiovascular disease	14.8	14.8
Injuries and accidents	19.3	20.3
Other causes	23.5	22.5

After Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994. N. Engl. J. Med. 330, 1029–1035.

case–control studies (Table 6.31). More than 60% of these have indicated reductions in the prevalence of cancers of the lung, colon/rectum, skin, and prostate; whereas, such protective effects have been found in less than 20% of prospective cohort or nested case–control studies. Nevertheless, the results of such surveys have fostered the hypothesis that β-carotene may have some beneficial effect unrelated to its role as a precursor of vitamin A.

That β-carotene may have antitumorigenic effects has been suggested by findings such as that from a case–control study nested within the Nurses' Health Study that found plasma β-carotene level to be inversely related to breast cancer risk in American women.[156] The cancer-chemopreventive potential of supplemental β-carotene/retinoids has been tested in at least five well-designed, placebo-controlled, double-blind clinical intervention trials none of which found significant reduction in cancer in high-risk subjects. In fact, the results of two studies found β-carotene treatment harmful. The first was the Carotene and Retinol Efficacy Trial (CARET), a 12-year study involving more than 18,314 Americans, found no protection against lung or prostate cancer in men, but an increase in lung cancer in women (mostly among former smokers).[157] The second was the Alpha-Tocopherol and Beta-Carotene trial (ATBC),[158] conducted in Finland with more than 29,000 men with histories of smoking, tracked the health impacts of modest supplements of β-carotene (20 mg/day) and/or α-tocopherol (50 mg/day). Within 5–8 years of follow-up, results showed significantly greater total mortality (8%) and lung cancer incidence (18%) among men taking β-carotene in comparison with those not taking that supplement (Table 6.32). The interpretation of these findings is not straightforward, as ample evidence also indicates that β-carotene at these levels is generally safe. It is possible that β-carotene, at these levels of intake, can redox cycle to act as a prooxidant and co-carcinogen in the lungs of smokers. It is also possible that resulting retinoid metabolites may activate RAR to produce antiapoptotic effects (PPARβ/γ target genes include those that activate survival pathways) and/or increase cell proliferation.

APL has been found to respond to all-*trans*-retinol. This hematological malignancy is associated with chromosomal translocations affecting RARα that impair retinoid signaling. Treatment with all-*trans*-retinol produced complete remission in most APL patients.[159] A multicenter trial demonstrated that combined treatment with all-*trans*-retinol and arsenic trioxide produces complete remission in virtually all patients.[160]

11. VITAMIN A TOXICITY

Hypervitaminosis A

The hepatic storage capacity for vitamin A tends to mitigate the development of intoxication due to intakes in excess of physiological needs. However, persistent large overdoses (more than 1000 times the nutritionally required

156. Eliasson, A.H., Liao, X., Rosner, B., et al., 2015. Am. J. Clin. Nutr. 101, 1197–1205.
157. The Beta-Carotene and Retinol Efficacy Trial (CARET) involved 18,314 American male and female current and ex-smokers and asbestos-exposed men supplemented with both β-carotene and retinyl palmitate for 4 years of treatment. (Omenn, G.S., Goodman, G., Thornquist, M., et al., 1996. IARC Sci. Publ. 136, 67–85).
158. Albanes, D., Hainonen, O.P., Huttenen, J.K., et al., 1995. Am. J. Clin. Nutr. 62, 1427S–1430S.
159. Avvisati, G., Tallman, M.S., 2003. Best Pract. Res. Clin. Haematol. 16, 419–427.
160. Lo-Coco, F., Avvisati, G., Vignetti, M., et al., 2013. N. Eng. J. Med. 369, 111–121. This and other studies noted the low prevalences of side effects including fever, dyspnea, weight gain, and hypotension, which was referred to as "*retinoic acid syndrome*." It now appears that these signs depend on the presence of malignant promyelocytes; hence, it is now called "*differentiation syndrome*."

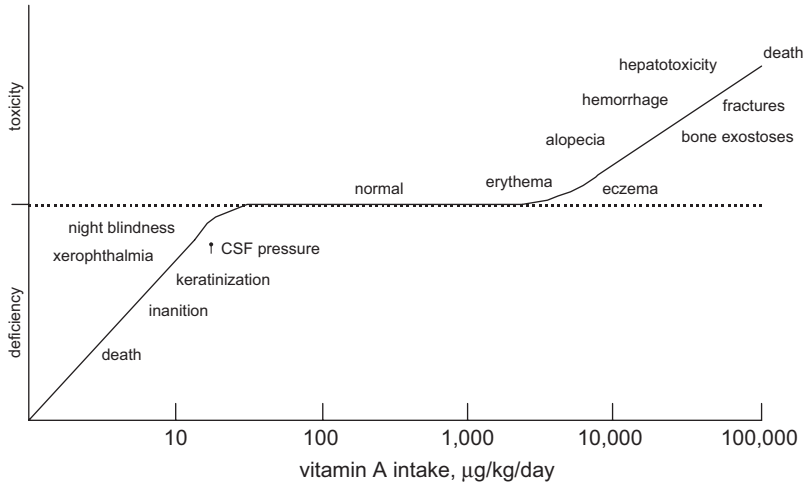


FIGURE 6.19 Both low and high habitual intakes of vitamin A can cause clinical signs.

amount) can exceed the capacity of the liver to store and catabolize and will, thus, produce intoxication[161] (Figs. 6.17 and 6.19). This is marked by the appearance in the plasma of high levels of retinyl esters that, because they are associated with lipoproteins rather than RBP4, are outside the normal strict control of vitamin A transport to extrahepatic tissues.

Five aspects of vitamin A metabolism tend to protect against hypervitaminosis:

1. relatively inefficient conversion of the provitamins A in the gut
2. the unidirectional oxidation of the vitamin to a form (retinoic acid) that is rapidly catabolized and excreted
3. excess capacity of CRBP(II) to bind retinol
4. hepatic storage of vitamin A
5. accelerated catabolism of retinoic acid.

Hypervitaminosis A, therefore, requires high exposures; signs (Table 6.29) are usually reversed on cessation of exposure to the vitamin. Signs of hypervitaminosis A are associated with plasma retinol levels >3 µmol/L and increases in serum retinyl ester levels without substantial increases in circulating holo-RBP4.

Who may be at risk of hypervitaminosis A?

- Children and adults inappropriately using of dietary supplements containing the preformed vitamin, particularly those consuming multiple sources of vitamins.
- Livestock fed incorrect diets in which mixing errors concerning vitamin A have been made.

TABLE 6.33 Signs of Hypervitaminosis A

Organ Affected	Signs
General	Muscle and joint pains, headache
Skin	Erythema, desquamation, alopecia
Mucous membranes	Cheilitis, stomatitis, conjunctivitis
Liver	Dysfunction
Skeletal	Thinning and fracture of long bones

Signs of Toxicity

Acute hypervitaminosis. In humans, signs can be manifest after single large doses (>660,000 IU for adults, >330,000 IU for children), or after doses >100,000 IU/day have been taken for several months (Table 6.33). Children experiencing hypervitaminosis A develop transient (1–2 days) signs: nausea, vomiting, symptoms associated with increased cerebrospinal fluid pressure (headache, vertigo, blurred or double vision), and muscular incoordination. Most field studies have found that 3–9% of children given high single doses (c.200,000 IU) for prophylaxis show transient nausea, vomiting, headache, and general irritability; a similar percentage of younger children may show bulging fontanelles[162] subsiding within 48–96 h.

Those reacting to extremely large doses of the vitamin show drowsiness, loss of appetite, and malaise followed by skin exfoliation, itching (circumocular), and recurrent vomiting. Several studies have found that about 30% of acne patients treated with 13-*cis*-retinoic acid show increased

161. It has been suggested that the hepatic damage following hepatitis B infection may be due to the toxic effects of retinoids, which accumulate in the cholestatic liver.

162. The convex displacement of the infant's "*soft spot*" (the membranous covering of the cranial sutures) caused by fluid accumulation in the skull cavity or increased intracranial pressure.

LDL/HDL ratios which, if persistent, would suggest increased risk of ischemic heart disease. Some 15% of such patients report arthralgia and myalgia.

Chronic hypervitaminosis. Recurrent exposures exceeding 12,500 IU (infants) to 33,000 IU (adult) typically produce many changes; the earliest are likely to involve the skin and mucous membranes (Table 6.31). Dry lips (cheilitis) are a common early sign in humans, often followed by dryness and fragility of the nasal mucosa, leading to dry eyes and conjunctivitis. Skin lesions include dryness, pruritis, erythema, scaling, peeling of the palms and soles, hair loss (alopecia), and nail fragility. Headache, nausea, and vomiting (signs of increased intracranial pressure) have also been reported.

Hypervitaminosis A can also reduce bone mineral density and increase fracture risk,[163] perhaps due to direct membranolytic activities of retinoids, or to their activation of abnormal gene expression. Infants and young children can show painful periostitis[164] and, rarely, premature closure of lower limb epiphyses (manifest as "*hyena disease*" in calves, characterized by shortened hind limbs). Chronic, high intakes of vitamin A may contribute to the pathogenesis of osteoporosis in adults. One study found reduced bone mineral density in association with intakes greater than 0.6 mg RE (2000 IU)/day,[165] a level consumed by at least half of American adults. An analysis of results from more than 72,000 postmenopausal women in the Nurses' Health Study found that individuals reporting intakes of at least 2000 IU/d had twice the risk of hip fracture due to mild or moderate trauma than those reporting intakes less than 500 IU/d.[166] Hypervitaminotic A animals frequently show bone abnormalities that apparently result from changes in impaired osteoclastic activities and enhanced osteoblastic activities resulting in overgrowth of periosteal bone in a nonvitamin D-dependent manner. This, in turn, can lead to impairments in visual function by restricting the optic foramina and pinching the optic nerve, and in motor function by increasing intracranial pressure. Intracranial hypertension is a well-known side effect of therapeutic doses of 13-*cis*-retinoic acid (for acne) and all-*trans*-retinoic acid (acute promyelitic leukemia). That this condition resolves more rapidly if patients undergo weight loss suggests that RBP4 of adipose origin may be involved in triggering these signs.

The accumulation of retinal condensation products is a significant initiating factor in retinal photodamage characterized by progressive retinal cell death. Impaired clearing of all-*trans*-retinal by photoreceptor cells can result in the accumulation of fluorescent bisretinoids by retinal pigment epithelial cells through phagocytosis of photoreceptor outer segments. This process may result in retinal dystrophy and degenerative diseases including Stargardt disease and age-related macular degeneration.

Therapeutic doses of all-*trans*-retinoic acid are generally well tolerated, although some patients experience headache, nausea, and visual changes. In rare cases, muscular stiffness and epileptiform seizures have been reported. A few cases of myocarditis have been reported patients with acute promyelitic leukemia given this vitamer.

Embryotoxic Potential of High Levels of Vitamin A

Retinoids can be toxic to maternally exposed embryos, which limits their therapeutic uses and raises concerns about the safety of high-level vitamin A supplementation for pregnant animals and humans. This is especially true for 13-*cis*-retinoic acid, which is very effective in the treatment of acne but can cause severe disruption of cephalic neural crest cell activity that results in birth defects characterized by craniofacial, central nervous system, cardiovascular, and thymus malformations. Similar effects have been induced in animals by high doses of retinol, all-*trans*-retinoic acid, or 13-*cis*-retinoic acid.

Teratogenicity. Animal model studies suggest that the teratogenic effects of excess vitamin A are due to the embryonic exposure to all-*trans*-retinoic acid (Table 6.34), although such effects can be induced without substantially increasing maternal plasma concentrations of that metabolite. It has been proposed that the mechanism of teratogenic action of retinoids involves elevated production of mRNAs for particular RARs that lead to an imbalance in heterodimers among the various RARs, RXRs, and other hormone receptors, consequently affecting the expression of genes not typically expressed in normal metabolism. Indeed, teratogenic doses of all-*trans*-retinoic acid have been found to produce prolonged increases in RARα_2 mRNA levels.

The critical period for fetal exposure to maternally derived retinoids is during organogenesis, i.e., before many women suspect they are pregnant. This is also before the fetus develops retinoid receptors and binding proteins, which serve to restrict maternal–fetal transfer of retinoids.

Fetal malformations of cranial–neural crest origin have been reported in cases of oral use of all-*trans*-retinoic acid

163. Ribaya-Mercado, J.D., Blumberg, J.B., 2007. Nutr. Rev. 65, 425–428. That these types of bone lesions have been identified in the fossilized skeletal remains of ancient humans suggests that excessive vitamin A is not a new phenomenon.

164. Inflammation of the periosteum, the membranous tissue surrounding bone.

165. The Rancho Bernardo Study of 570 women and 388 men found a U-shaped relationship of bone mineral density and vitamin A intake, with optimal bone mineral density occurring at 2000–2800 IU (0.6–0.9 mg RE) per day. (Promislow, J.H.E., Goodman-Gruen, D., Slymen, D.J., et al., 2002. J. Nutr. 129, 2246–2250).

166. Feskanich, D., Singh, V., Willet, W.C., et al., 2002. JAMA 287, 47–54.

TABLE 6.34 Teratogenicity of Vitamin A in Rodent Models

	Retinyl Palmitate[a]	All-*trans*-Retinoic Acid[a]	
Species	Highest Nonteratogenic	Lowest Teratogenic	Teratogenic
Rat[b]	30	90	6
Mouse[b]	15	50	3
Rabbit[c]	2	5	6
Hamster	–	–	7

[a]*Dosage level (mg/kg/day).*
[b]*Exposed on gestational days 6–15.*
[c]*Exposed on gestational days 6–18.*
After Kamm, J.J., 1982. J. Am. Acad. Dermatol. 64, 552–559.

in treating acne vulgaris and of regular prenatal vitamin A supplements in humans. The latter have generally been linked to daily exposures at or above 20,000–25,000 IU. A retrospective epidemiologic study imputed increased risk of birth defects associated with exposures of about ≥10,000 IU of preformed vitamin A per day;[167] however, the actual observed increase was in a small group of women whose vitamin A intakes exceeded 21,000 IU/day (Table 6.35).

Provitamin A Toxicity. In general, carotenoids have low toxicities. A small intervention study showed that a daily intake of 30 mg of β-carotene from carotene-rich foods produced accumulation of the carotenoid in the skin with consequent yellowing (**carotenodermia**) within 25–42 days of exposure;[168] the effect persisted for at least 14–42 days after cessation of carotene exposure. It is possible that, under highly oxidative conditions, asymmetric cleavage of β-carotene to yield apocarotenals and apocarotenoic acids that can diminish retinoic acid signaling by interfering with the binding of retinoic acid to RAR. This effect has been proposed as the basis for the finding that a regular daily dose of β-carotene increased lung cancer risk among smokers.

Recommended Upper Limits of Exposure

Both the US and the European Union have estimated upper tolerable limits of intakes of preformed vitamin A (Table 6.36).

167. Rothman and colleagues. (Rothman, K.J., Moore, L.L., Singer, M.R., et al., 1995. N. Engl. J. Med. 33, 1369–1373) estimated that threshold using regression techniques. That level of exposure to preformed vitamin A was associated with a birth defect risk of 1 in 57. This report has been criticized for suspected misclassification of malformations. Note: the current RDA for pregnant women of 800 µg of RE (2700 IU) per day.
168. Carotenodermia was diagnosed only after plasma total carotenoid concentrations exceeded 4.0 mg/L.

12. CASE STUDIES

Instructions

Review each of the following case reports, paying special attention to the diagnostic indicators on which the respective treatments were based. Then answer the questions that follow.

Case 1

The physical examination of a 5-month-old boy with severe marasmus[169] showed extreme wasting, apathy, and ocular changes: in the left eye, Bitot's spots, and conjunctival and corneal xerosis; in the right eye, corneal liquefaction and keratomalacia with subsequent prolapse of the iris, extrusion of lens, and loss of vitreous humor. The child was 65 cm tall and weighed 4.5 kg. His malnutrition had begun at cessation of breast feeding at 4 months, after which he experienced weight loss and diarrhea.

Laboratory Results

Parameter	Patient	Normal Range
Hb (hemoglobin)	10.7 g/dL	12–16 g/dL
HCT (hematocrit)	36 ml/dL	35–47 mg/dL
WBC (white blood cells)	15,000/µL	5000–9000/µL
Serum protein	5.6 g/dL	6–8 g/dL
Serum albumin	2.49 g/dL	3.5–5.5 g/dL
Plasma sodium	139 mEq/L	136–145 mEq/L
Plasma potassium	3.5 mEq/L	3.5–5.0 mEq/L
Blood glucose	70 mg/dL	60–100 mg/dL
Total bilirubin	1.1 mg/dL	<1 mg/dL
Serum retinol	5.5 µg/dL	30–60 µg/dL
Serum β-carotene	10.7 µg/dL	50–250 µg/dL
Serum vitamin E	220 µg/dL	500–1500 µg/dL

The child had an infection, showing **otitis media**[170] and *Salmonella* septicemia,[171] which responded to antibiotic treatment in the first week. The patient was given by nasogastric tube an aqueous dispersion of retinyl palmitate (with a nonionic detergent) at the rate of 3000 µg/kg per day for 4 days. This increased his plasma retinol concentration from 5 to 35 µg/dL by the second day, at which level it was

169. Marasmus is characterized by extreme emaciation or general atrophy. It occurs mostly in young children and is caused by extreme undernutrition, primarily lack of energy and protein.
170. Inflammation of the middle ear.
171. Presence in the blood of pathogenic, gram-negative, rod-shaped bacteria of the genus *Salmonella*.

TABLE 6.35 Teratogenic Risk of High Prenatal Exposures to Preformed Vitamin A

Retinol Intake			
(IU/day)	Pregnancies	Cranial–Neural Crest Defects	Total Defects
0–5000	6410	33 (0.51%)	86 (1.3%)
5001–10,000	12,688	59 (0.47%)	196 (1.5%)
10,001–15,000	3150	20 (0.63%)	42 (1.3%)
>15,000	500	9 (1.80%)	15 (3.0%)

After Rothman, K.J., Moore, L.L., Singer, M.R., et al., 1995. N. Engl. J. Med. 333, 1369–1373.

maintained for the next 12 days. The child responded to general nutritional rehabilitation with a high-protein, high-energy formula that was followed by whole milk supplemented with solid foods. He recovered but was permanently blind in the right eye and was left with a mild corneal opacity in the left eye. He returned to his family after 10 weeks of hospitalization.

Case 2

An obese 15-year-old girl, 152 cm tall and weighing 100 kg, was admitted to the hospital for partial jejunoileal bypass surgery for morbid obesity. She had a past history of obsessive eating that had not been correctable by diet. Except for massive obesity, her physical examination was negative.

Initial Laboratory Results

Parameter	Patient	Normal Range
Hb (hemoglobin)	10.5 g/dL	12–15 g/dL
RBC (red blood cell)	4.5×10^6/μL	$4–5 \times 10^6$/μL
WBC (white blood cell)	8000/μL	5000–9000/μL
Serum retinol	38 μg/dL	30–60 μg/dL
Serum β-carotene	12 μg/dL	50–300 μg/dL
Serum vitamin E	580 μg/dL	500–1500 μg/dL
Serum 25-OH-D_3	11 ng/dL	8–40 ng/dL

The following test results were within normal ranges: serum electrolytes, calcium, phosphorus, triglycerides, cholesterol, total protein, albumin, total bilirubin, copper, zinc, folic acid, thiamin, and vitamin B_{12}. The patient encountered few postoperative complications except for mild bouts of diarrhea and some fatigue. Over the next year, she lost 45 kg of body weight while ingesting a liberal diet. She reported having three to four stools daily but denied having any objectionable diarrhea or changes in stool appearance. Two years after surgery, she noted the onset of inflammatory horny lesions above her knees and elbows, and she experienced some difficulty in seeing at dusk. The skin lesions failed to respond to topical corticosteroids and oral antihistamine therapy. Because of intensification of these signs, she sought medical help; however, the cause was not determined.

She was readmitted to the hospital, complaining of her skin disorder and night blindness. At that time, she showed evidence of mild liver dysfunction and her serum concentrations of retinol and β-carotene were 16 and 14 μg/dL, respectively. Her fecal fat was 70 g/day (normal, <7 g/day). Biopsies of the skin of her left thigh and right upper arm each showed

TABLE 6.36 Recommended Upper Tolerable Intakes (ULs) of Preformed Vitamin A

US[a]		EU[b]	
Ages (years)	UL (μg RE/day)	Ages (years)	UL (μg RE/day)
0.1	600	1–3	800
1–3	600		
4–8	900	4–6	1100
9–13	1700	7–10	1500
14–18	2800	11–14	2000
		15–17	2600
Adults[c]	3000	Adults	3000

[a]*Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC, 773 pp.*
[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*
[c]*Including pregnant and lactating women.*

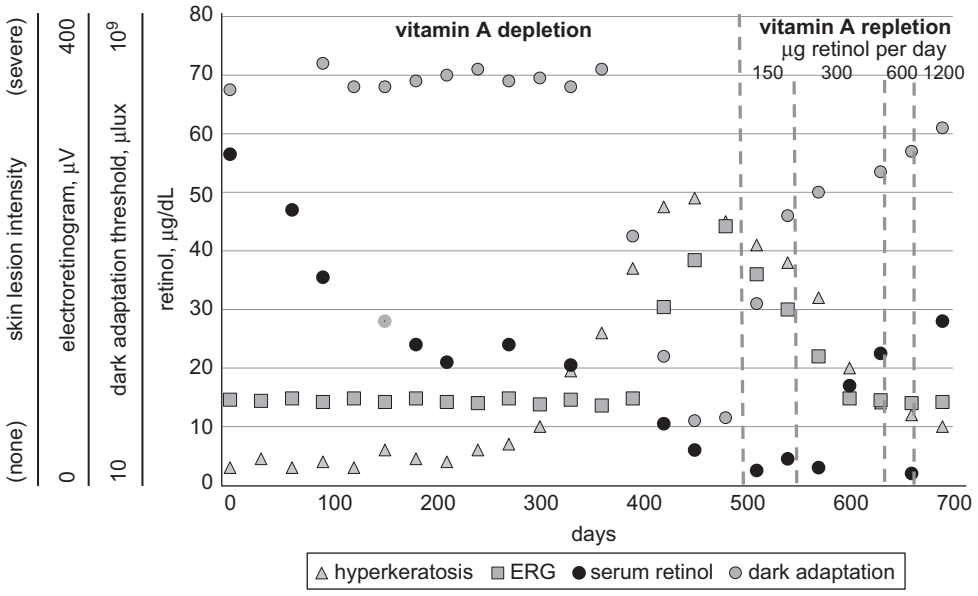


FIGURE 6.20 The volunteer's responses to chronic vitamin A deprivation followed by stepwise repletion with oral supplements of retinol. *After Russel. R.M., Multrak, R., Smityh, V., et al., 1973. Lancet 2, 161–1164.*

hyperkeratosis and horny plugging of dilated follicles. She was treated with 15,000 μg of retinyl palmitate given orally three times daily for 6 months. By 1 month, the follicular hyperkeratosis had cleared and healed with residual pigmentation. By 2 months, the night blindness had subsided. At that time, her serum retinol concentration was 54 μg/dL, β-carotene was 7 μg/dL, α-tocopherol was 1.6 μg/ml, and urinary [^{57}Co]B_{12} was 6.7% (normal, 7–8%). She has been well on a daily oral supplement of 1500 μg of retinyl palmitate.

Case 3

A 41-year-old man was housed in a metabolic ward for 2 years during a clinical investigation of vitamin A deficiency. He weighed 77.3 kg and was healthy by standard criteria (history, physical examination, and laboratory studies). For 505 days, he was fed a casein-based formula diet that contained <10 μg of vitamin A per day. His initial plasma retinol concentration was 58 μg/dL, and his body vitamin A pool, determined by isotope dilution, was 766 mg (10 mg/kg). At the end of 1 year, his plasma retinol had declined to 25 μg/dL and he began to show follicular hyperkeratosis (Fig. 6.20). On day 300, his plasma retinol was 20 μg/dL, and he showed a mild anemia (Hb 12.6 mg/dL). Two months later, by which time his plasma retinol had dropped to 10 μg/dL, he developed night blindness as evidenced by changes in dark adaptation and electroretinogram. When his plasma retinol reached 3 μg/dL, his body vitamin A pool was 377 mg and repletion with vitamin A was begun with increasing doses starting with 150 μg and increasing to 1200 μg of retinol per day over a 145-day period. After receiving 150 μg of retinol per day for 82 days, his night blindness was partially repaired, but his skin keratinization remained and his plasma retinol level was only 8 μg/dL. Then, after receiving 300 μg of retinol per day for 42 days, his follicular hyperkeratosis resolved and his plasma retinol level was 20 μg/dL. At the 600 μg of retinol per day level, his plasma retinol was in the normal range and all signs of vitamin A deficiency disappeared.

Case Questions

1. For each case, what signs/symptoms indicated vitamin A deficiency?
2. Propose hypotheses to explain why the patients of cases 1 and 2 each responded to oral vitamin A treatment even though they had very different medical conditions. Outline tests of those hypotheses.
3. Comment on the value of serum retinol concentration for the diagnosis of nutritional vitamin A status.

13. STUDY QUESTIONS AND EXERCISES

1. Discuss how the absorption, transport, tissue distribution, and intracellular activities of vitamin A relate to the concept of solubility.
2. Construct a flow diagram showing vitamin A, in its various forms, as it passes from ingested food, through the body where it functions in its various physiologic roles, and ultimately to its routes of elimination.
3. Construct a decision tree for the diagnosis of vitamin A deficiency in a human or animal.

4. Night blindness is particularly prevalent among alcoholics. Propose a hypothesis for the metabolic basis of this phenomenon and outline an experimental approach to test it.
5. Construct a figure detailing the mechanism by which vitamin A functions as a transcription factor.
6. Note three physiological mechanisms which may protect against hypervitaminosis A.
7. Discuss the points of control, and intervention possibilities for each, in the WHO conceptual framework for vitamin A deficiency.

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Chapter 7

Vitamin D

Chapter Outline

Anchoring Concepts

1. Vitamin D is the generic descriptor for **steroids** exhibiting qualitatively the biological activity of cholecalciferol (i.e., vitamin D_3).
2. Most vitamers D are hydrophobic and, thus, are insoluble in aqueous environments (e.g., plasma, interstitial fluids, cytosol).
3. Vitamin D is not required in the diets of animals or humans adequately exposed to sources of **ultraviolet light** (e.g., sunlight).
4. Deficiencies of vitamin D lead to structural lesions of *bone*.

By following the reasoning that vitamin D is not required in the diet under conditions of adequate ultraviolet irradiation of skin and that it is the precursor of a hormone, it is likely that the vitamin is not truly a vitamin but must be regarded as a pro-hormone. These arguments, however, are only semantic; the fact remains that vitamin D is taken in the diet and is an extremely potent substance which prevents a deficiency disease.

Hector DeLuca.[1]

1. Hector DeLuca is a prominent nutritional biochemist whose discoveries were seminal in elucidating the metabolic functions of vitamin D. These included identification of the active forms of the vitamin, 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3, and the characterization of the enzymes involved in those pathways. He is a professor Emeritus at the University of Wisconsin, where he has had a long and productive career.

LEARNING OBJECTIVES

1. To understand the nature of the various sources of vitamin D.
2. To understand the means of endogenous production of vitamin D.
3. To understand the means of enteric absorption of vitamin D.
4. To understand the transport and metabolism involved in the activation of vitamin D to its functional forms.
5. To understand the role of vitamin D and other endocrine factors in calcium homeostasis.
6. To understand the roles of vitamin D in noncalcified tissues.
7. To understand the genomic bases of vitamin D action.
8. To understand the physiologic implications of high doses of vitamin D.

VOCABULARY

Apolipoprotein E (apoE)
Cage layer fatigue
Calbindins
Calcidiol
Calcinosis
Calcipotriol
Calcitonin
Calcitriol
Calcitroic acid
Calcium (Ca)

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00007-1

Calcium-binding protein (CaBP)
Calcium-sensing receptor (CaR)
Calmodulin
Cathelicidin
Caveolae
Cholecalciferol
7-Dehydrocholesterol (7-DHC)
1,25-Dihydroxyvitamin D (1,25-$[OH]_2$-vitamin D)
24,25-Dihydroxyvitamin D (24,25-$[OH]_2$-vitamin D)
Diabetes
Diuresis
Epiphyseal plate
Ergocalciferol
Ergosterol
Genu varum
25-Hydroxyvitamin D (25-OH-vitamin D)
Hypercalcemia
Hyperphosphatemia
Hypersensitivity
Hypocalcemia
Hypoparathyroidism
Hypophosphatemia
Lead (Pb)
Lumisterol
Median erythemal dose (MED)
Melanin
Milk fever
25-OH-Vitamin D 1-hydroxylase
Osteoblast
Osteochondrosis
Osteoclast
Osteomalacia
Osteon
Osteopenia
Osteoporosis
Parathyroid gland
Parathyroid hormone (PTH)
Previtamin D
Privational rickets
Privational osteomalacia
Prolactin
Provitamin D
Pseudofracture
Pseudohypoparathyroidism
Psoriasis
Rickets
Sarcopenia
Steroid
Tachysterol
Tibial dyschondroplasia
Transcaltachia
Transcalciferin
24,25,26-Trihydroxyvitamin D (24,25,26-$[OH]_3$-vitamin D)
Varus deformity
Vitamin D-binding protein (DBP)
Vitamin D-dependent rickets types I and II
Vitamin D receptors (VDRs)
Vitamin D resistance
Vitamin D-responsive elements (VDREs)
Vitamin D_2
Vitamin D_3
Vitamin D 25-hydroxylase
Vitamin D-dependent rickets
Vitamin D-resistant rickets
Zinc fingers

1. SIGNIFICANCE OF VITAMIN D

Vitamin D,[2] the "sunshine vitamin," is actually a hormone produced from sterols in the body by the photolytic action of ultraviolet light on the skin; individuals who receive modest exposures to sunlight are able to produce their own vitamin D. However, this is not the case for most people: those living in northern latitudes; those spending most of their days indoors and/or having darker skin; animals being managed in controlled environments. Such individuals must obtain the nutrient from their diets; for them vitamin D is a vitamin in the traditional sense.

Vitamin D plays an important role, along with the essential minerals calcium (Ca), phosphorus (P), and magnesium (Mg), in the maintenance of healthy bones and teeth. Problems in those organs appear to have existed in different past populations throughout the world.[3] Today, nearly 40% of the world's adult population is of insufficient vitamin D status.[4] More than three-quarters of adults in Bangladesh, Scotland, Norway, and Estonia, and at least half of adults in several other countries (Denmark, Korea, New Zealand, Brazil), have been found to be of low-vitamin D status.[5] Huge numbers of infants in many countries are also known to be of low-vitamin D status, including most infants in Iran, Pakistan, India, and Turkey; but for many parts of the world, particularly, Africa and South America, little information is available. Vitamin D deficiency remains a global public health problem in all age groups, the most visible impacts being the increased risk of the metabolic bone diseases, rickets and osteoporosis.

2. The convention used in this chapter is that the terms "vitamin D" or "D" (without subscripts) are used in contexts in which the major vitamers (D_3, or **cholecalciferol**; D_2, or **ergocalciferol**) are equivalent.
3. Evidence of low bone mass has been found in many ancient populations. That such cases have not always been associated with osteoporotic fractures suggests that Ca intakes of earlier peoples may have been greater than those today (Nelson, D.A., Sauer, N.J., Agarwal, S.C., 2004. Evolutionary Aspects of Bone Health. In: Holick, M.F., Dawson-Hughes, B. (Eds.), Nutrition and Bone Health. Humana Press, Totowa, NJ, pp. 3–18).
4. Hilger, J., Friedel, A., Herr, R., et al., 2014. Br. J. Nutr. 111, 23–45; Palacios, C., Gonzalez, L., 2014. J. Steroid Biochem. Mol. Biol. 144, 138–145.
5. Have plasma 25-hydroxycholecalciferol concentrations <50 nM.

Rickets, the deforming and debilitating disease involving delayed or failed endochondral ossification (mineralization at the growth plates) of the long bones, remains a problem in many countries, having been reported at prevalences as great as 10% among infants exclusively fed breast milk and children with little sun exposure. Shockingly high prevalences have been reported in Yemen (27%), Ethiopia (42%), Tibet (66%), and Mongolia (70%).[6] This disability can significantly impair ambulation and work productivity; in some societies, it can be socially stigmatizing.

The global prevalence of **osteoporosis** (loss of bone leading to increased bone fragility) in adults is not well documented. The disease is estimated to affect 10 million Americans ≥50 years of age; the number with low bone mass (**osteopenia**) is estimated to exceed 61 million by 2020. Fracture incidence varies globally from about 50/100,000 for men and women in central Africa, to nearly 400 (men) and >900 (women) per 100,000 in northern Europe.[7] Two million osteoporosis-related fractures[8] occurred in the United States in 2005; by 2025, that number is expected to reach 3 million with an associated medical cost of $16–25 billion. Each year 250,000 Americans (mostly women) are hospitalized for hip fractures; three-fourths do not have full recoveries, half will be dependent on a cane or walker, 40% will require assisted care, and 20% will die of complications within a year. Women are increasingly susceptible to osteoporosis after the onset of menopause when their rate of bone loss can increase as much as 10-fold.[9] It is estimated that, among Americans over 50 years, women experience 75–80% of all hip fractures.

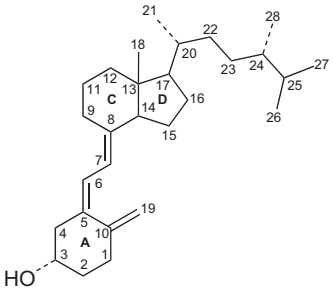
Vitamin D status affects more than bone. It also functions in the regulation of cellular development and differentiation of most cells, in the regulation of the parathyroid gland and the immune system function, in the skin, in cancer prevention, and in the metabolism of foreign compounds.

2. PROPERTIES OF VITAMIN D

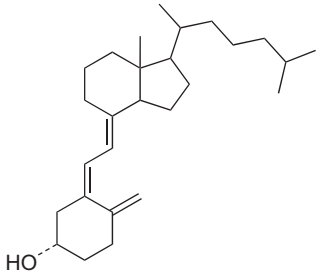
Vitamin D is the generic descriptor for all steroids qualitatively exhibiting the biological activity of **cholecalciferol**. These compounds contain the intact A, C, and D steroid rings, ultimately derived in vivo by photolysis of the B ring of 7-dehydrocholesterol (7-DHC). That process frees the A ring from the rigid structure of the C and D rings, yielding conformational mobility in which the A ring undergoes rapid interconversion between two chair configurations. Vitamin D-active compounds have either of two types of isoprenoid side chains attached to the steroid nucleus at C-17 of the D ring:

- A nine-carbon, monounsaturated (i.e., containing one double bond) side chain. Vitamin D-active compounds with this structure are derivatives of **ergocalciferol**, is also called **vitamin D_2**. This vitamer can be produced synthetically by the photolysis of plant sterols.
- An eight-carbon, saturated (i.e., containing no double bond) side-chain. Vitamin D-active compounds with this structure are derivatives of **cholecalciferol**, also called **vitamin D_3**, which is produced metabolically through a natural process of photolysis of 7-DHC on the surface of skin exposed to ultraviolet irradiation, e.g., sunlight. The metabolically active vitamers are side chain-substituted, open-ring **steroids** with a *cis*-triene structure with hydroxylated carbons at ring position 1 and side chain position 25.

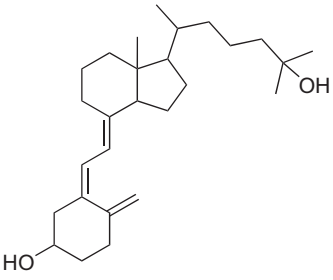
Chemical structures of the vitamin D group:



Vitamin D_3 (cholecalciferol)



Vitamin D_2 (ergocalciferol)



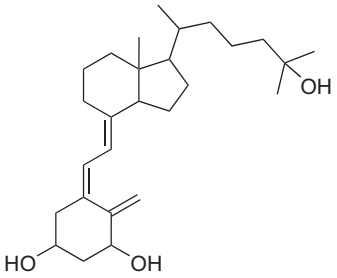
25-OH-Vitamin D_3

6. Prentice, A., 2008. Nutr. Rev. 66, S153–S164.

7. Dhanwal, D.K., Dennison, E.M., Harvey, N.C., et al., 2011. Indian J. Orthop. 45, 15–22.

8. Fractures of the vertebrae, hip, forearm, leg, and ankle, in that order, are the most common, although often asymptomatic. Canadian health statistics show the risk of radial fractures for men and women to be about 25/100,000 until the fifth decade of life, when it increases in women to more than 200/100,000 by the seventh and eighth decades.

9. Women can lose as much as one-fifth of their total bone mass within the first 5–7 years of menopause. By their eighth and ninth decades, many have lost 30–50% of their bone mass. Aging men also lose bone, but at a lower rate and starting from a generally larger bone mass. By their eighth and ninth decades, men typically have lost 20–30% of their peak bone mass.



1,25-(OH)$_2$-Vitamin D$_3$

Chemical Properties of Vitamin D

Unlike the ring-intact steroids, vitamin D-active compounds tend to exist in extended conformations due to the 180° rotation of the A ring about the 6,7 single bond (in solution, the stretched and closed conformations are thought to come to an equilibrium favoring the former). The hydroxyl group on C-3 is, thus, in the β-position (i.e., above the plane of the A ring) in the closed forms, and in the α-position (i.e., below the plane of the A ring) in the stretched forms. Rotation about the 5,6 double bond can also occur by the action of light or iodine to interconvert the biologically active 5,6-*cis* compounds to 5,6-*trans* compounds, which show little or no vitamin D activity.

Vitamers D_2 and D_3 are white-yellow powders that are insoluble in water, moderately soluble in fats, oils, and ethanol; and freely soluble in acetone, ether, and petroleum ether. Each shows strong ultraviolet (UV) absorption, with a maximum at 264 nm. Each is sensitive to O_2, light, and iodine. Heat or mild acidity can convert each to the respective 5,6-*trans* and other inactive forms. While the vitamin is stable in dry form, in organic solvents, and in most plant oils (owing to the presence of α-tocopherol, which serves as a protective antioxidant), its thermal and photo lability can result in losses during such preparatory procedures as saponification with refluxing. Therefore, it is often necessary to use inert gas environments, light-tight sealed containers, and protective antioxidants when isolating the vitamin. In solution, both D_2 and D_3 undergo reversible, temperature-dependent isomerization to previtamin D, each forming an equilibrium mixture of both vitamers.[10]

3. SOURCES OF VITAMIN D

Biosynthesis of Vitamin D_3

Cholecalciferol (D_3) can be biosynthesized by a two-step process involving ultraviolet light (UVB, 290–310 nm) acting on **7-DHC** in the skin (Fig. 7.1):

1. **Photoactivation**—The activation reaction involves light-induced electrolytic ring opening of 7-dehydrocholesterol due to absorption of light by the 5,7-diene of the B ring of the sterol nucleus[11] to produce s-*trans*, s-*cis*-previtamin D_3. This process is optimal with light in the UVB range (295–300 nm); however, shorter wavelength irradiation can also cause this photoisomerization.[12]
2. **Thermal isomerization**—Once formed, previtamin D_3 can photochemically convert to **lumisterol** or **tachysterol**, but its lowest energy prospect is to undergo a thermal rearrangement involving a C19 to C9 hydrogen shift to form **cholecalciferol** (vitamin D_3). The reversible nature of this rearrangement means that previtamin D_3 and vitamin D_3 are always in dynamic equilibrium; under physiological conditions, the equilibrium favors vitamin D_3.

This process in vivo appears to convert only 5–15% of the available 7-DHC to vitamin D_3.[13] Yield is affected by the physical properties of the skin and of the environment; thus, it differs between individuals and species and shows great variation according to time of day, season, and latitude. It has been estimated that adults can biosynthesize as much as 15 μg (0.6 IU) D_3 per day, i.e., 10–25% of their total vitamin D intakes at the summer peak.[14]

The provitamin D sterol, 7-DHC, is both a precursor to and product of cholesterol (via different pathways). It is synthesized in the sebaceous glands of the skin and secreted onto the surface, where it is reabsorbed into the various layers of the epidermis and dermis to be localized mostly in their membranes.[15] The skin contains high concentrations of the sterol (200 times that of liver); in humans the epidermis and dermis in humans have similar 7-DHC contents, although most previtamin D_3 is found in the epidermis. In humans, epidermal 7-DHC concentrations are greatest

10. For example, within 30 days a 93% D, 7% pre-D mixture is established at 20°C; at 100°C a 72% D, 28% pre-D is established in only 30 min.

11. For this reason, the vitamers D are called **secosteroids**, denoting the "broken" ring by the Latin prefix *seco* (to cut).

12. UVC light (200–280 nm) was shown to be effective in curing rickets in the rat (Knudson, A., Benford, F., 1938. J. Biol. Chem. 124, 287–299). It does not penetrate the atmosphere but is used for its germicidal properties.

13. Excess irradiation does little to increase the efficacy of this conversion. Instead, it increases the production of biologically inactive forms (e.g., lumisterol-3, tachysterol-3, and 5,6-*trans*-vitamin D_3).

14. Heaney, R.P., Armas, L.A.G., French, C., 2013. J. Nutr. 143, 571–575.

15. The skin is composed of three layers: the **epidermis**, the **dermis**, and a layer of insulating **subcutaneous fat**. The epidermis is comprised mostly of keratinocytes, which form its tough outer layer, the **stratum corneum** (horny layer) and are continually replaced from a basal layer of cells, the **Malpighian layer** (named for Marcello Malpighi, 1628–94, the "father of microscopic anatomy and histology"). The epidermis also contains a smaller numbers of melanocytes that produce melanin, which filters out UV irradiation from solar exposure and is the major contributor to skin color, and dendritic (antigen-presenting) Langerhans cells, named for the German anatomist Paul Langerhans, 1847–88, who first described them). The thick underlying dermis is comprised of fibrous and elastic tissue (collagen, elastin, and fibrillin); it also contains sensory nerve endings, sweat glands, sebaceous glands, hair follicles, and blood vessels.

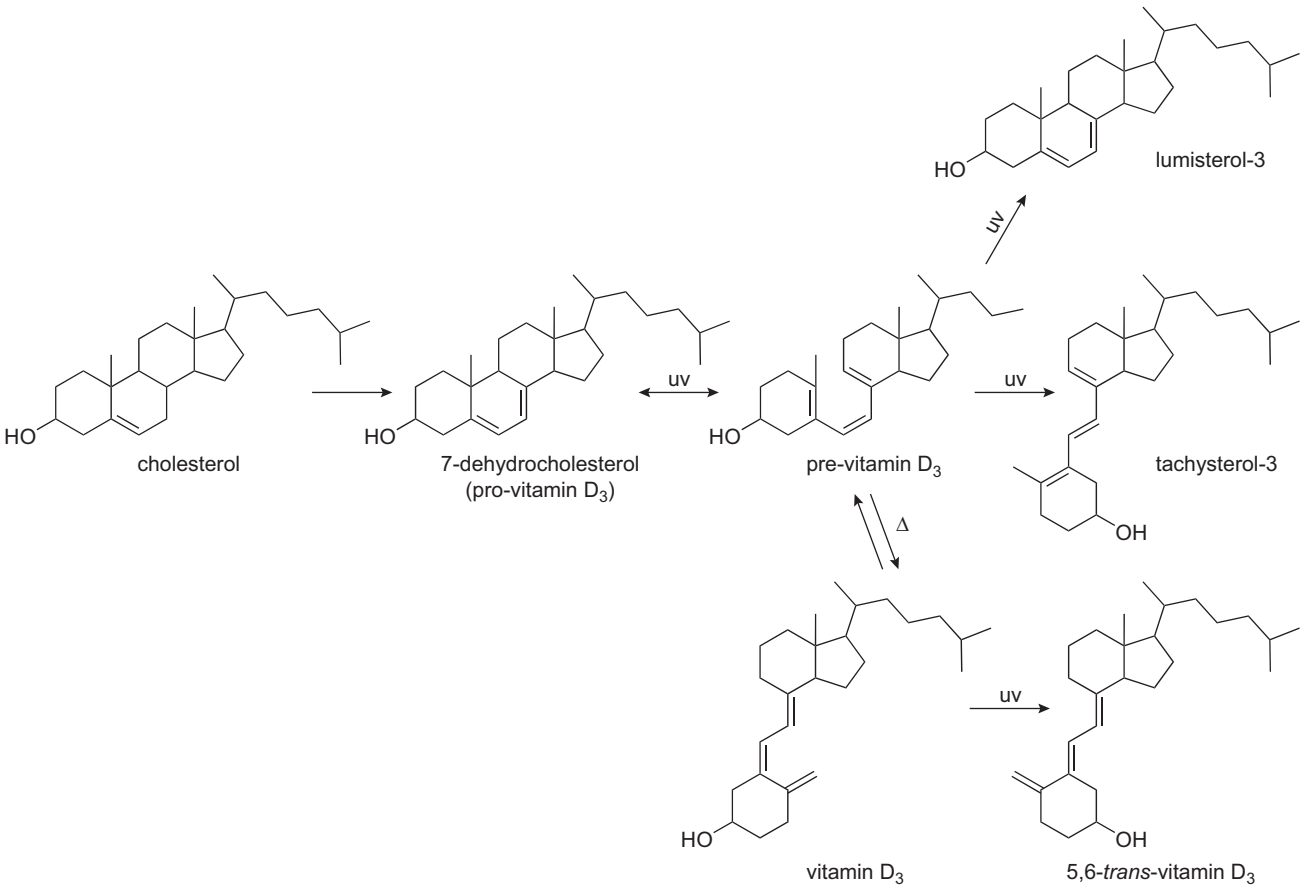


FIGURE 7.1 Biosynthesis of vitamin D_3 and its photolytic by-products.

in the deeper **Malpighian layer**; whereas, in the rat it is distributed more superficially in the **stratum corneum**. The associated differences in UVB penetrance mean that the D_3-biogenic effect of UVB is less efficient in humans than in the rat. Both the thickness and 7-DHC content of skin decline with age, reducing the D_3-biogenic capacity by twofold or more (Fig. 7.2).

Vitamin D biosynthesis is, thus, determined by environmental exposure to UV light, which also can increase risk to skin cancers in individuals experiencing episodes of severe burning.[16] The amount of sunlight required to support adequate vitamin D status is substantially less than that which increases skin cancer risk. Holick has estimated that exposure of only 6–10% of body surface to a **median erythemal dose (MED)**[17] of sunlight can be equivalent to consuming 600–1000 IU (15–25 mg) of vitamin D. He recommends exposures of one-quarter of that amount of

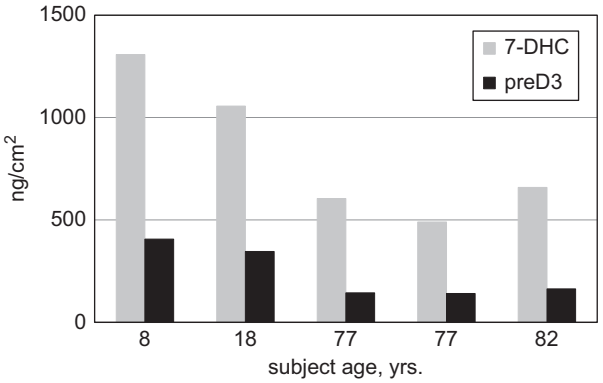


FIGURE 7.2 Epidermal contents of 7-dehydrocholesterol (7-DHC) and previtamin D_3 (pre-D3) of five subjects of varying ages. *After MacLaughlin, J., Holick, M.F., 1985. J. Clin. Invest. 76, 1536–1538.*

sunlight two to three times weekly to support the synthesis of physiologically relevant amounts, c.15,000 IU (375 mg) per week, of the vitamin.[18] Because sunlight exposure varies with latitude and season, D_3 biosynthesis also varies

16. UV exposure is a risk factor for skin cancers, 70–85% of which are basal cell carcinomas. Melanomas, while far less prevalent, account for most skin cancer deaths.

17. MED is a measure of skin sensitivity to light, 1 MED I the amount of light that causes the skin to turn slightly pink (**erythema**) due to infiltration of erythrocytes into the exposed skin—the sign of a mild sunburn.

18. Holick, M.F., 2001. Lancet 357, 4–6; Holick, M.F., Jenkins, M., 2003. The UV Advantage. iBooks, Inc., New York, p. 93.

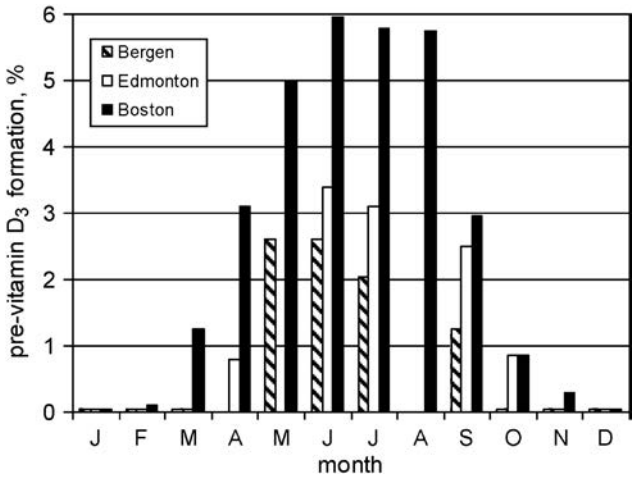


FIGURE 7.3 Seasonality of vitamin D biosynthesis (measures as conversion of 7-dehydrocholesterol in response to 1 h exposure to sunlight) at three different latitudes (Bergen: 60°N; Edmonton: 52°N; Boston: 42°N). *After Holick, M.F., 2008. Nutr. Rev. 66, S182.*

according to those factors. Vitamin D-producing UV irradiation depends on the zenith angle of the sun, being greatest at noon (60% occurs between 10 a.m. and 2 p.m.), reaching an annual peak at midsummer (Fig. 7.3), and declining with the distance from the earth's equator. In winter, individuals living above 40° N/S[19] have virtually no vitamin D_3 biogenesis.

Holick's Rule

Sun exposure of one-quarter MED over one-quarter of the body is equivalent to 1000 μg (40 IU) oral vitamin D_3.

The vitamin D_3-biogenic capacity of skin is diminished by factors that block UV penetrance. The epidermal pigment **melanin** efficiently absorbs UVB;[20] therefore, dark-skinned individuals can have lower circulating levels of 25-OH-D_3 and require greater UV doses for comparable vitamin D_3 biosynthesis compared to light-skinned individuals. Compared to a person with light, type 1 skin (easily sunburned; never tan), an individual with dark, type 5 or 6 skin (seldom or never burn; always tan) can require 5–10 times as much solar exposure to produce the same amount of vitamin D_3.[21] This difference would appear to have evolutionary basis, light skin being an adaptation of early humans dispersing into the higher latitudes where risks of suboptimal vitamin D status were significant.

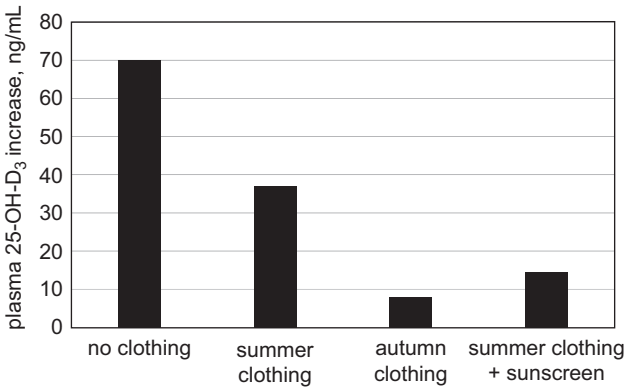


FIGURE 7.4 Effects of clothing and sunscreen on plasma 25-OH-D_3 responses of humans to 1 MED (median erythemal dose) of UVB. *After Matsuoka, L.Y., Wortsman, J., Dannenbaerg, M.J., et al., 1992. J. Clin. Endocrinol. Med. 75, 1099–1103.*

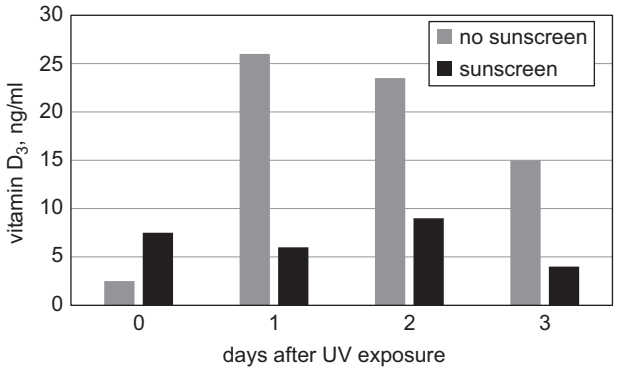


FIGURE 7.5 Effects of sunscreen (SPF8) application on circulating vitamin D_3 responses of humans to 1 MED (median erythemal dose) of UVB radiation. *After Matsuoka, L.Y., Wortsman, J., Hanifan, N., et al., 1987. J. Clin. Endocrinol. Med. 64, 1165–1168.*

Determinants of Vitamin D_3 Biogenesis

- *Exogenous factors* affecting sunlight exposure: season, latitude, time outdoors, sunscreen use
- *Endogenous factors* affecting UVB responsiveness: skin thickness, pigmentation.

Physical factors that reduce the exposure of the skin to UV light also reduce the biosynthesis of vitamin D_3 (Figs. 7.4 and 7.5). These include factors associated with lifestyle of humans (e.g., clothing, indoor living [glass and plexiglass absorb UV light], sunscreen use) and practical management of livestock (e.g., confined indoor housing). Properly applied topical sunscreens with sun

19. That is, the latitudes of Denver, Philadelphia, Toledo (Spain), Ankara and Beijing in the northern hemisphere, and San Martin de los Andes (Argentina) in the southern hemisphere. *Note*: the entire African and Australian continents lie north of 40°S.

20. Melanin absorbs radiation over a broad range, 290–700 nm. It is thought to be an evolutionary adaptation to protect against hypervitaminosis D induced by solar exposure in tropical latitudes—a trait that was lost in populations migrating to areas distant from the equator.

21. Bauer, J.M., Freyberg, R.H., 1946. J. Am. Med. Assoc. 130, 1208–1215.

TABLE 7.1 Contributions of Sun and Dietary Vitamin D to the Vitamin D Status of Older Women

	Low Sunlight Exposure		High Sunlight Exposure	
	Low Vitamin D	High Vitamin D	Low Vitamin D	High Vitamin D
Summer				
Vitamin D intake (mg/day)	3.58 ± 0.53	16.05 ± 1.38[a]	4.08 ± 0.65	14.53 ± 1.15[a]
Plasma 25-OH-D_3 (n*M*)	44 ± 6	80 ± 8[a]	57 ± 5	74 ± 6[a]
Winter				
Vitamin D intake (mg/day)	3.48 ± 0.45	16.33 ± 1.33[a]	4.40 ± 0.78	14.63 ± 1.43[a]
Plasma 25-OH-D_3 (n*M*)	35 ± 3	81 ± 7[a]	42 ± 4	64 ± 4[a]

[a]$p < 0.05$.
Adapted from *Salamone, L.M., Dallal, G.E., Zantos, D., et al., 1993. Am. J. Clin. Nutr. 58, 80–86.*

protection factors of 8 and greater have been shown to reduce cutaneous vitamin D_3 production by >95%.[22]

People living in northern or southern latitudes typically show seasonal changes in their serum 25-OH-D_3 levels. Greatest concentrations occur in the autumn, i.e., after a summer of relatively great solar exposure. This indicates that sunlight is generally more important than diet as a source of this critical nutrient (Table 7.1). However, many people do not spend sufficient time outside to meet their needs for vitamin D, particularly those with darker skin types and older people.

For individuals with adequate exposure to sunlight, vitamin D_3 cannot be considered a vitamin at all—it is a prohormone produced in the skin. However, environmental and lifestyle factors and, for livestock, management systems, can limit vitamin D_3 biogenesis, rendering them in need of exogenous (dietary) vitamin D. Under such conditions, in which endogenous synthesis is not sufficient to meet needs, vitamin D becomes a vitamin in the traditional sense.

Vitamin D Seasonality for free-living people.
- Plasma levels of 25-OH-D_3 and 24,25-$(OH)_2$-D_3 vary with the season: highest in late summer and lowest in late winter.
- Plasma levels of 1,25-$(OH)_2$-D_3 are fairly constant year-round.

Dietary Sources of Vitamin D

Vitamin D, as either **ergocalciferol** (**vitamin D_2**) or cholecalciferol (vitamin D_3), is not widely present in nature; however, its provitamins are common is both plants and animals. Ergocalciferol and its precursor **ergosterol** are found in plants, fungi,[23] molds, lichens, and some invertebrates (e.g., snails and worms). In fact, some microorganisms are quite rich in ergosterol, in which it may comprise as much as 10% of the total dry matter.[24] Ergosterol occurs in higher vertebrates only to the extent that they consume it and, then, only in low amounts. The actual distribution of ergocalciferol in nature is much more limited and variable than that of ergosterol (e.g., grass hays and alfalfa contain vitamin D only after they have been cut and left to dry in the sun). Cholecalciferol is widely distributed in animals but has an extremely limited distribution in plants. In animals, tissue cholecalciferol concentrations are dependent on the vitamin D_3 content of the diet and/or the exposure to sunlight. Few foods, however, are rich in the vitamin.

Animal Tissues

The richest natural sources are fish liver and oils[25] in which vitamin D occurs in free form and as long-chain fatty acid esters. Fatty fishes, which are high in food chains in which lower trophic level organisms feed on ergosterol-containing plants, can provide significant amounts of vitamin D.[26] In contrast, the amounts of vitamin D in farm-raised fish will depend on the levels of the vitamin added as a supplement

22. Holick pointed out that because few people apply sunscreens in more than half of recommended amounts, their use does little to limit cutaneous vitamin D production (Holick, M.F., 2004. Am. J. Clin. Nutr. 79, 362–371).

23. Ergosterol was named for the fungus, **ergot** (*Claviceps* spp.) that grows parasitically on rye and related grasses.

24. Provitamin D_3 accounts for virtually all of the sterols in *Aspergillus niger* and 80% of those in *Saccharomyces* cerevisiae (brewers' yeast).

25. Many fish oils have vitamin D_3 concentrations of c.50 μg/g; oils of cod, tuna, and mackerel can contain 20 times that amount. Marine mammals that consume large quantities of these cold water fish accumulate the vitamin in their livers, which is an important dietary source of vitamin D for people that consume seals and whales, e.g., Inuits, Faroe Islanders.

26. A meta-analysis found that the consumption of recommended amounts of fatty fishes increased plasma 25-OH-D_3 levels by some 6.8 nM; whereas, comparable intakes of lean fish had <30% of that effect (Lehmann, U., Gjessing, H.R., Hirche, F., et al., 2015. Am. J. Clin. Nutr. 102, 837–847).

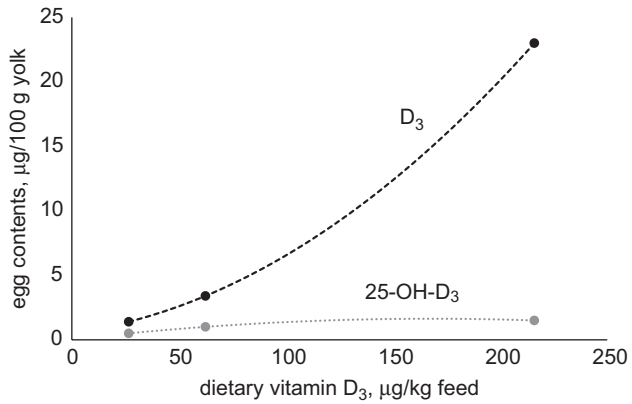


FIGURE 7.6 Effects dietary vitamin D level (X) on vitamin D contents of eggs (D_3,Y); $y = 0.0003x^2 + 0.0359x$. *Estimated from data presented by Mattila, P., Lehikoinen, K., Kiiskinen, T., et al., 1999. J. Agric. Food Chem. 47, 4089–4092.*

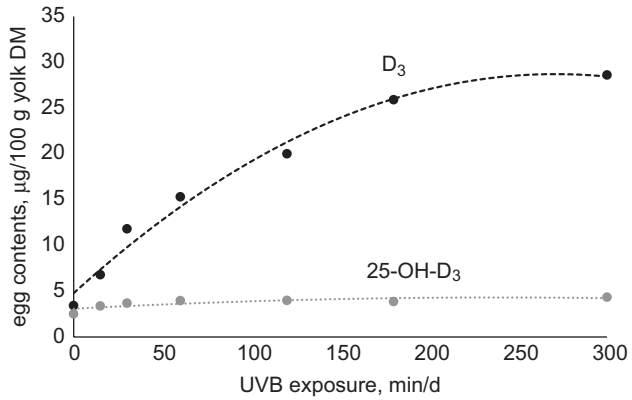


FIGURE 7.7 Effects of UVB exposure (4 weeks, X) on vitamin D contents of eggs (Y); for D_3, $Y = -0.0003x^2 + 0.1765x + 4.7809$ *(Estimated from Kühn, J., Schutkowski, A., Hirche, F., et al., 2015. J. Steroid Biochem. Mol. Biol. 148, 7–13.)*. This effect was seen in the three- to fourfold greater vitamin D_3 contents of eggs from free-range hens compared to those from confined hens but only during the late summer months. Adapted from *Kühn, J., Schutkowski, A., Kluge, H., et al., 2014. Nutrition 30, 481–484.*

to their formulated feeds. This is also true for other livestock. The vitamin D contents of eggs varies according to amounts of the vitamin added to the feed (Fig. 7.6) (addition of 25-OH-D_3 has only a small effect[27]), as well as the amount of solar exposure of the laying hens (Fig. 7.7). Variations in such management practices are likely to explain the differences in egg vitamin D_3 contents observed between individual farms and regions, and over time.[28]

Meats tend to have relatively low and variable vitamin D contents (chicken, 0–14 µg/kg; beef, 0–9 µg/kg; pork, 1–23 µg/kg; lamb, 1–61 µg/kg; veal, 0–50 µg/kg) as does milk (0.3–1 µg/kg) if not fortified.[29]

Plant Tissues

With a few notable exceptions, vitamin D_3 is not found in plants. Those exceptions include the species in the families Solanaceae (*Solanum glaucophyllum*, *Solanum malacoxylon*, *Solanum torvum*, *Solanum verbascifolium, Cestrum diurnum*, and *Nierembergia veitchii*) in which the vitamin occurs as water-soluble β-glycosides of vitamin D_3, 25-hydroxyvitamin D_3 (25-OH-D_3), and 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2$-D_3).[30] Vitamin D_3 has been identified species of the families Gramineae (*Trisetum flavescens* and *Dactylis glomerata*) and Leguminoseae (*Medicago sativa*).

Irradiated Mushrooms

Mushrooms are rich in ergosterol, which makes them a potential source of vitamin D_2 if exposed to sunlight or UVB.[31] That conversion is fairly rapid, requiring short exposures (60–95 min).[32] Accordingly, many mushroom producers use postharvest UVB exposure to produce edible mushrooms that contain significant amounts of D_2. Commercial species have been found to contain 0.03–63 µg (0.001–2.5 IU) vitamin D_2 per g fresh weight; the greatest amounts have been found in irradiated maitake (*Grifola frondosa*) and portabella (*Agaricus bisporus*).[33]

Breast Milk

There are relatively few published data on the vitamin D contents of breast milk, despite the fact that breastfeeding without adequate sunlight exposure or vitamin D supplementation appears to be a risk factor for vitamin

27. Mattila, P., Valkonen, E., Valaja, J., 2011. J. Agric. Food Chem. 59, 8298–8303.

28. Eggs produced commercially in 12 US states showed >12-fold variation in vitamin D_3 content. Between 2000–2001 and 2010, average vitamin D_3 content of US eggs increased by 60% (Exler, J., Phillips, K.M., Patterson, K.Y., et al., 2013. J. Agric. Food Chem. Anal. 29, 110–116). Hens in confined, environmentally managed conditions have virtually no exposure to sunlight; whereas, those managed in courtyard or free-range situations can have significant sun exposure.

29. Schmid, A., Walther, B., 2013. Adv. Nutr. 4, 453–462.

30. The search for the factor causing calcinosis in ruminants grazing on these Solanaceous species led to the discovery that plants could contain vitamin D. In addition, both D-25-hydroxylase and 25-OH-D-1-hydroxylase were found in *Solanum malacoxylon*, and the grass *Trisetum flavescens*, which produces vitamin D_3 when exposed to UVB. Thus, it appears that these species produce and metabolize vitamin D in ways similar to animals.

31. Mushrooms also contain 22,23-dihydroergosterol; hence, irradiated mushrooms also contain 22-dihydroergocalciferol, a metabolite of unknown physiological significance (Phillips, K.M., Horst, R.L., Koszewski, N.J., et al., 2012. PLoS One 7, 1–10).

32. Studies with the oyster mushroom (*Pleurotus ostreatus*) showed that optimal vitamin D_2 yield is realized within 60 min using 310–320 nm light at 11.5 W/m^2 at 20°C. Beyond that point, concentrations of lumisterol-2, tachysterol-2, and previtamin D_2 increased to the point of accounting for one-sixth of the total steroid products (Krings, U., Berger, R.G., 2014. Food Chem. 149, 10–14).

33. Phillips, K.M., Ruggio, D.M., Horst, R.L., et al., 2011. J. Agric. Food Chem. 59, 7841–7853.

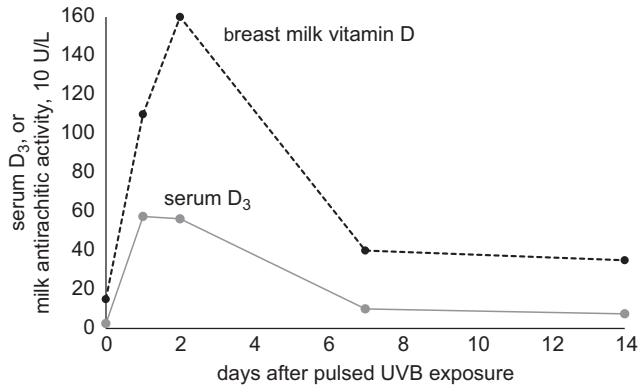


FIGURE 7.8 Related responses of vitamin D in breast milk and maternal serum to a pulsed exposure (1.5 ME.D) to UVB. *(After Specker, B.L., Tsang, R.C., Hollis, B.W., 1985. Am. J. Dis. Child. 139, 1134–1137.)*. Note: Antirachitic activity was calculated from analyzed vitamers: 1 U = 25 ng D_2 or D_3, or 5 pg 25-OH-D_2 and 25-OH-D_3.

D-deficiency rickets in children. Available reports have shown breast milk vitamin D concentrations to be highly variable between studies; this is likely due to the inclusion of samples from mothers with differing vitamin D intakes and with different UVB exposures. For example, breast milk from Finish mothers was ninefold greater in the summer compared to winter.[34] Specker and colleagues demonstrated that total breast milk vitamin D concentrations correlated with vitamin D intake are responsive to UVB exposure (Fig. 7.8) and can be lower for African-American women than for white American women (Table 7.2).

25-OH-D in Foods

Foods derived from animals also contain 25-OH-D, which is not measured in conventional food analyses. An expert panel of the NIH Office of Dietary Supplements found that including 25-OH-D in estimates of vitamin D intake of American adults would increase those estimates by 1.7–2.9 μg/day.[35]

Fortified Foods and Dietary Supplements

Even when consumed in appreciable amounts, the low amounts of vitamin D in foods (Table 7.2) make it very difficult to achieve adequate vitamin D nutriture from those foods. Thus, it has become the practice in many countries to fortify certain frequently consumed foods. Some countries mandate the addition of vitamin D (and vitamin A) to certain foods (e.g., margarine) and regulate the voluntary addition of vitamin A to others (e.g., baked goods, breakfast cereals, milk,[36] yogurt, cheeses, orange juice, and infant foods). Both D_2 and D_3 are used in such fortification. Some other foods may be enriched indirectly as the result of the supplementation of animal feeds with the vitamin. A meta-analysis of 16 clinical interventions with vitamin D_3 from fortified foods found significant heterogeneity in the plasma 25-OH-D_3 response due to such factors as dose, latitude, and baseline plasma 25-OH-D_3 concentration.[37] Overall, they found that every 1 μg vitamin D_3 ingested from fortified foods was associated with an average increase in plasma 25-OH-D_3 concentration of 1.2 nM.

Vitamin D is also added in many formulated nutritional supplements. Multivitamin supplements typically contain 400 IU (10 mg) vitamin D, and pharmaceutical preparations can contain as much as 50,000 IU (1250 mg) vitamin D_2 per capsule/tablet. These can provide important vitamin D nutrition; in many countries their use has increased in recent years. However, it can be difficult to identify the actual use of supplements and fortified foods; this can lead to food consumption surveys underestimating total intakes of vitamin D.

Vitamin D Analogues

Many nucleus or side chain derivatives of D_2 or D_3 have been developed for treating vitamin D refractory rickets, hypocalcemia and osteodystrophy secondary to chronic renal disease, and some types of cancer (Table 7.3). The goal has been to develop drugs active in vitamin D-dependent regulation of cell proliferation and growth while having low calcemic potential, thus avoiding the calcinosis caused by high levels of the vitamins.

Vitamin D Bioavailability

The vitamers D vary in bioavailability due to differences in biopotency, i.e., not concerning absorption or transport (Table 7.4). Vitamers requiring metabolic activation (cholecalciferol and ergocalciferol) are less biopotent than those proximal to the points of metabolic function (e.g., 25-OH-vitamin D). Humans and many other species have been thought not to discriminate between these vitamers. Indeed, many studies have found D_3 and D_2 in foods or pure form to produce equivalent responses in increasing plasma levels of their respective 25-hydroxylated intermediate, although a recent meta-analysis found bolus doses

34. Ala-Houhala, M., Koskinen, T., Parvainen, M.T., et al., 1988. Am. J. Clin. Nutr. 48, 1057–1060.
35. Taylor, C.L., Roseland, J.M., Coates, P.M., et al., 2016. J. Nutr. 146, 855–856.
36. In the late 1950s, the American Medical Association recommended that fluid milk be fortified with 400 IU (10 μg) per quart. The US Food and Drug Administration has specified that milk contains 400–600 IU/qt. However, a survey in the 1980s found that most fortified milk products failed to contain the specified amounts of vitamin D (Tanner, J.T., Smith, J., Defibaugh, P., et al., 1988. J. Assoc. Off. Anal. Chem. 71, 601–610). Most European countries to not fortify milk with vitamin D.
37. Black, L.J., Seamans, K.M., Cashman, K.D. et al., 2012. J. Nutr. 142, 1102–1108.

TABLE 7.2 Breast Milk Vitamin D Concentrations in American Women

Donors	D_3	25-OH-D_3	D_2	25-OH-D_2
African-American (n = 10)	36 (22–58)[a,b]	87 (75–101)[b]	54 (22–134)[b]	66 (52–85)[b]
White American (n = 14)	268 (127–567)	124 (97–159)	290 (164–512)	82 (64–105)

[a]*Mean (95% C.L.).*
[b]*Significantly different from White American, p < .05.*
Specker, B.L., Tsang, R.C., Hollis, B.W., 1985. Am. J. Dis. Child. 139, 1134–1137.

TABLE 7.3 Bioactive Vitamin D Analogues

1-OH-analogues	1-OH-D_3
Vitamin D_2 derivatives	1-OH-D_2; 1,25,28-$(OH)_3$-D_2; 1,24*S*-$(OH)_2$-D_2
Side-chain derivatives	Cyclopropane ring (side chain carbons 25, 26, and 27) derivatives, e.g., calcipitriol 20-epi and 20-methyl side chain derivatives analogues with one or more added carbons in the side chain or on the branching methyl groups unsaturated side chain derivatives, e.g., C_{16}=C_{17}, C_{22}=C_{23} oxa-containing (oxygen-for-carbon substitution), e.g., 22-oxa-calcitriol fluorinated derivatives, e.g., F_6-1,25-$(OH)_2$-D_3
Other derivatives	Dehydrotachysterol-2

TABLE 7.4 Relative Biopotencies of Vitamin D-Active Compounds

Compound	Relative Biopotency, %[a]
Vitamin D_3 (cholecalciferol)	100
Vitamin D_2 (ergocalciferol)	100 (mammals)[b]
	10 (birds)[c]
Dihydrotachysterol[d]	5–10
25-OH-Cholecalciferol[e]	200–500
1,25-$(OH)_2$-Cholecalciferol[e]	500–1000
1α-OH-Cholecalciferol[f]	500–1000

[a]*Results of bioassays of rickets prevention in chicks and/or rats.*
[b]*Biopotencies of vitamins D_2 and D_3 are equivalent for mammalian species.*
[c]*Biopotency of vitamin D_2 is very low for chicks, which cannot use this vitamer effectively.*
[d]*A sterol generated by the irradiation of ergosterol.*
[e]*Normal metabolite of vitamin D_3; the analogous metabolite of vitamin D_2 is also formed and is comparably active in nonavian species.*
[f]*A synthetic analog.*

of D_3 produced greater 25-OH-D responses than D_2 in humans.[38] Some species (avians) are known to distinguish between D_2 and D_3, greatly in favor of the latter; these species require D_3.

Expressing Vitamin D Activities

Because vitamin D exists in foods and supplements in different forms of differing biopotencies, the reporting of vitamin D activity in foods requires some means of standardization. For this purpose, an international unit (**IU**) has been defined:

1 IU = 0.025 mg of vitamin D2 or vitamin D3.

Foods Rich in Vitamin D

The richest food sources of vitamin D are oily fishes and their products, dairy products, irradiated mushrooms, and fortified foods oils (Table 7.5).

38. Tripkovic, L., Lambert, H., Hart, K., et al., 2012. Am. J. Clin. Nutr. 95, 1357–1364.

4. ENTERIC ABSORPTION OF VITAMIN D

Micelle-Dependent Passive Diffusion

Vitamin D is absorbed from the small intestine by nonsaturable passive diffusion that is dependent on micellar solubilization and, hence, the presence of fat[39] and bile salts. The fastest absorption appears to be in the upper portions of the small intestine (duodenum and ileum); however, the greatest amount of vitamin D is probably absorbed in the distal region where food has a longer transit time. Like other hydrophobic substances absorbed by micelle-dependent diffusion in mammals, vitamin D enters the lymphatic circulation[40] predominantly (90% of

39. The apparent absorption of D_3 by healthy adults was one-third less from a fat-free meal than from meals containing fat (Dawson-Hughes, B., Harris, S., Lichtenstein, A.H., et al., 2015. J. Acad. Nutr. Diet. 115, 225–230.

40. In birds, reptiles, and fishes, vitamin D, like other lipids, is absorbed into the portal circulation via portomicra.

TABLE 7.5 Vitamin D Activities in Foods

Food	Vitamin D, IU/100 g
Human milk	3
Dairy Products	
Milk	0–51[a]
Butter	0
Cheese	0–24
Cream	44
Eggs	82
Fish Products	
Cod	46
Cod liver oil	100,000
Herring	214
Mackerel	292
Salmon	670–685
Sardines	193
Shrimp	4–5
Beef liver	49
Meats	
Beef	1–16
Pork	1–104
Chicken	3–15
Chicken skin	24
Other	
Mushrooms	0–25,000[b]

[a]US regulations specify that milk be fortified with 400 IU of vitamin D_3 per quart (about 37 IU/100 ml).
[b]If UV-irradiated; upper limit from published literature.
USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

the absorbed amount) in association with chylomicra (or portomicra), with most of the balance being associated with α-globulins.[41] The efficiency of enteric absorption of dietary vitamin D appears to be about 50%. Newly absorbed vitamin D is released by enterocytes into the lymphatic circulation in chylomicra. The polar metabolites (25-OH-D; 1,25-$[OH]_2$-D) appear to be transported by DBP (vitamin D-binding protein) in the portal blood; only small amounts (13% of 25-OH-D; 1% of 1,25-$[OH]_2$-D) appear in the lymph in chylomicra.

41. probably the **vitamin D-binding protein (DBP)**.

5. TRANSPORT OF VITAMIN D

Vitamin D_3 formed in the skin is translocated into the dermal capillary bed, then into the general circulation, by a process that appears to be selective for D_3, leaving previtamin D_3 in the epidermis. A major player in this translocation is the specific **DBP** in plasma to which all of the vitamin of biogenic origin is bound (Table 7.6). In contrast, enterically absorbed vitamin D is present in the circulation bound to both DBP and lipoproteins in comparable amounts, apparently being transferred during the process of chylomicron degradation in the liver. Studies have demonstrated that circulating levels of the hepatic metabolite, 25-OH-D_3, are affected by **apolipoprotein E (apoE)** genotype (Fig. 7.9); humans with the *APOEε4*[42] allele have also been found to have greater serum 25-OH-D_3 concentrations than noncarriers.

Vitamin D-Binding Protein

Like other sterols, vitamin D is transported in the plasma largely in association with protein. While some birds and mammals transport vitamin D in association with albumin, and fishes with cartilaginous skeletons (e.g., sharks and rays) transport it in association with plasma lipoproteins, most species[43] use a protein that has been called **transcalciferin** or, more commonly, DBP (Table 7.6). DBP is in the same gene family as albumin and α-fetoprotein. In mammals, it is a glycosylated, cysteine-rich, α-globulin of 458 amino acids with a molecular weight of 55–58 kD, depending on its glycosylation state. It has three internally homologous α-helical domains and exists as multiple isoforms[44] due to differences in both the primary structure of the protein (involving the presence/absence of *N*-acetylneuraminic acid on a threonine residue at position 420) and the carbohydrate moiety that is added posttranslationally. Three alleles are common. The chicken has two distinct DBPs (54 kDa and 60 kDa) each of which preferentially binds vitamin D_3 and its metabolites versus the respective vitamin D_2 analogues.

DBP binds vitamins D and metabolites stoichiometrically, with ligand binding dependent on the *cis*-triene structure and C_3-hydroxyl grouping. In adequately nourished individuals, DBP binds some 88% of the 25-OH-D_3 in serum with an affinity and order of magnitude greater than those of 1,25-$(OH)_2$-D. The concentration of DBP in the plasma, typically 4–8 μM,

42. APOE is a key regulator of lipid metabolism. The APOE ε4 allele occurs in 3–40% of populations; in Western populations, it is a risk factor for age-related morbidity and mortality due to cardiovascular disease and late-onset Alzheimer's disease.
43. More than 140 species in five classes have been examined.
44. Humans DBP is identical to group-specific component ($G_c$protein), a genetic marker useful in epidemiologic and forensic studies.

TABLE 7.6 Distribution of Vitamin D Metabolites in Human Plasma

Metabolite	% Distribution			Normal Concentration
	DBP (Vitamin D-Binding Protein)	Lipoproteins	Albumin	
Vitamin D_3	60	40	0	2–4 ng/mL
25-OH-D_3	98	2	0	15–38 ng/mL
24,25-$(OH)_2$-D_3	98	2	0	—
1,25-$(OH)_2$-D_3	62	15	23	20–40 pg/mL

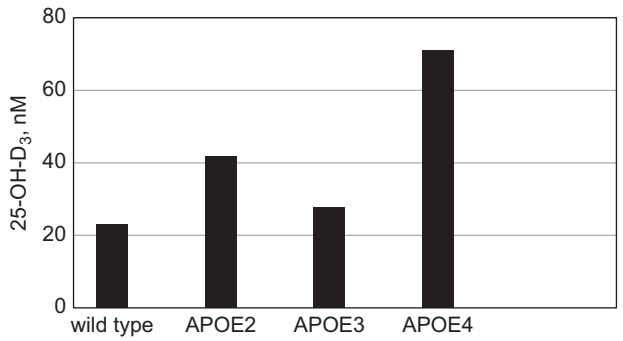


FIGURE 7.9 Relationship of apolipoprotein E genotype and serum 25-OH-D level in the mouse. *After Huebbe, P., Nebel, A., Siegert, S., et al., 2011. FASEB J. 25, 3262–3270.*

greatly exceeds that of 25-OH-D_3 (c.50 nM) and is remarkably constant, as it is unaffected by sex, age, or vitamin D status. The excess binding capacity means that only 5% of DBP actually carries the vitamin. That DBP can also bind the plasma actin monomer and fatty acids, which suggests that the vitamin D transport protein may also have other functions in metabolism.[45] The turnover of DBP in the plasma is much shorter than that of 25-OH-D_3 (1–3 days[46] versus 45 days[47]), indicating that the ligand is recycled. The extended half-life of 25-OH-D appears to be due to its megalin-dependent uptake and subsequent release by myocytes, such that muscle comprises a large extravascular pool protecting 25-OH-D from degradation.[48] DBP can be taken up endocytotically by the renal proximal tubule, being internalized from the glomerular filtrate via binding to the multiligand binding receptors megalin and cubilin.[49] This system results in delivery of 25-OH-D_3 to the cell with catabolism of DBP. Being synthesized by the liver, DBP is depressed in patients with hepatic disease. Its expression is increased by trauma, estrogen therapy, and pregnancy. It does not appear to cross the placenta; fetal DBP is immunologically distinct from the maternal protein.

In addition to facilitating the peripheral distribution of vitamin D obtained from the diet, DBP functions to mobilize the vitamin produced endogenously in the skin. Indeed, vitamin D_3 found in the skin is bound to DBP.[50] It has been suggested that the efficiency of endogenously produced vitamin D_3 is greater than that given orally for the reason that the former enters the circulation strictly via DBP, whereas the latter enters as complexes of DBP as well as chylomicra. This would indicate that oral vitamin D remains longer in the liver and is, thus, more quickly catabolized to excretory forms. In support of this hypothesis, it has been noted that high oral doses of vitamin D can lead to very high levels of 25-OH-D_3 (>400 ng/ml) associated with intoxication; whereas intensive UV irradiation can rarely produce plasma 25-OH-D_3 concentrations greater than one-fifth that level, and hypervitaminosis D has never been reported from excessive irradiation. The DBP protein has also been found on the surfaces of lymphocytes and macrophages, although the functional significance of such binding is not clear.

While DBP binds both D_2 and D_3 and their metabolites, genetic variants differ only in their binding of D_3.[51] The

45. DBP has a high affinity for the actin monomer. The DBP–actin complex (which can interfere with the assay of 25-OH-D-1-hydroxylase in kidney homogenates unless large amounts of 25-OH-D_3 are used) is cleared from the circulation at three times the rate of nonliganded DBP, suggesting that DBP may function in extracellular scavenging.

46. Haddad, J.G., Fraser, D.R., Lawson, D.E.M., 1981. J. Clin. Invest. 67, 1550–1560.

47. Clements, M.R., Davies, M., Fraser, D.R., 1987. J. Clin. Endocrinol. Metab. 73, 659–674.

48. Aboud, M., Gordaon-Thomson, C., Hoy, A.J., 2014. J. Steroid Biochem. Mol. Biol. 14, 232–236.

49. These multiligand receptors are expressed in the apical parts epithelial cells of renal proximal tubules, colocalized in endocytic compartments (coated pits and endosomes). Megalin is a 600 kDa transmembrane protein of the LDL-receptor family; cubilin is a 460 kDa peripheral membrane protein identical to the intrinsic factor–vitamin B_{12} receptor of in the small intestinal epithelium. Both proteins function in the renal reabsorption of proteins including VDB, RBP, transcobalamin (vitamin B_{12} transporter), and transferrin (iron transporter). In the thyroid, megalin appears to function in thyroid hormone transport; in the parathyroid, it is thought to be a Ca^{2+} sensor.

50. DBP has a very low affinity for lumisterols or tachysterols, thus not mobilizing from the skin these forms produced under conditions of excessive irradiation.

51. Nimitphong, H., Saetun, S., Chanprasertyotin, S., et al., 2013. Nutr. J. 12, 39–46.

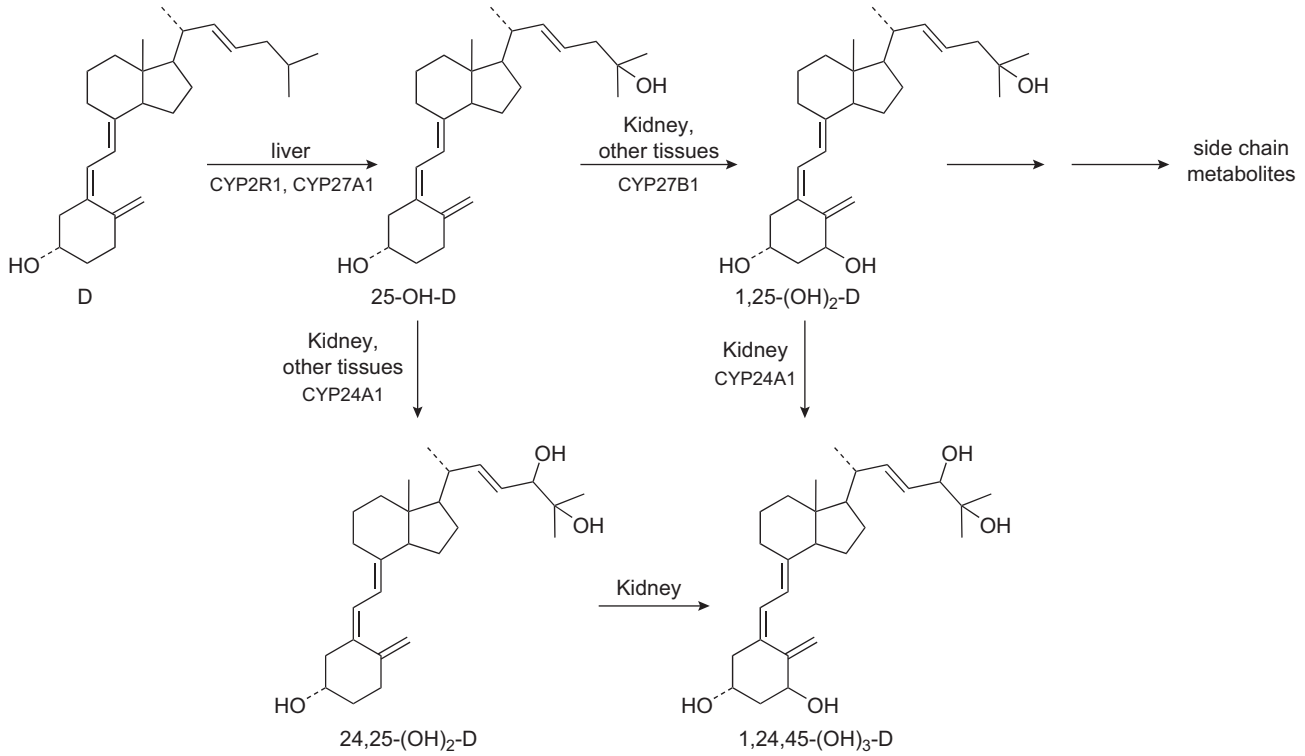


FIGURE 7.10 Vitamin D metabolism.

frequency of rare alleles of DBP polymorphisms has been found to be inversely related to circulating 25-OH-D_3 level, with effects comparable in magnitude to those of vitamin D intake.[52] Different patterns of DBP polymorphisms appear to explain 80% of the difference in plasma DBP levels and 10% of the difference in plasma 25-OH-D_3 levels between black and white subjects.

Tissue Distribution

Unlike the other fat-soluble vitamins, vitamin D is *not* stored by the mammalian liver except in some fishes. It reaches the liver within a few hours after being absorbed across the gut or synthesized in the skin, but from the liver it is distributed relatively evenly among the various tissues, where it resides in hydrophobic compartments. Therefore, fatty tissues such as adipose show slightly greater concentrations. However, in that tissue the vitamin is found in the bulk lipid phase, from which it is only slowly mobilized. About half of the total vitamin D in the tissues occurs as the parent vitamin D_3 species, with the next most abundant form, 25-OH-D_3, representing 20% of the total. In the plasma, however, the latter metabolite predominates by several fold.[53] Tissues including those of the kidneys, liver, lungs, aorta, and heart also tend to accumulate 25-OH-D_3.[54] It is thought that the uneven tissue distribution of vitamin D, in its various forms, relates to differences in both tissue lipid content and tissue-associated vitamin D-binding proteins, the latter fraction being the smaller of the two intracellular pools of the vitamin.

The concentrations of both 25-OH-D_3 and 1,25-$(OH)_2$-D_3 are lower in the cord sera of fetuses and newborn infants than in the sera of their mothers. That fetal 25-OH-D_3 levels correlate with maternal levels (and show the same seasonal variations) suggest that the metabolite crosses the placenta. Such a correlation is not apparent for 1,25-$(OH)_2$-D_3. The extent of transplacental movement of the latter metabolite is not known; however, the placenta appears to be able to produce it from maternally derived 25-OH-D_3.

6. METABOLISM OF VITAMIN D

Metabolic Activation

The metabolism of vitamin D involves its conversions to different hydroxylated products, each of which is more polar than its parent (Fig. 7.10).[55] The production of these

52. Sinotte, M., Diorio, C., Berube, S., et al., 2009. Am. J. Clin. Nutr. 89, 634–640.

53. The next most abundant is 24,25-$(OH)_2$-D_3.

54. These organs are susceptible to calcification under conditions of hypervitaminosis D.

55. It was the observation in the late 1960s of radioactive peaks migrating ahead of vitamin D in gel filtration of plasma from animals given radiolabeled cholecalciferol that first evidenced the conversion of vitamin D3 to other species some of which were ultimately found to be metabolically active.

metabolites, some of which are metabolically active forms of the vitamin, explains the lag time that is commonly observed between the administration of the vitamin and the earliest biological response.

25-Hydroxylation

Most of the vitamin D taken up by the liver from either DBP or lipoproteins is converted by hydroxylation of side chain carbon C-25 to yield 25-OH-D_3 (also called **calcidiol**), the major circulating form of the vitamin. This metabolism occurs in the liver in mammals but in both liver and kidney in birds. That activity, vitamin D 25-hydroxylase, involves at least six cytochrome *P*-450-dependent mixed-function oxygenases[56] of two general types: five low-affinity, high-capacity enzymes associated with the endoplasmic reticulum[57] (CYP2R1, CYP2J2/3, CYP3A4, CYP2D25, and CYP2C11) and one high-affinity, low-capacity enzyme located in the mitochondria (CYP27A1). CYP27A1 also occurs in kidney and bone suggesting extrahepatic 25-hydroxylation of vitamin D_3. The presence of two different mechanisms of 25-hydroxylation would appear to facilitate the maintenance of adequate vitamin D status under both deficient and excessive conditions of vitamin D intake/production. That CYP27A1 is not necessary for vitamin D function is indicated by the fact that, in the mouse, its genetic deletion does not impair bone health. The most physiologically important 25-hydroxylase, at least in humans, appears to be CYP2R1. An individual presenting with vitamin D-dependent rickets was found to have a transition mutation that abolished the 25-hydroxylase activity, and genome-wide studies that have found significant associations of *cyp2r1* polymorphisms and circulating 25-OH-D_3 levels.

25-Hydroxyvitamin D_3 is not retained within the cell but is released to the plasma where it accumulates by binding with DBP. At normal plasma concentrations of this metabolite, only small amounts of 25-OH-D_3 from this pool enter tissues. Therefore, the circulating level of 25-OH-D_3, normally 10–40 ng/mL (25–125 nM), is a good indicator of general vitamin D status.

1-Hydroxylation

25-OH-D_3 is further hydroxylated at the C-1 position of the A ring to yield 1,25-$(OH)_2$-D_3 (also called **calcitriol**). Being produced at a site, the kidney, distant from its target tissue to which it is transported in the blood, 1,25-$(OH)_2$-D_3 has been considered a hormone. The 1-hydroxylase uses $NADPH_2$ as the electron donor and has three constituent proteins: ferridoxin, ferridoxin reductase, and a cytochrome *P*-450 isoform CYP27B1. The complex is located primarily in renal cortical mitochondria but also in mitochondrial and microsomal fractions of at least some extrarenal tissues (e.g., bone cells, keratinocytes,[58] liver, and placenta[59]) where it is thought to provide capacity for local production of the active metabolite. Despite its key role in discharging the actions of vitamin D, the tightly regulated production and relatively fast turnover (4–6 h in serum) of 1,25-$(OH)_2$-D_3 render it not useful as a biomarker of vitamin D status. Circulating levels tend to be about 40 pg/mL (100 nM). An autosomal recessive defects in the *cyp27b1* gene produce **vitamin D-dependent rickets type I**. Affected individuals have normal plasma levels of 25-OH-D_3 but low levels of 1,25-$(OH)_2$-D_3.

Epimerization

25-Hydroxyvitamin D can undergo epimerization[60] at the C3 position[61] to 3-epi-25-(OH)-D_3 in which the C3-hydroxyl moiety extends above the plane of the steroid "A" ring instead of below that plane, as in the normal metabolite. 3-Epi-25-(OH)-D_3 accounts for some 25% of total circulating 25-(OH)-D_3,[62] leading to overestimation of vitamin D status by radioimmunoassay and mass spectrometric methods incapable of discriminating between the epimers. The mechanism of epimerization has not been characterized, but studies have shown that 3-epi-25-(OH)-D_3 is metabolized by the renal 1-hydroxylase to produce 3-epi-1,25-$(OH)_2$-D_3, which binds VDR (vitamin D receptor).

Catabolism

24-Hydroxylation

1,25-$(OH)_2$-D_3 is short-lived; it upregulates its rapid metabolism by the 24-hydroxylase, CYP24A1, which attacks its side chain to produce 1,24,25-$(OH)_3$-D_3 and 1,23,24,25-$(OH)_4$-D_3. CYP24A1 can also hydroxylate 25-OH-D_3 to produce 24,25-$(OH)_2$-D_3. The 24-hydroxylase has a 10-fold greater affinity for 1,25-$(OH)_2$-D_3 than for 25-OH-D_3, but the 1000-fold excess of the latter in the plasma suggests that the primary physiological significance of the

56. A mixed-function oxygenase uses molecular oxygen (O_2) but incorporates only one oxygen atom into the substrate.
57. In the rat, the microsomal hydroxylase is fivefold more active in males than females; in males it may involve cytochrome P4502C11, which is not expressed by females.
58. When exposed to sunlight/UVB, keratinocytes produce vitamin D_3, from which they can make 1,25-$(OH)_2$-D_3. Their contribution to the circulating pool of 1,25-$(OH)_2$-D_3 is substantially less than kidney but can be significant in patients with renal disease in whom reduced plasma 1,25-$(OH)_2$-D_3 levels induce increased expression of the skin 1-hydroxylase.
59. Maternal levels of 25-OH-D_3 increase during the third trimester of gestation. This presumably assists the mother in providing calcium for the mineralization of the fetal skeleton.
60. i.e., Changing configuration about a single asymmetric center.
61. The 3-epimer of 25-(OH)-D_3 has the C3-hydroxyl moiety extending above the plane of the steroid "A" ring; whereas, in the normal metabolite that grouping extends below the plane.
62. Karras, S.N., Shah, I., Petroczi, A., et al., 2013. Nutr. J. 12, 15–77.

hydroxylase may be in clearing excess 25-OH-D_3. Studies have shown that 24,25-$(OH)_2$-D_3 inhibits the 25-OH-D_3-mediated signaling of Ca and phosphate transport and promotes bone formation and mineralization. The greatest activity of CYP24A1 is found in renal mitochondria; its expression is upregulated in chronic renal disease and 1,25-$(OH)_2$-D_3. Both 1,25-$(OH)_2$-D_3 and 24,25-$(OH)_2$-D_3 are produced under conditions of vitamin D adequacy and normal Ca homeostasis. Calcitriol is a major biliary metabolite of the vitamin. That 24,25-$(OH)_2$-D_3 inhibits the stimulatory effect of parathyroid hormone (PTH)[63] on bone resorption by **osteoclasts** and suggests it may participate in local osteotropic control in bone.

Other Hydroxylations

More than 40 other hydroxylation metabolites of vitamin D have been identified. One, 24R,25-$(OH)_2$-D, appears to have a specific role in embryonic survival and fracture healing.[64] Most others appear to be physiologically inactive and are converted to excretable forms. Some 95% of vitamin D excretion occurs via the bile, with side chain hydroxylation products accounting for nearly one-fifth. Most do not bind DBP; they and their chain-shortened products are cleared from the circulation and converted to excretory forms including fatty esters and glucuronides. Two that are bound by DPB are 25,26-$(OH)_2$-D_3, detectable in the plasma after D_3, and the 26, 23-lactone of 25-OH-D_3 (apparently produced from 25-OH-D_3 by extrahepatic CYP24A1), which accumulates in hypervitaminotic D animals and is the major excretory metabolite in some species (guinea pig, opossum).

Regulation of Vitamin D Metabolism

Vitamin D has been found to have a relatively long physiological half-life, about 2 months. This likely reflects its partial sequestration in adipose, as the half-lives of its circulating (25-OH-D_3) and metabolically active (1,25-$[OH]_2$-D_3) forms are shorter, about 15–45 days and 15 h, respectively.[65] Circulating levels of 1,25-$(OH)_2$-D_3 are regulated at about 40 pg/mL (100 nM).

The 1-hydroxylase is the rate-limiting step in vitamin D metabolism. It is upregulated by the following:

- Low plasma Ca^{2+}—via Ca^{2+}-receptor-mediated stimulation of PTH release by the parathyroid[66]
- Low plasma P—via a pituitary signaling (superstimulation occurs if plasma Ca^{2+} is also low).

It is downregulated by the following:

- Its product, 1,25-(OH)2-D3;
- High plasma P—causes bone cells to secrete fibroblast growth factor 23 (FGF23),[67] which acts as a downregulator.

Other factors affect these responses. PTH and CT both stimulate the renal 1-hydroxylase; however, PTH has a rapid effect mediated by cAMP, while CT has a relatively slow effect apparently mediated by transcription.[68] Estrogen appears to have a role, as ovariectomy reduces the synthesis of 1,25-$(OH)_2$-D_3 by rat kidney. That effect appears to be mediated via PTH, as parathyroidectomy blocks the effect of estrogen in stimulating 1,25-$(OH)_2$-D_3 production. In contrast, regulation of the 1-hydroxylase in extrarenal tissues (e.g., macrophages) appears to be insensitive to PTH but to be stimulated by cytokines such as interferon-gamma and lipopolysaccharide.

The 25-hydroxylase is poorly regulated. It shows little or no feedback inhibition by 25-OH-D_3. Its expression increases with increasing hepatic vitamin D_3 level and exposure to inducers of cytochrome *P*-450 (phenobarbital, diphenylhydantoin)[69] but is inhibited by isoniazid.[70]

Catabolism of 1,25-$(OH)_2$-D_3 is tightly regulated to prevent hypercalcemia and hyperphosphatemia. This is accomplished by PTH, serum P, and factors affecting the principle catabolizing enzyme, the hepatic 24-hydroxylase (CYP24A1). The 1-hydroxylase is inhibited by strontium, the DPB–actin complex, and is feedback inhibited by 1,25-$(OH)_2$-D_3. Thus, when circulating levels of 1,25-$(OH)_2$-D_3 are high, its renal synthesis is low. The tight regulation of the 1-hydroxylase activity results in the maintenance of nearly constant plasma concentrations of 1,25-$(OH)_2$-D_3, which activates its own breakdown by stimulating the transcription of the 24-hydroxylase gene. That stimulation is suppressed in conditions of low serum P.

Epigenetic Regulation

Vitamin D metabolism appears to be regulated, in part, through epigenetic mechanisms. Methylation of the promoter of CYP2R1 (D_3 25-hydroxylase) was observed in leukocytes from vitamin D-deficient individuals; that effect was reversed upon return to vitamin D adequacy. Methylation of

63. PTH is a small (9.6 kDa) protein with high sequence homology across species.
64. St. Arnaud, R., 2010. J. Steroid Biochem. Mol. Biol. 121, 254–256.
65. Jones, G., 2008. Am. J. Clin. Nutr. 88, S582–S586.
66. It has been suggested that some elderly people who cannot adapt to low a Ca diet by increasing enteric Ca absorption may suffer impaired PTH-dependent 1,25-$(OH)_2$-D_3 upregulation.
67. Mutations in the *fgf23* gene are thought to be the cause of autosomal dominant hypophosphatemic rickets.
68. It has been suggested that the function of the CT-sensitive 1-hydroxylase, which is elevated in the fetus, may be to accommodate situations of increased need for 1,25-$(OH)_2$-vitamin D.
69. Antiepileptic agents such as these reduce the biological half-life of vitamin D apparently by enhancing its conversion to 25-OH-D and other hydroxylated products.
70. Patients on long-term isoniazid therapy are at risk to developing bone disease.

the *cyp27b1* gene appears to be the basis of downregulation of 25-OH-D_3 1-hydroxylase expression observed in many cancers. The 24-hydroxylase activity appears to involve tissue-dependent methylation of the *cyp24a1* gene. These effects appear to involve the liganded VDR transactivating or transrepressing genes through interactions with other nuclear factors (e.g., VDR-interacting repressor, histone deacetylase 2) to induce DNA methylation and histone acetylation.

Role of Vitamin D-Binding Protein

DBP is critical in the regulation of vitamin D metabolism, controlling the tissue distribution of vitamin D metabolites. Due to the relative excess of DBP (4–8 µM compared to 50 nM 25-OH-D_3), nearly 90% of circulating vitamin D metabolites are bound to DBP in vitamin D-adequate individuals, while occupying <5% of available binding sites. Further, because of its avid binding of 25-OH-D_3 in the plasma rather than other tissues, concentrations of the free metabolite are maintained at very low levels (10^{-13} M). Both 25-OH-D_3 and 1,25-(OH)2-D_3 can also bind to albumin, albeit with much lower affinities than DBP (Table 7.7). Accordingly, albumin binding is much less effective than DBP in protecting these metabolites against losses by renal filtration as the liganded DBP binds to megalin, which prevents its glomerular filtration. Thus, DBP functions to modulate the availability of these metabolites to the tissues, which appear to receive them from the small pools that are either not protein-bound, or are bound with low affinity to albumin and other non-DBP proteins. It is estimated that while <0.1% of the 25-OH-D_3 in plasma is not bound to proteins, the amount available to extrarenal tissues for in situ synthesis of 1,25-$(OH)_2$-D_3 (i.e., the non-DBP pools) comprises about 10% of total plasma total. Some authors have referred to this as the "bioavailable" pool and have pointed out that this pool appears to be unaffected by differences in circulating DBP and total 25-OH-D_3 levels (Table 7.8).

TABLE 7.7 Relative Affinities of DBP (Vitamin D-Binding Protein) and Albumin for 25-OH-D_3 and 1,25$(OH)_2$-D_3

Protein	Binding Constants, K_a	
	25-OH-D_3	1,25-$(OH)_2$-D_3
DBP	$7 \times 10^8\ M^{-1}$	4×10^7 M
Albumin	$6 \times 10^5\ M^{-1}$	$5.4 \times 10^4\ M^{-1}$

TABLE 7.8 Plasma DBP (Vitamin D-Binding Protein) and 25-OH-D_3 Levels in Black and White Americans

Subjects	DBP, µg/mL	25-OH-D_3, ng/mL	"Bioavailable" 25-OH-D3, ng/mL[a]
Black (n = 1181)	168 ± 3	15.6 ± 0.2	2.9 ± 0.1
White (n = 904)	337 ± 5[b]	25.8 ± 0.4[b]	3.1 ± 0.1

[a]*Not bound to DBP.*
[b]*Mean ± SEM; significantly different from Black mean, p < .05.*
Powe, C.E., Evans, M.K., Wenger, J., et al., 2013. N. Engl. J. Med. 369, 1991–2000.

Differential Metabolism of Vitamins D_2 and D_3

Although a minor dietary form, D_2 is metabolized analogously to D_3. The enzymes involved in the 1-, 24-, and 25-hydroxylations do not discriminate between these vitamers, and daily doses of modest amounts (1000 IU) of either produce comparable increases in serum 25-OH-D.[71] However, large single doses (50,000 IU) of 25-OH-D_2 are cleared more rapidly than 25-OH-D_3.[72] At high levels of exposure, D_2 is metabolized to a number of mono- (24-), di- (1,24-; 24,26-), and tri- (1,25,28-) hydroxylated metabolites that are not produced from D_3. One study found the half-life of 25-OH-D_2 to be significantly shorter than that of 25-OH-D_3 for subjects in Gambia but not for subjects in the U.K. Such discrepant results may be related to different distributions of genetic variants in DBP, which have been shown to affect responsiveness to D_3 but not to D_2 (Fig. 7.11). For humans and probably most other species, differences in biopotency between D_2 and D_3 are significant only at bolus doses.

Some species, including birds and some New World monkeys, strongly discriminate between D_2 and D_3. For them, D_2 is much less biopotent that D_3; they clear the mono- and dihydroxylated metabolites of D_2 faster than those of D_3. For example, in the chick, the plasma turnover rates of vitamin D_2, 25-OH-D_2, and 1,25-$(OH)_2$-D_2 are 1.5-, 11-, and 33-fold faster than those of the respective vitamin D_3 analogues. These differences in turnover rates are greater than those of the binding affinities to DBP (5-, 3.6-, and 3-fold, respectively), which are greater for D_3 and its metabolites than for D_2 and its metabolites.

7. METABOLIC FUNCTIONS OF VITAMIN D

Vitamin D_3 as a Steroid Hormone

At least some, if not all, of the mechanisms of action of vitamin D fit the classic model of a steroid hormone. That is, it has specific cells in target organs with specific receptor proteins; and the receptor–ligand complex moves to the

71. Holick, M.F., Biancuzzo, R.M., Chen, T.C. et al., 2008. J. Clin. Endocrinol. Metab. 93, 677–681.
72. Armas, L.A., Hollis, B.W., Heaney, R.P., 2004. J. Clin. Endocrinol. Metab. 89, 5387–5391.

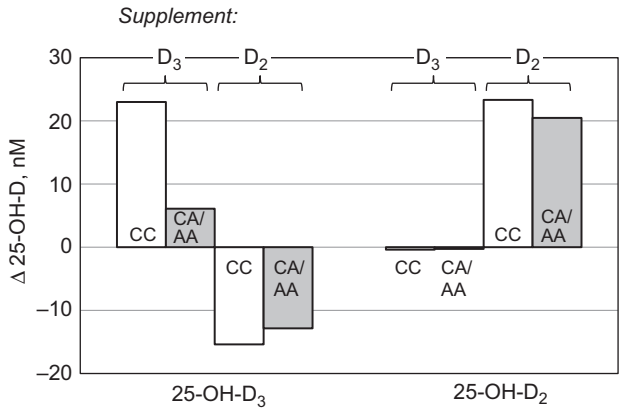


FIGURE 7.11 Changes in plasma 25-OH-D in response to vitamins D_3 and D_2 by humans with different DBP genotypes. *After Nimitphong, H., Saetung, S., Chanprasertyoptin, S., et al., 2013. Nutr. J. 12, 39–49.*

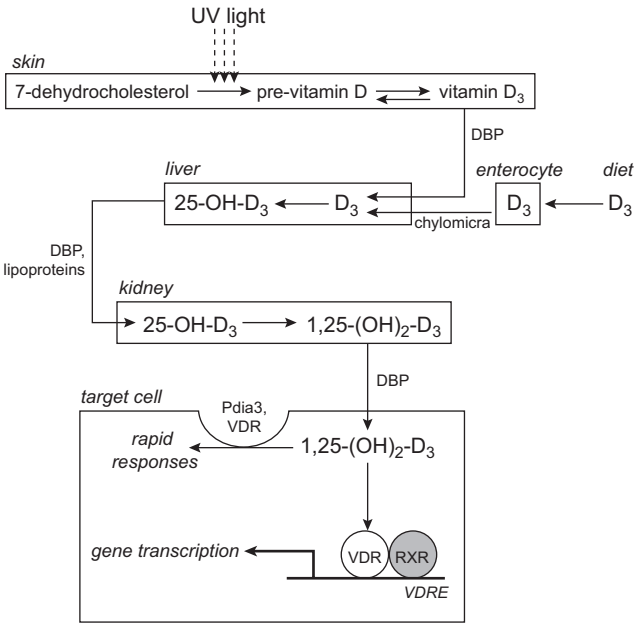


FIGURE 7.12 Metabolic activation of vitamin D (see text for abbreviations).

nucleus, where it binds to the chromatin at specific DNA sequences and stimulates the transcription of certain downstream genes to produce specific mRNAs that encode the synthesis of specific proteins (Fig. 7.12). Vitamin D, itself, is not metabolically active; it must be converted to the active metabolite, 1,25-$(OH)_2$-D_3, which functions in two general ways: by regulating gene expression and by nongenomic signaling of key physiological processes.

Genomic Pathways of Vitamin D Function

Vitamin D Receptor

Target tissues for vitamin D contain a specific nuclear receptor, VDR, which binds 1,25-$(OH)_2$-D_3 with high affinity and 25-OH-D_3 with lower affinity.[73] Autoradiographic studies have shown that 1,25-$(OH)_2$-D_3 is localized in the nuclei of many cell types that contain the VDR. VDRs have been identified in more than 30 different cell types including cells involved in Ca homeostasis (bone, kidney, intestine), immune function, endocrine function, hematopoiesis, skin, and tumors (Table 7.9). Such findings indicate a wide breadth of genomic functions.[74] A rare autosomal recessive defect in VDR manifests as vitamin D-resistant rickets type II characterized by high plasma 1,25-$(OH)_2$-D_3 (differentiating the condition from vitamin D-resistant rickets type I) and, in some families, alopecia. Allelic variation in the VDR gene is associated with reduced bone mineral density and increased fracture risk.[75] In neonates, VDR is absent until weaning.[76] In at least some organs, VDRs appear to be inducible by estrogen. In the guinea pig (and presumably in humans, who also require vitamin C), the number of VDRs can be reduced by deprivation of ascorbic acid.[77]

The VDR is a 51 kDa protein classified in the family of steroid, thyroid hormone, and retinoic acid receptor genes on the basis of its similar primary amino acid structure. VDRs of different species vary in size (e.g., human, 48 kDa; avian, 60 kDa), but their DNA-binding domains show >95% homology between species. Each is a sulfhydryl protein with an N-terminal recognition domain containing a cysteine-rich cluster comprising two "zinc finger" structures.[78] Their ligand-binding domains show less homology between species; each consists of 12 α-helices configured to create a three-layered sandwich structure that encompasses 1,25-$(OH)_2$-D_3 with high affinity in a hydrophobic core. Binding of 1,25-$(OH)_2$-D_3 causes a conformational change in the receptor that allows it to interact with other players in transcription. Multiple polymorphic variations have been identified in the human VDR gene; by affecting mRNA expression, stability, and patterns, variants can have different VDR protein concentrations.

73. The ability of VDR to bind 25-OH-D_3 is likely to be the basis for the ability of anephric patients to increase calcium absorption in response to high doses of vitamin D.

74. That VDR also binds lithocholic acid has caused some to suggest that it may also function as a bile acid sensor.

75. An 18-month study of elderly subjects found VDR *BB* homozygotes more likely to lose bone mineral than *bb* homozygotes (78% versus 31% lost at rates >0.48%/year) (Ferrari, S., Rizzoli, R., Chevalley, T., et al., 1995. Lancet 345, 423–424).

76. VDR abundance varies among various tissues in the range of 200–25,000 copies/cell; VDR contents of intestinal cells are surprisingly low, <2000 copies/cell.

77. Guinea pig intestinal 1,25-$(OH)_2$-D_3 receptors (occupied and unoccupied) were reduced by vitamin C deprivation. This may relate to the development of rickets-like bone changes in vitamin C deficiency.

78. "Zinc fingers" are finger-like structures folded around a zinc atom tetrahedronally coordinated through the sulfhydryls of cysteine residues in the primary structure of the protein. This motif is similar to DNA-binding motifs found in other transcriptional regulating proteins.

TABLE 7.9 Distribution of Known Nuclear Vitamin D Receptors

Organ System	Cell Type
Bone	Osteoblasts
Connective tissue	Cartilage chrondrocytes, fibroblasts, stroma
Alimentary tract	Enterocytes, colonocytes
Liver	Hepatocytes
Kidney	Epithelium (proximal and distal); glomerular podocytes
Heart	Atrial myoendocrine cells, cardiomyocytes
Lung	Bronchial epithelium
Skeletal, smooth muscle	Myocytes
Thymus	Epithelium
Hematolymphopoietic	Activated T and B cells, macrophages, monocytes, spleenocytes, thymus reticular cells, lymphocytes, lymph nodes, tonsillary dendritic cells
Reproductive	Amnion, chorioallantoic membrane, epididymus, ovary, oviduct, placenta, testis, Sertoli and Leydig cells, uterus, yolk sac
Skin	Epidermis, fibroblasts, hair follicles, keratinocytes, melanocytes, sebaceous glands
Nervous	Hippocampus, cerebellar Purkinje and granule cells, bed nucleus, stria terminalis, amygdala central nucleus, sensory ganglia, spinal cord
Immune	Thymus, bone marrow, B cells, T cells
Other endocrine	Adrenal medulla and cortex, pancreatic β cells, pituitary epithelium, thyroid follicles and C cells, parathyroid epithelium, parotid epithelium
Testes	Germ cells
Prostate	Epithelium
Mammary gland	Mammary alveolar and ductal cells
Adipose tissue	Adipocytes
Other	Bladder epithelium, choroid plexus epithelium

These include a *FokI* polymorphism in exon II, *BsmI* and *ApaI* allelic variants in the intron between exons VIII and IX, a *Taq1* restriction fragment polymorphism in exon IX, and a repeat mononucleotide polymorphism in the 3′ untranslated region. Studies have demonstrated contributions of these polymorphisms, as well as epigenetic silencing of VDR expression, to interindividual variations in circulating levels of 25-OH-D_3; to differences in enteric Ca^{2+} absorption and bone mineral density; and to risks of rheumatoid arthritis, osteoarthritis, type 2 diabetes, autoimmune disease, Parkinson's disease, fracture, and cancer.[79]

The liganded VDR is thought to be translocated from the cytosol into the nucleus via interactions with microtubules. This mechanism is thought to involve a sequence followed by other sterols: stimulation of protein kinase C (PKC), PKC-activation of guanylate cyclase, phosphorylation of microtubule-associated proteins, and association of VDR with importins. Like other steroid hormone receptors, the VDR is a *trans*activator of the 1,25-$(OH)_2$-D_3-dependent transcription of mRNAs for various proteins involved in Ca transport, the bone matrix, and cell cycle regulation. The VDR of intestinal epithelial cells can also bind bile acids, ultimately leading to the detoxification of those inducers through upregulation of drug-metabolizing CYP3A enzymes.

Vitamin D-Responsive Elements

Specific DNA promotor sequences act as VDREs. These are similar to the responsive elements mediating gene expression responses of thyroid hormone or retinoic acid; each consists of imperfect direct repeats of six-base pair half-elements.[80] Binding of the 1,25-$(OH)_2$-D_3–VDR complex to VDREs involves one of the retinoid X receptors (RXRs). The preferred active species appears to be a VDR–RXR heterodimer.[81] Most cells contain both VDRs and RXRs; therefore, the availability of 9-*cis*-retinoic acid may determine which set of genes is regulated by 1,25-$(OH)_2$-D_3. Transcriptional regulation of gene expression by vitamin D acting through this system is thought to involve a conformational change in VDR effected by the phosphorylation of a specific serinyl residue upon the binding of 1,25-$(OH)_2$-D_3. This facilitates the recruitment of coactivator proteins that induces chromatin remodeling and exposes domains of the protein capable of interacting with VDREs to influence RNA polymerase-mediated transcription.

Genes Regulated by Vitamin D

Some 50 genes have been identified as being regulated by vitamin D (Table 7.7). These include genes associated with many aspects of metabolism to include vitamin D metabolism as well as cell differentiation and proliferation, energy

79. Utterlinden, 2005. In: Feldman, D., Pike, J.W., Glorieux, F.H. (Eds.), Vitamin D, second ed. Elsevier, New York, NY, pp. 1121–1157.
80. Owing to their direct repeats, these lack the dyad symmetry of the classic steroid hormone-responsive elements.
81. Interaction of these receptor proteins is thought to involve C-terminal dimerization interfaces in both.

metabolism, hormonal signaling, mineral homeostasis, oncogenes, and chromosomal proteins. For most of these, the regulation appears to involve 1,25-$(OH)_2$-D_3-dependent modulation of mRNA levels (i.e., regulation of transcription and/or message stability). To date, 1,25-$(OH)_2$-D_3-regulated transcription has been established for less than a dozen of these genes, and VDREs have been reported for only a few (e.g., calbindin$_{9K}$, integrin$_{\alpha\gamma\beta3}$, osteocalcin, and the plasma membrane Ca^{2+} pump). Evidence for posttranscriptional regulation of calbindin$_{9K}$ has been presented. From this emerging picture, it is clear that changes in vitamin D status have potential for pleiotropic actions.

The first gene product to be recognized as inducible by 1,25-$(OH)_2$-D_3 was for many years called **Ca-binding protein (CaBP)**. Two forms of CaBP have subsequently been described; these are now called **calbindins**.[82] They are widespread in animal tissues, with greatest concentrations found in avian and mammalian duodenal mucosa, where they can comprise 1–3% of the total soluble protein of the cell. Calbindins function in the enteric absorption of Ca by facilitating the movement of Ca through the enterocytic cytosol while keeping the intracellular concentration of the free Ca^{2+} ion below hazardous levels. Calbindin-D_{9k} can bind two Ca^{2+} atoms, while calbindin-D_{28k} can bind four Ca^{2+} atoms. Calbindin-D_{9k} occurs primarily in mammalian intestinal mucosa but also in kidney, uterus, and placenta; calbindin-D_{28k} occurring in mammalian kidney (distal convoluted tubules), pancreas (β cells) and brain, and avian intestine and kidney. Calbindins are not expressed in vitamin D deficiency but are expressed in response to 1,25$(OH)_2$-D_3. That such treatment increases the expression of the protein without affecting its message indicates that vitamin D regulation of calbindin occurs at the translational level.

VDR also downregulates the expression of some genes. These include genes encoding PTH (Table 7.10). This appears to involve binding of VDR homodimers or VDR–RXR heterodimers to a negative response element (nVDRE), which is transcriptionally active in the absence of 1,25$(OH)_2$-D_3. It is thought that downregulation occurs by VDR directly binding an activator of the nVDRE.

Vitamin D also appears to have extratranscriptional effects on gene expression. Studies have revealed that 1,25-$(OH)_2$-D can affect DNA methylation, histone acetylation, and microRNA generation, thus, affecting physiological functions via epigenetic effects. The active metabolite has also been found to affect pre-mRNA constitutive and alternative gene splicing by recruiting nuclear receptor

82. Calbindins are members of a large family of Ca^{2+}-binding proteins each with a distinctive helix–loop–helix sequence, the "EF hand." They have been identified in many species. The mammalian form (calbindin-D_{9k}) is a 10 kDa protein with two high-affinity Ca^{2+}-binding sites. Avian calbindin-D_{28K} is larger, 30 kDa; it is also expressed in the shell gland (uterus) of laying hens.

TABLE 7.10 Genes Known to Be Regulated by Vitamin D

Gene	Tissue in Which Regulation Has Been Demonstrated
Upregulated	
Aldolase subunit B	Chick kidney
Alkaline phosphatase	Chick and rat intestine
ATP synthase	Chick and rat intestine
Calbindin-$D_{28\ kDa}$	Chick brain, kidney, uterus, intestine; mouse kidney; rat kidney, brain
Calbindin-$D_{9\ kDa}$	Chick kidney, skin, bone; rat intestine, skin, bone
Carbonic anhydrase	Marrow; myelomonocytes
CCAT enhancer-binding protein β	Mouse intestine, osteoblasts
Cytochrome *c* oxidase, subunits I, II, and III	Chick and rat intestine
Cytochrome P450 isoform CYP3A	Mouse intestine
Fibronectin	MG-63, TE-85, HL-60 cells
c-Fms	HL-60 cells
c-Fos	HL-60 cells
Glyceraldehyde-3-phosphate dehydrogenase	BT-20 cells
Heat shock protein 70	Peripheral blood monocytes
Integrin$_{\alpha\gamma\beta3}$	Chick osteoclasts
Interleukin 1	U937 cells
Interleukin 6	U937 cells
Interleukin 3 receptor	MC3T3 cells
c-Ki-Ros	BALB-3T3 cells
Matrix Gla protein	UMR106-01, ROS cells
Metallothionein	Rat keratinocytes
NADH dehydrogenase, subunits II and III	Chick intestine
Nerve growth factor	L-929 cells
Neutrophil-activating polypeptide	HL-60 cells
c-Myc	MG-63 cells
Osteocalcin	ROS cells
Osteopontin	ROS cells
1-OH-D_3 24-hydroxylase	Rat kidney

Continued

TABLE 7.10 Genes Known to Be Regulated by Vitamin D—cont'd

1,25-$(OH)_2$-D_3 receptor	Mouse fibroblasts
Plasma membrane Ca^{2+} pump	Chick intestine
Prolactin	Rat pituitary cells
Protein kinase C	HL-60 cells
Tumor necrosis factor α	U-937, HL-60 cells
Vascular endothelial growth factor (VEGF)	Mouse fibroblasts
Vitamin D receptor	Rat intestine, pituitary
Downregulated	
ATP synthase	Chick kidney
Calcitonin	Rat thyroid gland
CD-23	Peripheral blood monocytes
Collagen, type I	Rat fetal calvaria
Cytochrome *b*	Chick kidney
Cytochrome *c* oxidase. Subunits I, II, and III	Chick kidney
Cytochrome P450 isoform CYP2B1	Mouse liver
Fatty acid-binding protein	Chick intestine
Ferridoxin	Chick kidney
Granulocyte–macrophage colony-stimulating factor	Human T lymphocytes
Histone H_4	HL-60 cells
Interleukin 2	Human T lymphocytes
Interferon γ	Human T lymphocytes
c-Myb	HL-60 cells
c-Myc	HL-60, U937 cells
NADH dehydrogenase subunit I	Chick kidney
25-OH-D_3 1-hydroxylase	Rat kidney
Prepro-PTH (parathyroid hormone)	Rat, bovine parathyroid
Protein kinase inhibitor	Chick kidney
PTH	Rat parathyroid
PTH-related protein	T lymphocytes
Transferrin receptor	PBMCs (peripheral blood mononuclear cells)
α-Tubulin	Chick intestine

TABLE 7.11 Rapid Responses to 1,25-$(OH)_2$-D_3

Response	Organ/Cell
Ca^{2+} transport	Intestinal mucosa, CaCo-2 cells
Protein kinase C (PKC) activation	Intestinal mucosa, chondrocytes, liver, muscle
MAPK activation	Intestinal mucosa, liver, leukemia cells
Ca^{2+} signaling	Adipocytes, pancreatic β cells
Ca^{2+} channel opening	Osteoblasts
G protein activation	Intestinal mucosa
Cell differentiation	Promyelocytic NB4 cells
Src activation	Keratinocytes
Raf activation	Keratinocytes
Insulin secretion	Pancreatic β cells
Increased contraction/ relaxation	Cardiomyocytes

Mizwicki, M.T., Norman, W.N., 2009. Sci. Signal. 2, 1–14.

coregulators (e.g., heterogeneous nuclear ribonucleoprotein C, hnRNPC).[83]

Nongenomic Pathways of Vitamin D Function

Some responses to vitamin D occur within seconds to minutes of 1,25-$(OH)_2$-D_3 exposure and are membrane-mediated. This suggests signaling independent of genomic responses, which typically take hours to days. The first such response to be recognized is **transcaltachia**, the rapid transport of Ca^{2+} across the intestinal mucosa. Several other transcription-independent responses have since been demonstrated (Table 7.11). That these rapid responses depend on VDR is indicated by their absence in VDR-null mice. However, evidence indicates the involvement of another receptor, a membrane-associated rapid response steroid-binding protein. That appears to be a protein localized in plasma membrane **caveolae**,[84] protein disulfide isomerase 3 (Pdia3). Convincing evidence shows that binding of 1,25-$(OH)_2$-D_3 to Pdia3 triggers a protein phosphorylation cascade that begins with interaction with phospholipase A_2-activating protein

83. Zhou, R., Chun, R.F., Lisse, T.S., et al., 2015. J. Steroid Biochem. Mol. Biol. 148, 310–317.

84. Caveolae (Latin, "little caves") are small (50–100 nm) invaginations of the plasma membrane in endothelial cells, adipocytes, and other cell type. They are comprised of a type of "lipid raft," which are organized microdomains within plasma membranes comprised of glycosphingolipids and protein receptors, and serving as sites for assembling signaling molecules, influencing membrane protein trafficking, etc.

in membrane caveolae, and leads to activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) and, ultimately, phospholipase A_2 (PLA_2).[85] The resulting rapid release of arachidonic acid activates PKC either directly or indirectly via its metabolite prostaglandin E_2 (PGE_2). In this view, the binding of 1,25-$(OH)_2$-D_3 to VDR, also in caveolae, stimulates its interaction with and rapid activation of the tyrosine kinase Src.

Calcium and Phosphorus Metabolism

The most clearly elucidated function of vitamin D is in the homeostasis of Ca^{2+} and phosphate. This is effected by a multihormonal system involving the controlled production of 1,25-$(OH)_2$-D_3, which functions in concert with PTH and calcitonin (CT). Regulation of this system occurs at the points of intestinal absorption, bone accretion and mobilization, and renal excretion.

Intestinal Absorption of Ca^{2+}

Calcium is absorbed in the small intestine[86] by both transcellular and paracellular mechanisms. The former is an active, saturable process, occurring in mammals primarily in the duodenum and upper jejunum and constitutes the most important means of absorbing Ca under conditions of low intake of the mineral; the latter is a nonsaturable process occurring throughout the intestine and is the most important means of absorbing Ca when Ca intake is high. The active metabolite, 1,25-$(OH)_2$-D_3, stimulates the enteric absorption of Ca through roles in both mechanisms, although its mechanism in the paracellular process is unclear. The availability of Ca for both processes is affected by both exogenous (e.g., inhibition by food phytates or P) and endogenous (e.g., gastric acid secretion) factors.

The transcellular absorption of Ca^{2+} progresses in three steps, each dependent on 1,25-$(OH)_2$-D_3:

1. **Uptake of Ca^{2+} from the intestinal lumen**. At the microvillus brush border, Ca^{2+} diffuses through a channel or integral membrane transporter (CaT1) gated by the intercellular Ca^{2+} concentration, which affords controlled movement of Ca^{2+} down a steep electrochemical gradient.[87] Vitamin D treatment of cells in culture increases Ca^{2+} uptake, with increased expression of CaT1- and Ca^{2+}-binding proteins (calbindins), which appear to control the microvillar Ca^{2+} channel. Vitamin D also shifts synthesis from phosphatidylethanolamine to phosphatidylcholine, affecting membrane fluidity and increasing the association of the Ca^{2+}-binding protein calmodulin[88] with the brush border.
2. **Translocation of Ca^{2+} across the mucosal cell**. Movement of Ca^{2+} across the enterocyte appears to be facilitated by both calbindins and vesicular transport.
3. **Extrusion of Ca^{2+} into the circulation**. Ca^{2+} moves across the basolateral membrane against a substantial thermodynamic gradient (a 50,000-fold differential in Ca^{2+} concentration and a positive electrical potential)[89] facilitated by a Ca^{2+}-ATPase.[90] This Ca^{2+} pump is stimulated by calmodulin and calbindin. Ca^{+2} is also extruded by a membrane $Na+/Ca^{2+}$ exchanger.[91]

The paracellular absorption of Ca^{+2} is less well understood. Evidence suggests that it is nonsaturable but stimulated by 1,25-$(OH)_2$-D_3 and regulated by the proteins comprising the tight junction complex at the apical region. Diffusion of Ca^{+2} through this pathway occurs when intralumenal Ca^{+2} concentrations exceed 2–6 n*M*. Some researchers have found vitamin D to promote Ca uptake by this pathway, perhaps by 1,25-$(OH)_2$-D_3-mediated activation of PKC, which is known to increase paracellular permeability.

Intestinal Phosphate Absorption

Healthy individuals absorb dietary phosphate (P_i)[92] with efficiencies of 60–65%, most absorption occurring in the duodenum and jejunum. Phosphate is absorbed by two mechanisms: a nonsaturable paracellular pathway and a saturable, energy-requiring, Na^+-dependent process on the mucosal surface that is driven by a Na^+ gradient maintained by the Na^+, K^+-ATPase on the basolateral membrane. The latter process appears to be rate limiting to P_i absorption. Vitamin D, as 1,25-$(OH)_2$-D_3, increases net P_i

85. Doroudi, M., Schwart, Z., Boyan, B.D., 2015. J. Steroid Biochem. Mol. Biol. 147, 81–87.
86. Some 70–80% of enteric calcium absorption occurs in the ileum. The colon may be responsible for 3–8% of Ca absorption.
87. Luminal concentrations of Ca^{2+} can be in the mM range; whereas, intracellular concentrations of the free ion are in the range of 50–100 nM.
88. Calmodulin is a 17 kDa acidic protein (also of the "EF hand" family) expressed in many cell types and subcellular compartments. It can bind as many as four Ca^{2+} ions, which causes conformational change and posttranslational modifications that allow it also to bind to more than 100 target proteins. In this way, calmodulin serves as a major transducer of Ca^{2+} signals in the control of cellular metabolism.
89. It is estimated that the movement of 1 mole of Ca^{2+} against this gradient requires about 9.3 kcal.
90. The CaATPase spans the membrane with a Ca^{2+}-binding domain on the cytoplasmic side. It appears that phosphorylation-induced conformational changes in the protein allow it to form a channel-like opening through which Ca^{2+} is expelled, thus serving as a Ca "pump."
91. The efflux of 1 mole of Ca^{2+} is linked to the influx of 3 moles of Na^+, thus generating negative cytosolic electropotential.
92. P_i is an essential constituent of bone and teeth, which account for some 85% of total body P_i. It is also important in regulating the genes, *PHEX* and *FGF23*. P_i comprises 1% of adult body weight.

uptake by increasing Na^+/P_i cotransport across the mucosal brush border, apparently through upregulation of the transporter.

Renal Resorption of Calcium and Phosphate

Vitamin D, as 1,25-$(OH)_2$-D_3, stimulates the resorption of both P_i and Ca^{2+} in the renal tubule.[93] The quantitative significance of this effect is greater for P_i some 60% of which is reabsorbed in the proximal tubule by a Na^+/P_i cotransport mechanism analogous to those in the intestinal epithelium and in bone. The Na^+/P_i cotransporter, Npt2a, is expressed in proximal tubular cells where the major portion of P_i is reabsorbed; its expression is also upregulated by both PTH and P_i supply. In contrast, Ca^{2+} is reabsorbed mostly in the distal tubule, primarily (80%) by passive, vitamin D-independent, paracellular routes in the proximal tubules and ascending loop of Henle. Some 8000 mg of Ca are filtered at the glomerulus daily,[94] 98% of which is reabsorbed in the tubules. The transcellular process resembles that of the intestine in having a Ca^{2+} channel component, cytosolic Ca^{2+}-binding proteins (calbindins-D_{9k} and -D_{28k}), and a plasma membrane Ca^{2+} ATPase, all of which are located in the distal portions of 1,25-$(OH)_2$-D_3-responsive nephrons.

Calcium and Phosphate Homeostasis

Homeostatic control of Ca and P is dependent on functions of the parathyroid and thyroid glands (Fig. 7.13). Each gland senses serum Ca^{2+} level[95] by a cell surface G protein-like, **Ca^{2+}-sensing receptor (CaR)** in chief cells of the parathyroid and parafollicular cells (C cells) of the thyroid.[96] The CaR appears sensitive to fluctuations in plasma Ca^{2+} of a few percent, responding by regulating the synthesis of two calcitropic hormones: PTH by the parathyroid and CT by the thyroid, which elevate and lower plasma Ca^{2+}, respectively. When Ca^{2+} levels drop the parathyroid loses VDR, which reduces its sensitivity to 1,25-$(OH)_2$-D_3. It also secretes PTH into the circulation, which acts on target tissues to restore plasma Ca^{2+} by stimulating renal tubular Ca^{2+} reabsorption and renal 1-hydroxylation of 25-OH-D_3.[97] The resulting increase in circulating 1,25-$(OH)_2$-D_3 stimulates enteric Ca^{2+}

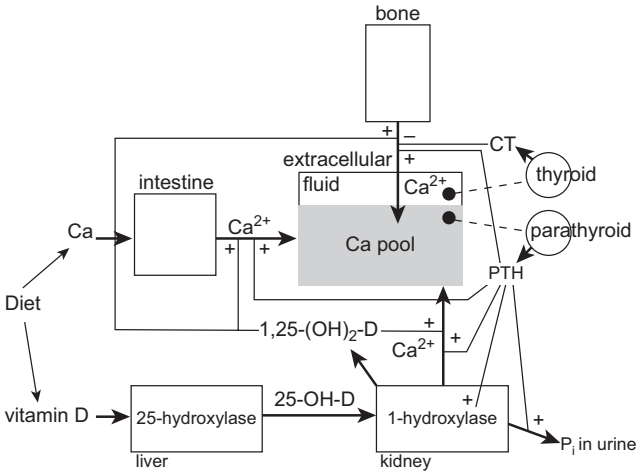


FIGURE 7.13 Calcium homeostasis.

absorption and osteoclastic activity. Demineralization of bone serves to mobilize Ca^{2+} and P_i from that reserve, thus maintaining the homeostasis of those minerals in the plasma.[98] Increasing plasma Ca^{2+} levels evoke signaling by CaR in thyroid C cells to increase the expression of CT, which inhibits bone resorption and, at high doses, increases urinary Ca^{2+} excretion. This system feeds back to regulate the synthesis of PTH through inhibition by 1,25-$(OH)_2$-D_3 binding a negative VDRE near the promotor of the PTH gene. A similar mechanism has been proposed for the downregulation of CT by 1,25-$(OH)_2$-D_3. The interplay of these hormones with Ca^{2+} and 1,25-$(OH)_2$-D_3 produces fine control of circulating Ca^{2+} levels at 4–5.6 mg/dL.[99]

Under **hypercalcemic** conditions, CT is secreted by the thyroid. The hormone suppresses bone mobilization and is also thought to increase the renal excretion of both Ca^{2+} and P_i. In that situation, the 25-OH-vitamin D 1-hydroxylase may be feedback inhibited by 1,25-$(OH)_2$-D_3, which may actually convert to the catalysis of the 24-hydroxylation of 25-OH-D_3. In the case of egg-laying birds, which show relatively high circulating concentrations of 1,25-$(OH)_2$-D_3, the 1-hydroxylase activity remains stimulated by the hormone prolactin.

Secondary Hyperparathyroidism

It is characterized by elevated serum PTH concentrations, is common among elderly people. The condition can reflect some degree of renal insufficiency, with associated reduction in renal 25-OH-vitamin D_3 1-hydroxylase activity. Serum concentrations of PTH can also increase owing to

93. Each day the human kidney filters some 8 g calcium at the glomerulus, 98% of which is reabsorbed.

94. The concentration of ultrafilterable Ca in plasma, c. 1.35 mM (c.55% of total plasma Ca), is similar to that of the glomerular fluid.

95. Although tightly maintained, serum Ca^{2+} levels are normally 10,000 times greater than intracellular ones. Perturbation of this ion gradient by transient increases in intracellular Ca^{2+} concentrations can signal different cellular responses (e.g., transcriptional control, neurotransmitter release, muscular contraction) through the actions of binding proteins.

96. CaR is also expressed in renal tubules.

97. Parathyroidectomized animals cannot mount this 1-hydroxylase response unless treated with PTH.

98. The normal ranges of these parameters in human adults are Ca, 8.5–10.6 mg/dL; P, 2.5–4.5 mg/dL.

99. This corresponds to 8–10 mg total Ca per deciliter, about half of which is present in ionized form (Ca^{2+}).

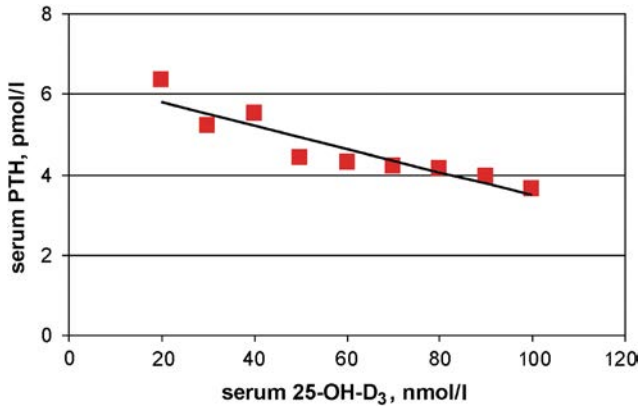


FIGURE 7.14 Relationship of serum PTH and vitamin D status. *Need, A.G., Horowitz, M., Morris, H.A., Nordin, B.C., 2000. Am. J. Clin. Nutr. 71, 1577–1581.*

privational vitamin D deficiency, in which case it is manifested by low circulating levels of 25-OH-D_3 (Fig. 7.14). Accordingly, the PTH levels of people living in northern latitudes are highest during the winter for subjects not taking supplemental vitamin D.

Bone Mineral Turnover

Bone is the predominant target organ for vitamin D, accumulating more than one-quarter of a single dose of the vitamin within a few hours of its administration. That lesions in bone mineralization (rickets, osteomalacia) occur in vitamin D deficiency has long indicated its vital function in the metabolism of this organ.[100] The pattern of vitamin D metabolites in bone differs from that in intestine; whereas the latter contains mainly 1,25-$(OH)_2$-D_3, bone contains mainly 25-OH-D_3 (accounting for >50% of the vitamin D metabolites present, with 1,25-$(OH)_2$-D_3 comprising less than 35%). As in plasma, the level of 24,25-$(OH)_2$-D_3 in bone is fairly constant relative to that of 25-OH-D_3.

Vitamin D contributes to both the formation (mineralization) and the mobilization of bone mineral (demineralization). Bone mineralization is epitaxial, occurring by codeposition of Ca^{2+}, P_i (i.e., PO_4^{3-}), and hydroxyl (OH^-) ions at multiple sites on the surfaces of preexisting crystals or on topographically similar protein/lipid surfaces. The resulting structure is one comprised of small (<200 Å) crystals with an average chemistry resembling that of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Bone mineral may also contain magnesium, sodium, potassium, fluoride, strontium, phosphate, and citrate. The amount of bone mineral is, therefore, a function of the balance of the laying down of mineral[101] by bone-forming cells called **osteoblasts**, and the dissolution of bone crystal[102] by bone-resorbing cells called **osteoclasts**. Calcium deposition in the skeleton involves the intracellular synthesis of collagen and fibrils by osteoblasts, which extrude these fibrils to form the extracellular matrix of bone, portions of which can be mineralized. Bone demineralization is directed by multinucleated osteoclasts that release proteins and lysosomal enzymes that dissolve bone mineral and lyse its organic matrix. The accretion/mobilization of bone Ca^{2+}, therefore, involves the relative activities of osteoblasts and osteoclasts with the bone surface serving, in effect, as a Ca buffer. Bone growth results from the dominance of osteoblastic activity, which in the long bones is organized by the arraying of chondrocytes to affect periosteal apposition along epiphyseal growth plates (Fig. 7.15). Balanced demineralization–mineralization affects the coordinated growth ("modeling") of skeletal bone and the "remodeling" (primarily in the endosteal area) of mature bone to replace damage and prevent senescence.

While the ultimate effects on bone involve both osteoblasts and osteoclasts, vitamin D targets only osteoblasts and osteoprogenitor cells, both of which have VDRs that are stimulated by glucocorticoids. 1,25-Dihydroxyvitamin D_3 affects the expression of several osteoblast genes (Table 7.12). It is generally thought that 24,25-$(OH)_2$-D_3, which is concentrated in the epiphyseal cartilage, may also be involved in this process. While the mechanism remains unclear, it appears to involve PTH[103] and, because PTH stimulates adenylate cyclase activity, perhaps act via cAMP. Vitamin D affects osteoclasts only by indirect means, as osteoclasts are activated by osteoblasts through the induction of the membrane-associated receptor activator of nuclear factor κB (NFκB) ligand (RANKL). Vitamin D also has a role in the differentiation of macrophages to osteoclasts; the number of these giant multinucleated bone-degrading cells is very low in bone from vitamin D-deficient animals.

In the absence of adequate levels of 1,25-$(OH)_2$-D_3, the failure of mineralization and/or net excess of osteoclastic demineralization have structural and functional consequences to bone, as bone density is a primary determinant of bone strength. This ultimately results in the well-known clinical signs (see section Signs of Vitamin D deficiency). Inadequate vitamin D status may also be associated with fracture risk, particularly in older individuals

100. Thus, the involvement of vitamin D in the metabolism of Ca and P was clear, as structural bone contains 99% of total body Ca and 85% of total body P, i.e., 1200 g Ca and 770 g P in a 70 kg man.

101. Mineralization is preceded by secretion of the bone matrix, unmineralized osteoid, by chondrocytes.

102. Dissolution of bone mineral is followed by depolymerization of glycosaminoglycans and digestion of collagen and other bone matrix proteins.

103. Because PTH is secreted in response to hypophosphatemia, deprivation of P_i can also lead to bone demineralization with increases in the activity of 25-OH-vitamin D 1-hydroxylase and accumulation of 1,25-$(OH)_2$-D_3 in target tissues.

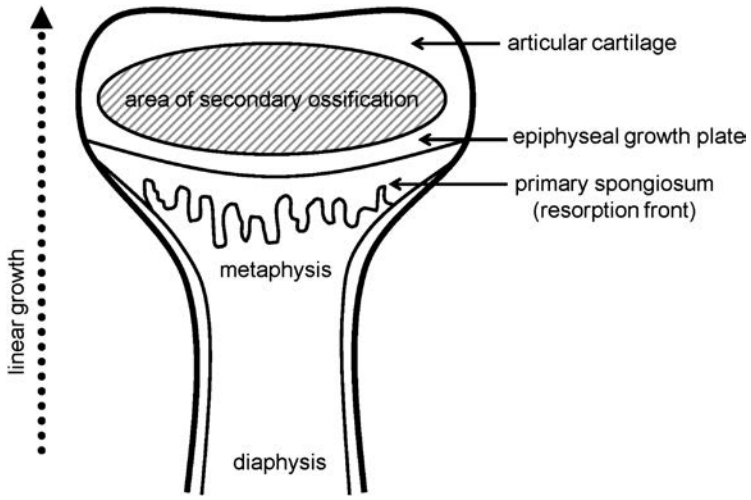


FIGURE 7.15 Linear growth of long bones occurs by deposition of bone along the epiphyseal growth plates comprised of arrays of chondrocytes apical of a highly vascularized zone of spongiform bone.

TABLE 7.12 Vitamin D-Responsive Osteoblast Genes

Collagen
Alkaline phosphatase
Osteocalcin
Osteopontin
Bone sialoprotein-transforming growth factor-β (TGF-β)
Vascular endothelial growth factor (VEGF)
Matrix metalloproteinase-9 (MMP-9)
β3-integrin
Receptor activity of NFκB (RANKL)
Osteopetegrin

TABLE 7.13 Abnormalities in Vitamin D Status of Older Hip Fracture Patients

Parameter	Controls[a] n = 78	Patients[b] n = 120
Dietary Intakes		
Ca (mg/day)	696 ± 273	671 ± 406
Vitamin D (IU/day)	114 ± 44	116 ± 63
Serum Analytes		
Ca (m*M*)	2.35 ± 0.12	2.13 ± 0.16
P_i (m*M*)	1.09 ± 0.15	1.11 ± 0.26
Alkaline phosphatase (units)	2.1 ± 0.5	2.0 ± 0.7
Albumin (g/liter)	41.9 ± 2.8	32.5 ± 4.8[b]
Vitamin D-binding protein (DBP) (mg/liter)	371 ± 44	315 ± 60[b]
25-OH-D_3 (n*M*)	32.9 ± 13.6	18.5 ± 10.6[b]
24,25-$(OH)_2$-D_3 (n*M*)	1.8	0.5[b]
1,25-$(OH)_2$-D_3 (p*M*)	105 ± 3 1	79 ± 46[b]
Parathyroid hormone (PTH) (μg Eq/liter)	0.12 ± 0.05	0.11 ± 0.05

[a]*Subjects in both groups were in their eighth decade and had similar histories of sun exposure (one-third with high exposure).*
[b]$p < .05$.
Lips, P., van Ginkel, F.C., Jongen, M.H.M., et al., 1987. Am. J. Clin. Nutr. 46, 1005–1010.

(Tables 7.13 and 7.14). A meta-analysis of 12 randomized, controlled trials revealed that vitamin D doses of 700–800 IU (17.5–20 mg) per day reduced the relative risk of hip fracture by 26% and of any nonvertebral fracture by 23%.[104] Risk reductions were not observed for trials that used a lower vitamin dose (400 IU [10 mg]/day).

African-Americans exhibit lower circulating levels of 25-OH-D_3 than white Americans (Fig. 7.16) suggesting increased risk of fracture relative to whites. However, the reverse is true: the prevalences of both hip fracture and osteoporosis of African-Americans are about half that of White Americans.[105] This has been explained on the basis of multiple characteristics of African-Americans that affects fracture risk.[106] Compared to whites, African-Americans tend to have greater peak bone mass, greater

104. Bischoff-Ferrari, H.A., Willett, W.C., Wong, J.B., et al., 2005. J. Am. Med. Assoc. 293, 2257–2264.
105. Barrett-Conner, E., Siris, E.S., Wehren, L.E., et al., 2005. J. Bone Miner. Res. 20, 185–194.
106. Cosman, F., Nieves, J., Dempster, D., et al., 2007. J. Bone Miner. Res. 22, V34–V38; Aloia, J.F., 2008. Am. J. Clin. Nutr. 88, 545S–550S.

TABLE 7.14 Reduction of Fracture Risk in Women by 1,25-$(OH)_2$-Vitamin D_3

Treatment	Year	Women in Study	Women With New Fractures	Number of New Fractures
1,25-$(OH)_2$-D_3	1	262	14	23
	2	236	14	22
	3	213	12	21
Ca	1	253	17	26
	2	240	30[a]	60[b]
	3	219	44[b]	69[b]

[a]$p < .01$.
[b]$p < .001$.
Tilyard, M.W., Spears, G.F.S., Thomson, J., Dovey, S., 1992. N. Engl. J. Med. 326, 357–362.

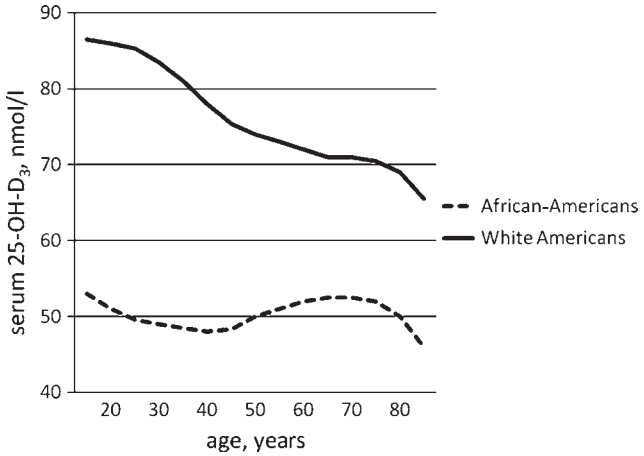


FIGURE 7.16 Serum 25-OH-D_3 levels in African-American and White Americans in the third National Health and Nutrition Examination Survey.

muscle mass, and lower bone turnover rates. They are also more likely to be obese, which gives their bone mineralization the positive stimulation of loading. While the risks of falls is not different in African-Americans from whites, their shorter hip axis length protects against osteoporotic fractures caused by falls. Studies with adolescent girls have found that African-Americans have better enteric Ca absorption and renal Ca conservation that whites. Further, African-American adults have higher levels of PTH without associated bone loss, indicating skeletal resistance to PTH. That these advantages diminish with age are evidenced by the fact that, like other groups, elderly African-Americans with elevated PTH experience bone loss.

VDR genotype appears to contribute significantly to the variation observed in bone mineral density in populations, 80% of which is thought to be due to genetic factors. Two VDR polymorphisms, Fok1 and Bsm1, have been identified as independent risk factors for stress fracture. Individuals with the B- or f-containing genotypes with respect to the each polymorphism were more likely to develop fractures than individuals without those alleles.[107]

Roles of Other Minerals

Vitamin D function can be affected by several other mineral elements:

- **Zinc.** Deprivation of zinc reduces the 1,25-(OH)2-D_3 response to low Ca intake.[108] It has been suggested that zinc may indirectly affect the renal 25-OH-D_3 1-hydroxylase.
- **Iron.** Iron deficiency is associated with low-serum 24,25-(OH)2-D3 levels and reduced 25-OH-D3 responses to supplemental D3. It has been suggested that iron deficiency, which is known to impair the enteric absorption of fat and vitamin A, may also impair the absorption of vitamin D.
- **Lead.** Exposure to lead appears also to impair the 1-hydroxylation of 25-OH-D3. That effect increases the enteric absorption of lead, which can be bound by the vitamin D-responsive protein, calbindin. In children, blood levels of 1,25-(OH)2-D3 and Ca^{2+} have been found to be inversely related to blood lead concentration.[109] 1,25-(OH)2-D3-mediated lead absorption is a chief contributor to the elevated body lead burden observed in Ca deficiency (Table 7.15), although chronic ingestion of lead by Ca-deficient animals reduces 1,25-(OH)2-D3 production. This combination of effects allows the hormone system impaired by chronic lead ingestion to contribute to susceptibility to lead toxicity.[110]

107. Chatzipapas, C., Boikos, S., Drosos, G.I., et al., 2009. Horm. Metab. Res. 41, 635–640; Diogenes, M.E.L., Bezerra, F.F., Cabello, G.M.K., et al., 2010. Eur. J. Appl. Physiol. 108, 31–38.
108. This finding may have clinical significance, as in many parts of the world children are undernourished with respect to both Zn and Ca.
109. The prevalence of lead toxicity among children is seasonal, i.e., greatest in the summer months.
110. Lead poisoning is a serious environmental health issue. In the United States alone, high-blood lead levels are estimated in as many as five million school children and 400,000 pregnant women.

TABLE 7.15 Vitamin D-Stimulated Uptake and Retention of Lead by the Chick

Diet		Kidney		Tibia Ash	
Vitamin D	Pb, %	Ca, ppm	Pb, ppm	Ca, ppm	Pb, ppm
—	0	69.0 ± 8.8	0	33.2 ± 0.4	23.4 ± 14.3
1,25-$(OH)_2$-D_3	0	64.1 ± 1.5	0	36.0 ± 0.2	10.9 ± 6.7
—	0.2	56.6 ± 3.8	4.8 ± 0.5	33.5 ± 0.3	133.1 ± 24.1
1,25-$(OH)_2$-D_3	0.2	80.2 ± 13.4	13.7 ± 2.4	35.5 ± 0.2	335.1 ± 15.8
—	0.8	62.4 ± 2.4	9.2 ± 0.9	32.8 ± 0.2	299.8 ± 4.8
1,25-$(OH)_2$-D_3	0.8	90.1 ± 7.6	32.4 ± 7.6	34.7 ± 0.4	1008.8 ± 71.2

Fullmer, C.S., 1990. Proc. Soc. Exp. Biol. Med. 194, 258–264. Means with like superscripts are not significantly different ($p < .05$.).

TABLE 7.16 Experimental Evidence for Vitamin D Functions in Noncalcified Tissues

Putative Role	Observations
Cell differentiation	Promotion by 1,25-$(OH)_2$-D_3 of myeloid leukemic precursor cells to differentiate into cells resembling macrophages
Membrane structure	Alteration of the fatty acid composition of enterocytes, reducing their membrane fluidity
Mitochondrial metabolism	Decrease in isocitrate lyase and malate synthase (shown in rachitic chicks)
Muscular function	Stimulation of Ca^{2+} transport into the sarcoplasmic reticulum of cultured myeloblasts by 1,25-$(OH)_2$-D_3 Easing, on treatment with vitamin D, of electrophysiological abnormalities in muscle contraction and relaxation in vitamin D-deficient humans Reduction, on treatment with vitamin D, of muscular weakness in humans
Pancreatic function	Stimulation of insulin production by pancreatic β cells in rats by 1,25-$(OH)_2$-D_3 Impairment of insulin secretion, unrelated to the level of circulating Ca, shown in vitamin D-deficient humans
Immunity	Stimulation of immune cell functions by 1,25-$(OH)_2$-D_3 Control of inflammation by 1,25-$(OH)_2$-D_3-dependent regulation of cytokine production
Neural function	Region-specific enhancement of choline acetyltransferase in rat brain by 1,25-$(OH)_2$-D_3
Skin	Inhibition of DNA synthesis in mouse epidermal cells by 1,25-$(OH)_2$-D_3
Parathyroid function	Inhibition of transcription of PTH gene via interaction of 1,25-$(OH)_2$-D_3 and DNA in parathyroid cells

Vitamin D Functions in Noncalcified Tissues

That 1,25-$(OH)_2$-D_3 and nuclear VDRs occur in tissues not directly involved in Ca^{+2} homeostasis (e.g., pancreatic β cells,[111] skin Malpighian layer cells, specific brain cells, pituitary, muscle, mammary gland, endocrine cells of the stomach, the chorioallantoic membrane surrounding chick embryos) suggests that the vitamin functions in the regulation and differentiation of many cell types (Table 7.16). These functions occur via VDR: at least 100 proteins (including several oncogenes) are known to be regulated by 1,25-$(OH)_2$-D_3. Responses to 1,25-$(OH)_2$-D_3 are observed at concentrations two to three orders of magnitude greater than circulating levels; hence, it is possible that under normal circumstances they may be limited to specific sites of local production of the active metabolite.

Muscular Function

That vitamin D plays an important role in muscle is evidenced by the muscle weakness typically experienced by vitamin D-deficient subjects, the presence of VDR in myocytes, and the lack of muscle development in VDR-knockout mice. 1,25-$(OH)_2$-D_3 is essential for the

111. It is of interest to note that circulating insulin levels are reduced in vitamin D deficiency and respond quickly to treatment with 1,25-$(OH)_2$-D_3.

homeostatic control of intracellular Ca^{+2}, affecting both muscle contractility and myogenesis. The former effect involves the transcriptional regulation of various Ca^{+2}-binding proteins, including calbindin-D_{9K}, as well as enzymes related to the synthesis of phosphatidylcholine. Myocytes treated with 1,25-$(OH)_2$-D_3 respond by activating PKC and transporting Ca^{2+} in the sarcoplasmic reticulum, an effect necessary for muscle contraction. They also show upregulation of tyrosine phosphorylation of the MAP kinase cascade,[112] stimulating proliferation and growth. Rapid responses of skeletal muscle have been observed for 1,25-$(OH)_2$-D_3: Ca^{+2} uptake through voltage-dependent channels and Ca^{2+} release by intracellular stores. Thus, it is not surprising that muscular weakness has been noted in subjects with low plasma 25-OH-D_3 levels, and plasma 25-OH-D_3 predicts the rate of strength recovery after exercise-induced muscular injury.[113] This effect may involve activation of mTOR signaling[114] in muscle, which has been shown in 25-OH-D_3-stimulated muscle growth in the chicken.[115] Patients with rickets experience weakness, hypotonia[116], and atrophy. Emerging evidence suggests that, in vitamin D-adequate individuals, skeletal muscle may serve as a storage site for 25-OH-D_3. By supporting muscular function, vitamin D reduces risks of falling and, consequently, fractures.[117]

Immune Function

Studies have shown that 1,25-$(OH)_2$-D_3 supports normal function of both the innate and adaptive components of the immune system as well as the inflammatory cascade.[118] VDRs have been identified in most immune cells, including most antigen-presenting cells (e.g., macrophages, dendritic cells) and $CD4^+$, $CD8^+$ T-lymphocytes. Many of these cells also express the 25-OH-D_3 1- and -24-hydroxylases and are, thus, capable of producing and catabolizing 1,25-$(OH)_2$-D_3. Dendritic cells also express the vitamin D_3-25-hydroxylase. The emerging picture is one of 1,25-$(OH)_2$-D_3, obtained either from local immune cell metabolism or by transport from the kidney, modulating the phenotype and function of dendritic cells by downregulating the expression of costimulatory molecules (CD40, CD80, CD86) and the cytokine IL-12, while upregulating the expression of IL-10. This limits Th1 cell development, promotes the activity of $CD4^+$ suppressor T-cells, and promotes recruitment of regulatory T-cells (Tregs). VDR and 1,25-$(OH)_2$-D_3 also modulate the differentiation of B cell precursors and limit the proinflammatory action of the nuclear factor kappa B (NFκB) pathway. Inflammation is also affected by a 1,25-$(OH)_2$-D_3-induced protein,[119] which forms inflammasome complexes[120] that regulate IL-1β processing. Human trials have found inverse associations of inflammatory markers and plasma 25-OH-D_3 levels.[121] Vitamin D supplementation has been found to upregulate genes in immune response and inflammation pathways in the colon in ways that are mitigated by supplemental dietary calcium.[122]

Macrophages, dendritic cells, and T cells can regulate the production and turnover of 1,25-$(OH)_2$-D_3, which is required for the production of antimicrobial peptides, the **cathelicidins**. They comprise a family of peptides of 12–80 amino acid residues; most have 23–37 residues that form amphoteric α-helices enabling them to enter and disrupt bacterial and fungal membranes and viral envelopes. They also affect the permeability of cell membranes and contribute to the endothelial barrier to pathogens by increasing cell stiffness at sites of infection. They have been shown to reduce susceptibility to several pathogens[123] in both animals and humans. Cathelicidin induction involves initial activation of macrophage Toll-like receptors,[124] which upregulates expression of both VDR and the 25-OH-D_3 1-hydroxylase (CYP27B1), leading to induction of downstream targets of VDR, including cathelicidin which has a VDRE in its promotor. Cathelicidins can also synergize the proinflammatory effects of endogenous mediators. Promotion of cathelicidin secretion in the lungs appears to a means whereby locally generated 1,25-$(OH)_2$-D_3 protects from respiratory infection.

Vitamin D affects the regulation of autoimmunity. This may involve VDR and 1,25-$(OH)_2$-D_3-suppressing

112. This chain of proteins transduces signals from cell surface receptors to DNA in the nucleus. Originally called ERK (extracellular signal-related kinases), it is now call MAP for a prominent constituents, MAPK (mitogen-activated protein kinases).
113. Barker, T., Henricksen, V.T., Martins, T.R., et al., 2013. Nutrients **5**, 1253–1275.
114. mTOR (mammalian target of rapamycin) is a serine/threonine protein kinase that serves as a master regulator of cell growth and proliferation.
115. Vignale, K., Greene, E.S., Caldas, J.V., et al., 2015. J. Nutr. 145, 855–863.
116. Low muscle tone.
117. Falls cause >90% of hip fractures, which increase with age from 30% in subjects >65 years to 50% in subjects >80 years.
118. See reviews: Cantorna, M.T., Snydeer, L., Lin, Y.D. et al., 2015. Nutrients 7, 3011–3021; Baeke, F., Takiishi, T., Korf, H., et al., 2010. Curr. Opin. Pharmacol. 10, 482–496.
119. vitamin D-upregulated protein 1 (VDUP1), a multifunctional, stress-induced protein of the arrestin family of proteins.
120. i.e., Multiprotein oligomers in myeloid cells that promote maturation of inflammatory cytokines.
121. Zanetti, M., Harris, S.S., Dawson-Hughes, B., 2014. Nutr. Rev. 72, 95–98.
122. Protiva, P., Pendyala, S., Nelson, C., et al., 2016. Am. J. Clin. Nutr. 103, 1224–1231.
123. e.g., *Mycobacterium tuberculosis*, Influenza A, *Pseudomonas aeruginosa*, *Bordetella bronchiseptica*, *Aggregatibacter actinomycetemcomitans*, *Candida albicans*.
124. These are membrane-spanning receptors expressed in macrophages and dendritic cells. Their name comes from the toll gene, and the 1985 comment of Christiane Nüsslein-Volhard, "Das ist ja toll!" (That's amazing!) who shared a 1995 Nobel Prize for that discovery.

inflammation and promoting the development NKT and CD8αα T cells, as well as direct effects of cathelicidins.[125]

- **Asthma**. Several, but not all, studies have found low 25-OH-D_3 levels to be associated with increased risk of asthma.[126] It has been suggested that locally produced 1,25-$(OH)_2$-D_3 may regulate sensitivity of lymphocytes and monocytes to glucocorticoids by influencing the expression of the immune response protein FOXP3 in Tregs. This is supported by in vitro studies that have shown D_3 treatment to reduce proliferation of airway smooth muscle cells, a common feature in asthma. That VDR-knockout mice do not show inflammatory responses to experimentally induced asthma suggests that 1,25-$(OH)_2$-D_3 may be immunosuppressive, inhibiting signs/symptoms of T-helper 1 (Th1) cell-driven autoimmune disease.
- **Rheumatoid arthritis (RA)**. One study observed an inverse relationship of vitamin D intake, particularly from supplements, and risk of developing RA.[127] D_3 treatment has been found to prevent experimentally induced RA in animal models, and VDR is known to be expressed by articular chondrocytes in osteoarthritic cartilage.
- **Multiple sclerosis (MS)**. It has been long recognized that MS, which is characterized by immune attacks on the myelin sheaths of nerves, is more prevalent in northern latitudes than in the tropics, being inversely correlated to the numbers of hours of annual or winter sunlight. MS patients tend to have lower plasma 25-OH-D_3 levels than controls. Two studies found D_3 supplements to reduce MS risk by as much as 40%.[128] One study with MS subjects found D_3 supplementation to increase circulating levels of the antiinflammatory cytokine transforming growth factor 1 (TGF-1), suggesting potential for alleviating symptoms.
- **Inflammatory bowel disease**. Patients with Crohn's disease or ulcerative colitis have been found to have lower plasma 25-OH-D_3 levels than unaffected controls. Deficiency of VDR or vitamin D deprivation has been found to exacerbate the development of diarrhea and cachexia in experimental IBD with IL-10 knockout mice; VDR signaling is needed to prevent the proliferation of pathogenic CD8+ T cells.[129]
- **Insulin-dependent diabetes (type 1 diabetes, T1D)**. Vitamin D may play a role in reducing risk of developing T1D, which results from the T-cell dependent destruction of insulin-producing pancreatic β cells by cytokines and free radicals from inflammatory infiltrates. This hypothesis follows from the findings that 1,25-$(OH)_2$-D_3 inhibits its production of IL-12, thus suppressing the activity of IL-12-dependent Th1 cells in activating cytotoxic $CD8^+$ lymphocytes and macrophages. T1D prevalence is positively associated with latitude and negatively associated with hours of sunlight. High doses of 1,25-$(OH)_2$-D_3 have been shown to arrest diabetes in the nonobese diabetic mouse.[130] One prospective trial found D_3 doses of 50 μg (2 IU)/day to reduce T1D risk;[131] another detected no benefits using lower doses (<10 μg [0.4 IU]/day).[132] A large, multicenter case–control study[133] and a cohort study[134] both found vitamin D supplementation in infancy to reduce T1D development later in life.
- **Atopic dermatitis**. Low-plasma 25-OH-D_3 levels are associated with increased sensitivity to common food allergins (especially milk and wheat) in infants with atopic dermatitis.

Antioxidant Regulation

Vitamin D appears to function in the regulation of cellular antioxidant balance. Its genomic functions support antioxidant protection. Liganded VDR signals expression of different factors active in scavenging reactive oxygen species (ROS) produced intracellularly;[135] it also inhibits expression of ROS-generating factors.[136] Vitamin D can also have prooxidative effects through both genomic action in signaling expression of NADPH oxidase, and through nongenomic promotion of Ca^{2+} influx into cells, which stimulates the production of reactive nitrogen species. These actions can increase drug sensitivity of cancer cells but also may promote vascular calcification and fat accumulation in the adipocyte (major sources of ROS).

125. Marques, C.D.L., Danta, A.T., Fragoso, T.S., et al., 2010. Braz. J. Rhematol. 50, 67–80.
126. Kerley, C.P., Elnazir, B., Faul, J., et al., 2015. Pulm. Pharmacol. Ther. 32, 75–92.
127. Merlino, L.A., Curtis, J., Mikuls, T.R., et al., 2004. Arthritis Rheumatol. 50, 72–77.
128. Munger, K.L., Zhang, S.M., O'Reilly, E., et al., 2004. Neurology 62, 60–65; Munger, K.L., Levin, L.I., Hollis, B.W., et al., 2006. J. Am. Med. Assoc. 296, 2832–2838.
129. Chen, J., Bruce, D., Cantorna, M.T., 2014. BMC Immunol. 15, 6–17.
130. A 28% reduction in the autoimmune NOD mouse was reported (Mathieu, C., Waer, M., Laureys, J., et al., 1994. Diabetologia 37, 552–558), but no effect was observed in the BB rat model (Mathieu, 1997. In: Feldman, D., Glorieux, F., Pike, J. (Eds.), Vitamin D and Diabetes. Academic Press, San Diego, CA, pp. 1183–1196).
131. Hyppönen, E., Läärä, E., Reunanen, A., et al., 2001. Lancet 358, 1500–1503.
132. Stene, L.C., Ulriksen, J., Magnus, P., Joner, G., 2003. Am. J. Clin. Nutr. 78, 1128–1134.
133. EURODIAB substudy 2 study group, 1999. Diabetologia 42, 51–54.
134. Hyppönen, E., Läärä, E., Reunanen, A., et al., 2001. Lancet 358, 1500–1503.
135. Superoxide dismutase, glutathione peroxidase, catalase, thioredoxin reductase, nuclear regulatory factor 2.
136. e.g., Inducible nitric oxide synthase (iNOS), nitric oxides (NOX).

Cardiovascular Health

Vitamin D may contribute to cardiovascular health. Epidemiological studies have shown that low-circulating 25-OH-D_3 levels, as well as factors known to affect vitamin D status (latitude, altitude, season), are associated with the prevalence of coronary risk factors, with long-term risk of stroke, and with cardiovascular disease (CVD) mortality. Severe vitamin D deficiency is associated with in-hospital cardiovascular mortality in patients with acute coronary disease. These effects may be the result of vitamin D function in the proliferation of vascular smooth muscle, Ca^{2+} homeostasis, regulation of inflammation, and regulation of the renin–angiotensin system.

Neurologic Function

A role of vitamin D in brain development and function was first indicated by the finding of 25-OH-D_3 in cerebrospinal fluid and of expression of both VDR and 25-OH-D_3-1-hydroxylase in neurons and glial cells.[137] Maternal deprivation of vitamin D can alter in brain morphology, stem cell proliferation, and expression of neurotrophic factors in rat pups. Low-vitamin D status in infants has been linked to abnormalities manifest in adulthood, including sensitivity to psychosis-inducing drugs, schizophrenia, and Parkinson's disease; and adequate vitamin D status has been associated with reduced risk of cognitive decline and dementia in older adults.[138] These effects are thought to reflect needs for vitamin D in maintaining dopaminergic neurotransmitter systems, regulating intracellular Ca^{2+} homeostasis, and prevention of oxidative damage.[139] It has been suggested that vitamin D may act as a neurosteroid to protect the brain from secondary insults.

Skin Health

Vitamin D has a paracrine function in the skin. Keratinocytes express 25-(OH)2-D3-1-hydroxylase. Therefore, they cannot only produce D_3 with solar exposure, they can also metabolize it to 1,25-$(OH)_2$-D_3, which has been shown to confer photoprotection by minimizing UV-induced DNA damage, inflammation, and cell death in that organ. Studies have shown that 1,25-$(OH)_2$-D_3 can inhibit proliferation and induce terminal differentiation of cultured keratinocytes. These effects appear to be mediated by VDRs, which are expressed throughout the epidermis as well as in hair follicles and skin immune cells.[140] Among the gene products induced by VDR activation in the skin is cathelicidin,[141] which functions both in the direct killing of pathogens as well as a host response involving cytokine release, inflammation, and cellular immune response. Cathelicidin abnormalities result in skin diseases including atopic dermatitis, rosacea, and psoriasis. Decreased levels of cathelicidin have been observed in atopic dermatitis, and abnormal processing of the cathelicidin peptide has been found to be involved in the inflammatory and vascular responses in rosacea. Patients with hereditary vitamin D-resistant rickets have VDR mutations and show alopecia; however, the basis of that symptom is unclear, as alopecia is not seen in subjects with simple vitamin D deficiency or loss of CYP27B1.

Gut Microbiome

Recent studies have demonstrated a role of vitamin D in regulating the gut microbiome. That vitamin D may protect against enteric infection was indicated by the findings of higher circulating 25-OH-D_3 levels associated with reduced risk to *Clostridium difficile* infection in patients with inflammatory bowel syndromes, and more moderate symptoms in patients with Crohn's disease. Genetic deletion of CYP27B1 (hence, the ability to produce 1,25-$(OH)_2$-D_3) or VDR in the mouse has been shown to increase numbers of *Bacteroidetes* and *Proteobacteria* species at the expense of beneficial members of the *Firmicutes* and *Deferribacteria* phyla. This shift in microbial composition appears to amplify the host response to injury, as was manifest as increased risk to colitis induced by dextran sodium sulfate.[142] The basis of the action of vitamin D in supporting a healthy gut microbiome may be the gut epithelial signaling of VDR, which has been shown to protect the mucosal barrier by inhibiting inflammation-induced epithelial cell apoptosis and resisting the effects of an inflammation-induced VDR-targeting microRNA[143] With emerging evidence of signaling between the microbiome and the gut, the body's largest immune organ, it is possible that effects

137. Interestingly, a few cells appear to express the 1-hydroxylase but not VDR: macrocellular cells in the nucleus basalis of Meynert, and Purkinje cells of the cerebellum.

138. Eyles, D.W., Burne, T.H.J., McGrath, J.J., 2013. Front. Neuroendocrinol. 34, 47–64.

139. Cui, X., Gooch, H., Groves, N.J., et al., 2015. J. Steroid Biochem. Mol. Biol. 148, 305–309; Wrzosek, M., Kakaszkiewicz, J., Wrzosek, M., et al., 2013. Pharmacol. Rep. 65, 271–278.

140. VDRs are expressed by the majority of Langerhans cells, macrophages, and T lymphocytes in skin.

141. The cathelicidins are among 20 or more antimicrobial proteins, including the β-definsins, calprotectin and adrenomedullin. Only the cathelicidins are known to be modulated by vitamin D (*see* Schauber, J., Gallow, R.L., 2008. Exp. Dermatol. 17, 633–639).

142. Ooi, J.H., Li, Y., Rogers, C.J., et al., 2014. J. Nutr. 143, 1679–1686.

143. Li, Y.C., Chen, Y., Du, J., 2015. J. Steroid Biochem. Mol. Biol. 148, 179–183.

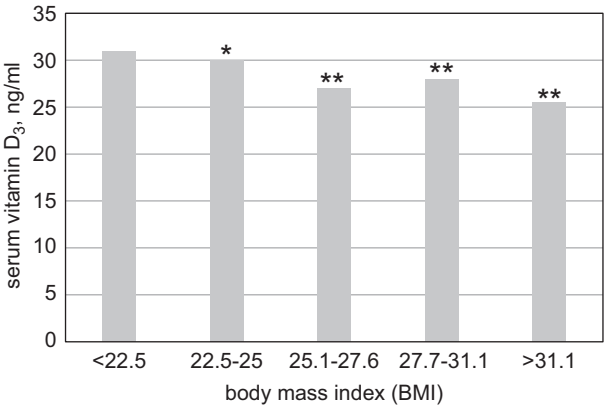


FIGURE 7.17 Inverse relationship of vitamin D status and body mass index apparent in National Health and Nutrition Examination Survey (NHANES) III subjects. Comparisons with lowest quintile: $*p < .05$, $**p < 0.01$. *After Black, P.N., Scrugg, R., 2005. Chest 128, 3792–3798.*

of vitamin D on gut microbial homeostasis may affect immune function of the host.

Adipose Function

Vitamin partitions into bulk lipid depots in adipose tissue where 1,25-$(OH)_2$-D_3 can be produced and functions in the regulation of cytokine production. Sequestration of 25-OH-D_3 is dependent on the degree of adiposity, which is manifest as a reduction of circulating 25-OH-D_3 level (Fig. 7.17). It has been suggested that 25-OH-D_3 evolved as a UVB indicator for species with low-dietary vitamin D intakes; for them, shortening autumnal day length would be detected by declining plasma 25-OH-D_3 levels, which would stimulate accumulation of fat and induce a winter metabolism, which in contemporary health would be the metabolic syndrome. Therefore, it has been proposed that vitamin D deficiency may contribute to obesity[144] and that, by stabilizing 25-OH-D_3 levels, supplemental D_3 prevent obesity. This is supported by findings that 1,25-$(OH)_2$-D_3 can induce apoptosis in adipocytes; that effect appears to involve VDR competing with RXR for binding PPARγ (peroxisome-proliferator activation receptor) and suppressing fat deposition by promoting expression of the steroid-metabolizing enzyme 11β-hydroxysteroid hydroxylase. Studies have shown that serum 25-OH-D_3 levels are inversely correlated with BMI[145], body fat mass and insulin resistance, and directly associated with weight loss from caloric restriction.[146] That vitamin D functions in supporting insulin sensitivity is indicated by the findings that 1,25-$(OH)_2$-D_3 upregulates the glucose transporter 4 (GLUT4) translocation and insulin signaling in adipocytes,[147] and that VDR polymorphisms have been associated with increased visceral adipose tisssue mass, increased plasma adipokine concentrations, high fasting glucose levels and elevated risk to noninsulin-dependent diabetes (type 2 diabetes, T2D).[148] While a few clinical trials have found weight loss to improve 25-OH-D_3 levels and D_3 supplements to improve insulin sensitivity, such effects have not been observed in most trials. As such, the efficacy of vitamin D in preventing T2D may depend on an individual's degree of adiposity as well as chronic inflammatory status.

Pregnancy

Circulating levels of 1,25-$(OH)_2$-D_3 increase by two- to three-fold in the first trimester of pregnancy, apparently due to the expression of the 1-hydroxylase and suppression of the 24-hydroxylase in placental and decidual cells, which also express VDR. It has been suggested that locally produced 1,25-$(OH)_2$-D_3 functions in the placenta to suppress Th1-dependent immunity to facilitate immune tolerance to implantation, affecting successful fetal maintenance; it also appears to support antimicrobial and antiinflammatory functions. These functions are thought to underlie the observation of lower levels of HIV transmission by vitamin D-adequate mothers to their fetuses. That vitamin D functions in normal fetal development is indicated by meta-analyses that found low-maternal serum 25-OH-D_3 levels to increase risks of preeclampsia, gestational diabetes, small-for-gestational age births, and bacterial vaginosis;[149] and studies that have found maternal vitamin D status to predict growth and later bone mass of children.

8. BIOMARKERS OF VITAMIN D STATUS

The most informative indicator of vitamin D status is the concentration of 25-OH-D_3 in serum/plasma.[150] Those levels are normally in the range of 10–40 ng/mL (25–125 nM). Serum PTH levels can also be a useful biomarker of vitamin D status. Maximal PTH levels are seen at plasma 25-OH-D_3 levels of c.100 nM; PTH expression is downregulated at increasing 25-OH-D_3 levels. PTH levels less than 45–65 nM are

144. Foss, Y.J., 2009. Med. Hyp. 72, 314–321.
145. Body mass index; BMI = weight (kg)/height (m^2), or = [weight (lb)/height (in^2)] × 705.
146. Arunabh, S., Pollack, S., Yeh, J., Aloia, J.F., 2003. J. Clin. Endocrinol. Metab. 88, 157–161; Parikh, J., Edelman, G.I., Uwaifo, R.J., et al., 2004. J. Clin. Endocrinol. Metab. 89, 1196–1199.
147. Manna, P., Jain, S.K., 2012. J. Biol. Chem. 287, 42324–42332.
148. Hitman, G.A., Mannan, N., McDermott, M.F., et al., 1998. Diabetes 47, 688–690; Oh, J.-Y., Barrett-Connor, E., 2002. Metabolism 51, 356–359; Ortlepp, J.R., Metrikat, J., Albrecht, M., et al., 2003. Diabet. Med. 20, 451–454; Khan, R.J., Riestra, P., Gebreab, S.Y., 2016. J. Nutr. 146, 1476–1482.
149. Harvey, N.C., Holroyd, C., Ntani, G., et al., 2014. Health Technol. Assess. 18, 1–190; Aghajafari, F., Nagulesapillai, T., Ronksley, P.E., et al., 2013. Br. Med. J. 346, 1169–1183.
150. Neither 1,25-$(OH)_2$-D_3 or DBP are informative as biomarkers of vitamin D status. Circulating levels of 1,25-$(OH)_2$-D_3 are tightly regulated at about 40 pg/mL (100 nM), reflecting vitamin D status only under conditions of severe deficiency and not reflecting the local production of 1,25-$(OH)_2$-D_3 by many extrarenal tissues. Most (>95%) of DBP in the plasma is not bound to vitamers D; it is present in a 100-fold excess compared to 25-OH-D_3.

TABLE 7.17 Criteria of Vitamin D Status

25-OH-D_3 Level		Status
nM	ng/ml	
<50	<20	Deficient
50–75	20–30	Insufficient
76–250	31–100	Adequate
>250	>100	Excess (risk of hypervitaminosis)

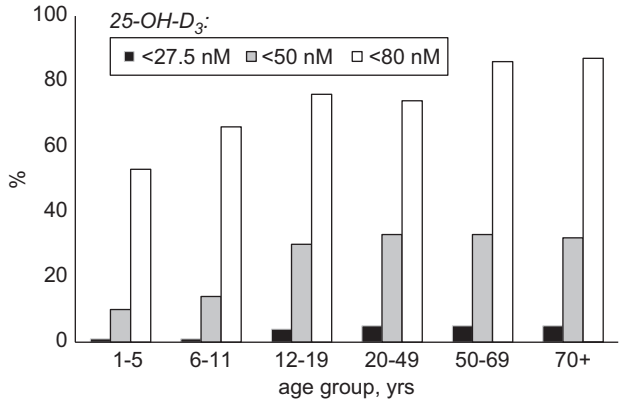


FIGURE 7.18 Prevalence of low-serum 25-OH-D3 levels in Americans, from National Health and Nutrition Examination Survey (NHANES) 2000–2004. *Yetley, E.A., 2008. Am. J. Clin. Nutr. 88, 558S–564S.*

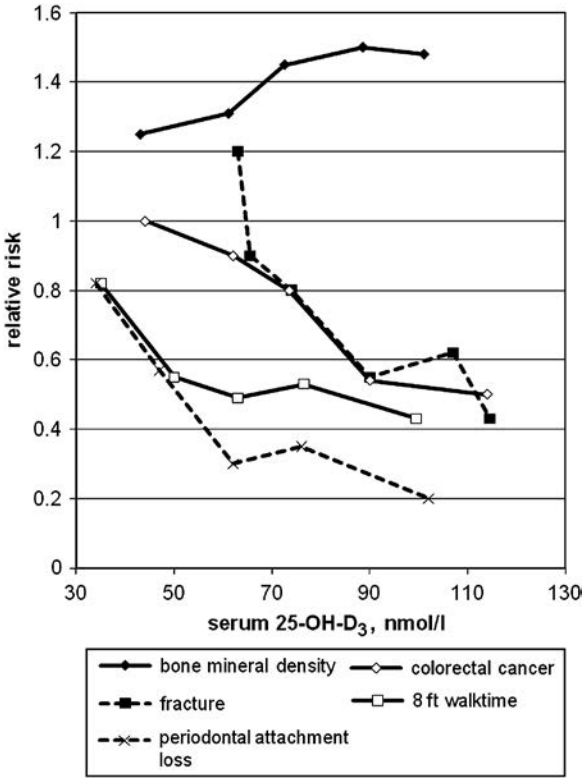


FIGURE 7.19 Relationship of vitamin D status and multiple health risks. *After Bischoff-Ferrari, H.A., Giovannuci, E., Willett, W.C., et al., 2006. Am. J. Clin. Nutr. 84, 18–28.*

associated with secondary hyperparathyroidism. A network analysis of 12 VDR target genes and 12 clinical and biochemical parameters related to vitamin D status showed PTH level to correlate with most of the other endpoints, and all of the target genes to correlate with serum 25-OH-D_3 level.

The IOM considered 50 nM adequate for bone health in adults and children,[151] although an expert consensus considered the level of 70–80 nM as optimal (Table 7.17, Fig. 7.18).[152] An analysis of multiple endpoints related to bone and dental health, lower extremity function, risks of falls, fractures, and cancer found the optimal serum 25-OH-D_3 level to be 90–100 nM (Fig. 7.19). Such levels require regular daily vitamin D intakes of greater than 1000 IU (25 mg).[153] The maintenance of such serum 25-OH-D_3 levels requires the use of solar radiation, vitamin D supplements, and/or D_2-fortified foods.

Plasma 25-OH-D_3 measurement can be affected by factors in addition to those related to biogenesis and intake of vitamin D. These include polymorphisms of 7-dehydrocholesterol reductase, DBP, and CYP2R1; in some cases their effects can be great as that of deprivation of the vitamin.[154] Pregnancy appears to suppress serum 25-OH-D_3 levels; a study in Boston found that 76% of new mothers (and 81% of their newborns) had serum 25-OH-D_3 levels <20 ng/ml.[155] Plasma 25-OH-D_3 levels are also negatively associated with BMI and percentage body fat, low magnesium status, and recent inflammatory insult.

Recent studies have suggested decline in the vitamin D status of Americans in recent decades. Comparisons of serum 25-OH-D_3 levels measured in sera of participants in the National Health and Examination Surveys (NHANES) conducted in 1988–1994 and 2000–2004 showed an apparent 7.1 nM (c.9%) decline among men. It is likely that methodological changes may have contributed to that difference, as the

151. Institute of Medicine, 2011. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.

152. Dawson-Hughes, B., Heaney, R.P., Holick, M.F., et al., 2005. Osteoporosis Int. 16, 713–716.

153. An analysis indicated that vitamin D intakes of at least 1000 μg/day are required to bring 50% of healthy American adults above the serum 25-OH-D_3 level of 75 nM (Bischoff-Ferrari, H. A., Giovannucci, E., Willett, W.C., et al., 2006. Am. J. Clin. Nutr. 84, 18–28). Current recommended intakes for vitamin D are 200 and 600 IU/day in younger and older adults, respectively.

154. Sinotte, M., Diorio, C., Berube, S., et al., 2009. Am. J. Clin. Nutr. 89, 634–640; Wang, T.J., Zhang, F., Richards, J.B., et al., 2010. Lancet 376, 180–188.

155. Lee, J.M., Smith, J.R., Philipp, B.L., et al., 2007. Clin. Pediatr. 46, 42–44.

TABLE 7.18 Recommended Vitamin D Intakes

Age–Sex	US RDA[a], µg/day	Age–Sex	FAO/WHO RNI[b], µg/day
0–11 mos.	10	0–11 mos.	5
1–70 years	15	1–18 years	5
>70 years	20	19->65 years, females	7.5
		Males	10
Pregnancy	15	Pregnancy	–
Lactation	15	Lactation	–

[a]*Recommended Dietary Intakes; Food and Nutrition Board, 2010. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.*
[b]*Recommended Nutrient Intakes, Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*

manufacturer's reformulation of the assay (an enzyme-linked immunosorbent assay, ELISA) used in those surveys produced 25-OH-D_3 results that 1% lower than the previous method.[156] That the ELISA overestimates 25-OH-D_3 and underestimated 25-OH-D_2 can lead to misclassification of vitamin D status in subjects using supplements containing D_2.[157]

9. VITAMIN D DEFICIENCY

Causes of Vitamin D Deficiency

Vitamin D deficiency can result from inadequate irradiation of the skin, from insufficient intake from the diet, or from impairments in the metabolic activation of the vitamin. Although sunlight can provide the means of biosynthesis of D_3, it is well documented that many people, particularly those in extreme latitudes during the winter months, do not receive sufficient solar irradiation to support adequate vitamin D status. Even people in sunnier climates may not produce adequate D_3 if their lifestyles or health status keep them indoors, or if factors such as air pollution or clothing reduce their exposure to sunlight. Most people, therefore, show strong seasonal fluctuations in plasma 25-OH-D_3 concentration; for some, this can be associated with considerable periods of suboptimal vitamin D status if not corrected by dietary sources of the vitamin. Until the practice of vitamin D fortification of foods became widespread, at least in technologically developed countries, it was difficult to obtain adequate vitamin D from the diet (Table 7.18), as most foods contain only minuscule amounts.[158] Therefore, vitamin D deficiency can have primary (privational) and secondary (nonprivational) causes:

- **Primary causes** involve inadequate vitamin D supply
  - inadequate exposure to sunlight
  - insufficient consumption of foods containing vitamin D
- **Secondary causes** relate to impaired absorption, metabolism, or nuclear binding of the vitamin
  - gastrointestinal diseases (e.g., small bowel disease, gastrectomy, pancreatitis)—involving malabsorption of the vitamin
  - hepatic diseases (biliary cirrhosis, hepatitis)—that reduce activity of the 25-hydroxylase
  - renal diseases—that reduce the activity of the 1-hydroxylase (nephritis, renal failure) or cause loss of 25-OH-D_3 into the urine (nephrotic syndrome)[159]
  - exposure to drugs (e.g., the anticonvulsants phenobarbital, diphenylhydantoin)—that induce the catabolism of both 25-OH-D_3 and 1,25-$(OH)_2$-D_3[160]
  - **hypoparathyroidism**—impairs the ability to respond to hypocalcemia[161] by increasing activity of the 1-hydroxylase
  - *cyp27b1* mutations—resulting in loss of 25-OH-D_3 1-hydroxylase activity in **vitamin D-dependent rickets type I**[162]
  - VDR mutations—impair transcription of vitamin D-regulated genes in **vitamin D-dependent rickets type II**[163]
  - PTH resistance—results in **pseudohypoparathyroidism**, i.e., hypocalcemia without compensating renal retention or bone mobilization of Ca, despite normal PTH secretion[164]
  - **vitamin D resistance**[165]—impairments in both enteric absorption and renal tubular reabsorption of phosphate, hypersensitivity to PTH, and reduced 1-hydroxylation of 25-OH-D_3[166]

156. Looker, A.C., Pfeiffer, C.M., Lacher, D.A., et al., 2008. Am. J. Clin. Nutr. 88, 1519–1527.
157. Nguyen, V.T.Q., Li, X., Castellanos, K.J., et al., 2014. J. AOAC Int. 97, 1048–1055.
158. Eggs are the notable exception. Even cows' milk and human milk contain only very small amounts of vitamin D.
159. Renal tubular degeneration characterized by edema, albuminuria, hypoalbuminemia, and, usually, hypercholesterolemia.
160. Vitamin D supplements (up to 4000 IU/day) are recommended to prevent rickets in children undergoing long-term anticonvulsant therapy.
161. In affected individuals, this leads to hyperphosphatemia. These conditions typically respond to treatment with 1,25-$(OH)_2$-D_3 or high levels of vitamin D_3.
162. This condition can be managed using low doses of 1,25-$(OH)_2$-D_3 or 1-α-OH-D_3.
163. This condition can be managed using high doses of 1,25-$(OH)_2$-D_3 or 1-α-OH-D_3.
164. This condition can be managed using low doses (0.25–3 mg/day) of 1,25-$(OH)_2$-D_3 or 1-α-OH-D_3.
165. Also called **hypophosphatemic rickets/osteomalacia** or **phosphate diabetes**.
166. The condition can be managed using phosphate plus D_3 (25,000–50,000 IU/day) or low doses of 1,25-$(OH)_2$-D_3 or 1-α-OH-D_3.

TABLE 7.19 Signs of Vitamin D Deficiency

Organ System	Rickets	Osteomalacia	Osteoporosis
General	Occurs in young children; loss of apetite, retarded growth	Occurs in older children and adults	Most common in postmenopausal women and older men
Bone/teeth	Failed mineralization, deformation, swollen joints, paint, tenderness, delayed tooth eruption	Demineralized formed bone (e.g., spine, shoulder, ribs, pelvis); fractures (wrist, pelvis); bone pain, tenderness	Loss of trabecular bone with retained structure, high fracture incidence
Skin	Not affected	Not affected	Not affected
Muscle	Weakness, myotonia, pain	Weakness, sarcopenia, pain	Not affected
Vital organs	Not affected	Not affected	Not affected
Nervous	Tetany, ataxia	Not affected	Not affected
Reproductive	Birds: thin eggshell	Low sperm motility and number	Not affected
Ocular	Not affected	Not affected	

FIGURE 7.20 Rachitic child with beaded ribs.

Signs of Vitamin D Deficiency

Vitamin D deficiency affects several systems, most prominently skeletal and neuromuscular (Table 7.19).

Vitamin D Deficiency in Humans

The most obvious signs of vitamin D deficiency are failures in mineralization of bone; but vitamin D deficiency also increases risks to CVD, respiratory disease, and infection.

Rickets

Rickets first appears in 6–-24-month- old children, but can manifest at any time until the closure of the bones' epiphyseal growth plates. It is characterized by impaired mineralization of the growing bones with accompanying bone pain, muscular tenderness, and hypocalcemic tetany. Tooth eruption may be delayed, the fontanelle may close late, and knees and wrists may appear swollen. Affected children develop deformations of their softened, weight-bearing bones, particularly those of the rib cage and legs, characteristic signs being "beaded ribs" (Fig. 7.20), **bow-leg (Genu varum)**, **knock knee**, and **sabre tibia** (forward curvature of the tibia) (Figs. 7.21 and 7.22), which occur in nearly half of cases. Radiography reveals enlarged epiphyseal growth plates resulting from their failure to mineralize and continue growth. Rickets is most frequently associated with low dietary intakes of Ca, as in the lack of access to or avoidance of milk products.[167]

Osteomalacia

Osteomalacia occurs in older children and adults with formed bones whose epiphyseal closure has rendered that region of the bone unaffected by vitamin D deficiency. The signs and symptoms of osteomalacia are more generalized than those of rickets, e.g., muscular weakness and bone tenderness and pain, particularly in the spine, shoulder, ribs, or pelvis. Lesions involve the failure to mineralize bone matrix, which continues to be synthesized by functional osteoblasts; therefore, the condition is characterized by an increase in the ratio of nonmineralized bone to mineralized bone. Radiographic examination reveals abnormally low

167. Despite the use of vitamin D-fortified foods, rickets has reemerged as a public health problem, being reported in at least 22 countries. Cases in Africa and South Asia appear to be caused primarily by deficiencies of calcium, which some have suggested may increase the catabolism of vitamin D. Other cases, however, appear to be due to insufficient vitamin D. These include cases in the United States, 83% of which were described as African-American or black, and 96% of which were breastfed, with only 5% vitamin D supplementation during breastfeeding.

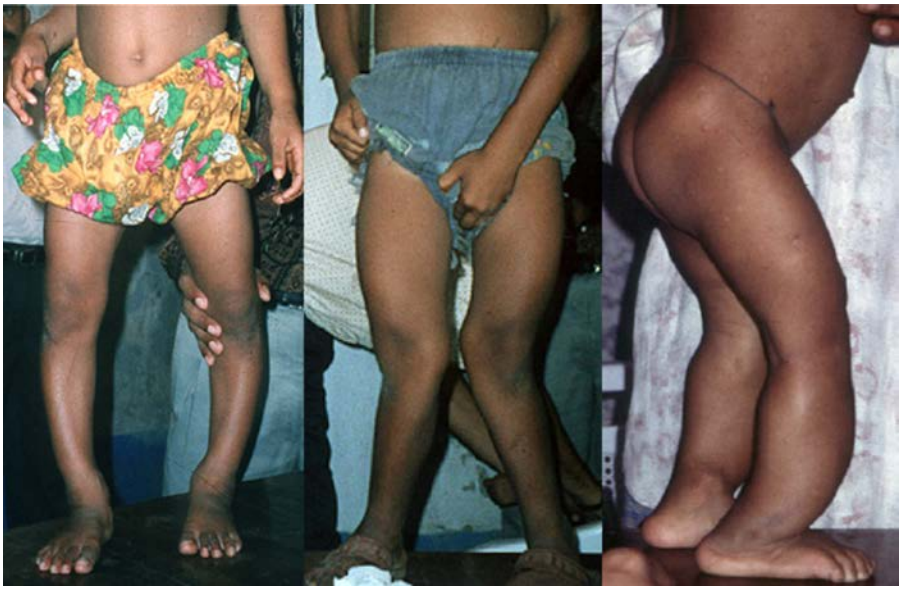


FIGURE 7.21 Rachitic children with genu varum (left), genu valgum (center) and sabre tibia (right).

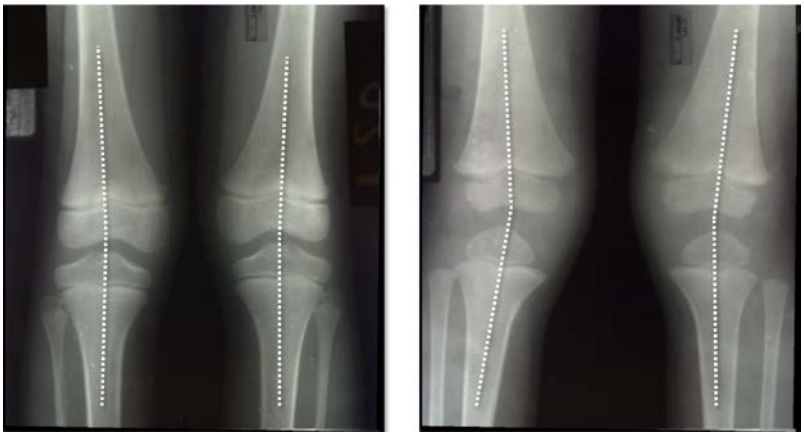


FIGURE 7.22 Knee radiographs of normal (left) and rachitic (right) children. Note the lateral displacement of the lower legs (165° in the child with genu valgum versus 171° in the unaffected child).

bone density (osteopenia) and the presence of pseudofractures, especially in the spine, femur, and humerus. Patients with osteomalacia are at increased risk of fractures of all types, but particularly those of the wrist and pelvis, which are typically caused by falls. Patients with osteomalacia frequently show myopathy primarily of type II muscle fibers (the first to be recruited to avoid falling) resembling the sarcopenia[168] of aging, which is associated with reduction in muscle VDR.

Osteoporosis

Although it is sometime confused with osteomalacia, osteoporosis is a very different disease, being characterized by decreased bone mass with retention of normal histological appearance. Its etiology (loss of trabecular bone with retention of bone structure) is not fully understood; it is a multifactorial disease associated with aging and involving impaired vitamin D metabolism and/or function associated with low or decreasing estrogen levels. It is the most common bone disease of postmenopausal women and also occurs in older men[169] (e.g., nonambulatory geriatrics, postmenopausal women) and in people receiving chronic steroid therapy. In women, bone loss generally begins in the third and fourth decades and accelerates after menopause;

168. Loss of muscle.

169. Osteoporosis affects one-third of women 60–70 years of age, and two-thirds over 80 years, for a total of some 25 million Americans, costing the US economy some $13–18 B per year.

in men, bone loss begins about a decade later. These groups show high incidences of fractures, particularly of the vertebrae, hip, distal radius, and proximal femur. Osteoporosis has two types:

- **Type I osteoporosis**—characterized by distal radial and vertebral fractures; occurring primarily in women of 50–65 years and probably related to postmenopausal decreases in the amount of calcified bone at the fracture site.
- **Type II osteoporosis**—characterized by fractures of the hip, proximal humerus, and pelvis, i.e., where there has been loss of both cortical and trabecular bone; occurring primarily among individuals over 70 years. In women, osteoporosis is characterized by rapid loss of bone (e.g., 0.5–1.5% per year) in the first 5–7 years after menopause.[170] The increased skeletal fragility observed in osteoporosis appears not to be due solely to reductions in bone mass but also involves changes in skeletal architecture and bone remodeling (e.g., losses of trabecular connectivity as well as inefficient and incomplete microdamage repair). Affected individuals show abnormally low circulating levels of 1,25-$(OH)_2$-D_3, suggesting that estrogen loss may impair the renal 1-hydroxylation step, i.e., that the disease may involve a bihormonal deficiency. Studies of the use of various vitamers D in the treatment of osteoporotic patients, most of which have involved low numbers of subjects, have produced inconsistent results. Results of the Nurses' Health Study showed that adequate vitamin D intake (12.5 μg/day) was associated with a 37% lower risk of osteoporotic hip fracture compared to lower intakes of the vitamin.[171]

Musculoskeletal Pain

Deep pain is common among rickets and osteoporosis patients. Some reports have indicated persistent, nonspecific musculoskeletal pain among asymptomatic adults with low circulating levels of 25-OH-D_3; however, a systematic review of published data found no convincing evidence of either low-vitamin D status or latitude being associated with chronic pain prevalence in noncases.[172] Similarly, well-controlled intervention trials have largely yielded negative findings.

Vitamin D Deficiency in Renal Patients

Low circulating levels of 25-OH-D_3 are frequently observed in patients with chronic renal disease and those with nephrotic syndrome and normal renal function. Some studies have found treatment with vitamin D analogues to reduce proteinuria in patients with chronic renal disease. Chronic renal disease can lead to bone disease, **renal osteopathy**, a frequent complication in renal patients in which the severity is related to the degree of loss of glomerular filtration rate. The condition is more common in children than adults, probably due to their greater need of growing bone for metabolically active vitamin D, along with phosphate and PTH circulating levels of which also decline in the disease. A meta-analysis of 16 clinical trials concluded that such treatments are effective in increasing serum Ca^{2+} and decreasing serum PTH but did ineffective in reducing either the need for dialysis or survival.[173]

Nonskeletal Effects of Hypovitaminosis D

Studies have shown risks to several nonskeletal diseases to be inversely related to vitamin D status in subjects with plasma 25-OH-D_3 levels <20–50 ng/mL regardless of sun exposure (Table 7.20). Meta-analyses of prospective cohort studies found a nonlinear decrease in all-cause mortality risk with increasing concentrations of plasma 25-OH-D_3 to an apparent optimum in the range of 75–87.5 nM.[174]

Low plasma levels of 25-OH-D_3 are inversely associated with risk factors for falling: postural balance and strength (e.g., leg extension power, quadriceps strength, arm muscle strength, handgrip strength, ability to climb stairs, physical activity).[175] A meta-analysis of 28 observational and cross-sectional studies found elderly fallers to have lower plasma 25-OH-D_3 levels than nonfallers.[176] Other meta-analyses of the eight published randomized trials found supplementation with 700–1000 IU D_3/day to reduce risks of falling by older subjects by 14–19%,[177] although individual studies have reported reductions by nearly 50%. The greatest benefits were observed when vitamin D was given with

170. Therefore, the primary determinant of fracture risk from postmenopausal or senile osteoporosis in older people is the mass of bone each had accumulated during growth and early adulthood. This includes cortical bone, which continues to be accreted after closure of the epiphyses until about the middle of the fourth decade.
171. Feskanich, D., Willett, W.C., Colditz, G.A., et al., 2003. Am. J. Clin. Nutr. 77, 504–511.
172. Straube, Andrew Moore, R., Derry, S., et al., 2009. Pain 141, 10–13.
173. Palmer, S.C., McGregor, D.O., Craig, J.C., et al., 2009. Cochrane Database Syst. Rev. CD008175.
174. Zittermann, A., Iodice, S., Pilz, S., et al., 2012. Am. J. Clin. Nutr. 95, 91–100; Sempos, C.T., Durazo-Arvizu, R.A., Dawson-Hughes, B., et al., 2013. J. Clin. Endocrinol. Metab. 98, 3001–3009. The latter results suggested a reversal of risk reduction at levels >99 nM.
175. Mowé, M., Haug, E., Bohmer, T., 1999. J. Am. Geriatr. Soc. 47, 220–226; Bischoff, H.A., Stahelin, H.B., Urscheler, N., et al., 1999. Arch. Phys. Med. Rehabil. 80, 54–58; Annweiler, C., Schott-Petelaz, A.M., Berrut, G., et al., 2009. J. Am. Geriatr. Soc. 57, 368–369; Annweiler, C., Montero-Odasso, M., Schot, A.M., et al., 2009. J. Nutr. Health Aging 13, 90–95.
176. Annweiler, C., Beauchet, O., 2014. J. Intern. Med. 277, 16–44.
177. Bischoff-Ferrari, H.A., Dawson-Hughes, B., Staehelin, H.B., et al., 2009. Br. Med. J. 339, 3692–3603; Kalyani, R.R., Stein, B., Valiyil, R., et al., 2010. J. Am. Geriatr. Soc. 58, 1299–1310.

TABLE 7.20 Relationship of vitamin D Status and Mortality

Cause	Serum 25-OH-D Category, nM					P-linear Trend
	<30	30–<50	50–<70	70–<90	90	
All causes	1	0.90 (0.79, 1.03)	0.78 (0.68, 0.90)	0.80 (0.68, 0.94)	0.73 (0.59, 0.90)	<0.0001
Cardiovascular	1	0.95 (0.74, 1.21)	0.84 (0.65, 1.09)	0.82 (0.61, 1.11)	0.73 (0.49, 1.09)	<0.03
Cancer	1	1.11 (0.89, 1.40)	0.94 (0.74, 1.19)	0.96 (0.73, 1.25)	0.90 (0.65, 1.26)	NS
Respiratory diseases	1	0.48 (0.33, 0.70)	0.36 (0.24, 0.55)	0.42 (0.26, 0.69)	0.24 (0.11, 0.54)	<0.0001

Khaw, K.T., Luben, R., Wareham, N., 2014. Am. J. Clin. Nutr. 100, 1361–1370.

supplemental Ca (Fig. 7.27), and when plasma 25-OH-D_3 levels were raised to ≥60 nM.

Vitamin D deficiency appears to increase risk to noninsulin-dependent diabetes (type 2 diabetes, T2D). Subclinical, chronic inflammation has been associated with insulin resistance, which has been found to be inversely related to serum 25-OH-D_3 concentrations over a wide range,[178] and serum 25-OH-D_3 levels have been found to be inversely correlated with insulin resistance, body fat mass, and BMI. The Third National Health and Nutrition Examination Survey (NHANES III) showed serum of 25-OH-D_3 level to be inversely associated with diabetes risk in a multiethnic sample of over 6000 adults.[179] Swedish researchers have found T2D incidence to be highest during the winter months when circulating 25-OH-D_3 levels are lowest.[180] Two VDR polymorphisms, *BsmI* and *ApaI*, as well as low-plasma VDB levels have been associated with high fasting glucose levels, hyperinsulinemia, and elevated T2D risk.[181] The relationship of vitamin D status and T2D risk appears to be greatest among overweight/obese individuals, as prediabetics with low-plasma 25-OH-D_3 levels shows the greatest insulin resistance.[182] It has been suggested that the exacerbation of the T2D risk of low-vitamin D status by adiposity may be due to the sequestration of vitamin by partitioning into bulk lipid depots in adipose tissue (Fig. 7.30). Indeed, the 25-OH-D_3 of subcutaneous white adipose tissue correlates with serum plasma 25-OH-D_3 levels in obese adults. This sequestration has been proposed as suppressing the plasma 25-OH-D_3 response to dietary or biogenic vitamin D, reducing the apparent bioavailability of dietary sources of the vitamin.

Low-vitamin D status has also been associated with increased susceptibility to infections,[183] as well as increased risks of autoimmune and CVD, depressive symptoms, schizophrenia and Parkinson's disease, cognitive decline and dementia, periodontal disease, and ocular diseases.[184] Hospital mortality has been found greater for patients with low 25-OH-D3 levels (<20 ng/mL) than of vitamin D-adequate patients.

Serum 25-OH-D_3 levels are more frequently low in pregnant women than in nonpregnant women. A study in Boston found that 76% of new mothers (and 81% of their newborns) had serum 25-OH-D_3 levels <20 ng/mL.[185] Low-vitamin D status is generally more common among black than white pregnant women; black infants are four times as likely as white infants to be exposed in utero to vitamin D deficiency.[186] Systematic reviews and meta-analyses of observational studies have found low maternal serum 25-OH-D_3 levels to be associated with increased risks of preeclampsia, gestational diabetes, small-for-gestational age births, and bacterial vaginosis, although there is clear heterogeneity in many of these responses.[187] Some studies have found maternal vitamin D status to predict growth and later bone mass of children.

Vitamin D Deficiency in Animals

Rickets

Vitamin D-deficient, growing animals show rickets (Figs. 7.23 and 7.24). Species at greatest risk are those that experience rapid early growth, such as the chick. Rachitic signs

178. Chiu, K.C., Chu, A., Go, V., Saad, M.F., 2004. Am. J. Clin. Nutr. 79, 820–825.
179. Scragg, R., Sowers, M., Bell, C., 2004. Diabetes Care 27, 2813–2818.
180. Berger, B., Stenstrom, G., Sundkist, G., 1999. Diabetes Care 22, 773–777.
181. Hitman, G.A., Mannan, N., McDermott, M.F., et al., 1998. Diabetes 47, 688–690; Oh, J.-Y., Barrett-Connor, E., 2002. Metabolism 51, 356–359; Ortlepp, J.R., Metrikat, J., Albrecht, M., et al., 2003. Diabet. Med. 20, 451–454.
182. Abbasi, F., Blasey, C., Feldman, D., et al., 2015. J. Nutr. 145, 71–719.
183. e.g., Pneumonia, and infections of *Mycobacterium tuberculosis*, *Leishmania major*, hepatitis C, Varicella-Zoster virus, and HIV.
184. e.g., Myopia, age-related macular degeneration, diabetic retinopathy, and uveitis.
185. Lee, J.M., Smith, J.R., Philipp, B.L., et al., 2007. Clin. Pediatr. 46, 42–44.
186. Bodnar, L.M., Simhan, H.N., Powers, R.W., et al., 2007. J. Nutr. 137, 447–452.
187. Harvey, N.C., Holroyd, C., Ntani, G., et al., 2014. Health Technol. Assess. 18, 1–190; Aghajafari, F., Nagulesapillai, T., Ronksley, P.E., et al., 2013. Br. Med. J. 346, f1169–f1183.

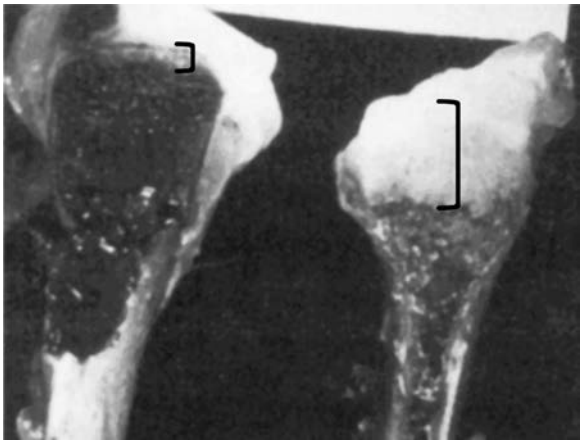


FIGURE 7.23 Tibiae of normal (*left*) and rachitic (*right*) chicks dissected to show epiphyseal growth plates (brackets). Note proliferation cartilage replacing vascularized spongiform bone in the affected tibia.

FIGURE 7.24 Rachitic puppy.

are similar in all affected species: impaired mineralization of the growing bones with structural deformation in weight-bearing bones.

Osteoporosis

Older vitamin D-deficient animals show the undermineralization of bones that characterizes osteoporosis. This can be a practical problem in the high-producing laying hen, in which it is called **cage layer fatigue.** The condition is associated with reductions in egg production, feed intake, and efficiency of feed utilization and survival.[188]

188. In well-managed flocks, it is common for a hen to lay >300 eggs in a year, with 40 of these laid during the first 40 days after commencing egg laying. As each eggshell contains about 2 g Ca, and the hen is able to absorb only 1.8–1.9 g Ca from the diet each day, she experiences a Ca debt of 0.1–0.2 g/day during that period. She accommodates this by mobilizing medullary bone; but, as her total skeleton contains only about 35 g Ca, chronic demineralization at that rate without either decreasing the rate of egg production or increasing the efficiency of Ca absorption leads to osteoporosis characterized by fractures of the ribs and long bones.

Tibial Dyschondroplasia

There appear to be other situations of impaired renal 1-hydroxylation of 25-OH-D_3, thus limiting the physiological function of the vitamin. One is the failure of bone mineralization seen in rapidly growing, heavy-bodied chickens, and turkeys called tibial dyschondroplasia. The disorder is similar to the condition called osteochondrosis in rapidly growing pigs and horses; it is characterized by the failure of vascularization of the proximal metaphyses of the tibiotarsus and tarsalmetatarsus. It occurs spontaneously, but can be produced in animals made acidotic, that condition reducing the conversion of 1-hydroxylation of 25-OH-D_3. Both the incidence and severity of tibial dyschondroplasia can be reduced by treatment with 1,25-$(OH)_2$-D_3 or 1α-OH-D_3 but not by higher levels of vitamin D_3 alone. That lesions in genetically susceptible poultry lines cannot be completely prevented by treatment with vitamin D metabolites suggests that tibial dyschondroplasia may involve a functional impairment in VDRs.

Milk Fever

High-producing dairy cows can become hypocalcemic at the onset of lactation when they have been fed Ca-rich diets before calving. The condition, called milk fever, occurs when plasma Ca levels decrease to less than about 5.0 mg/dL; it is characterized by tetany and coma, which can be fatal. Milk fever results from the inability of the postparturient cow to withstand massive lactational Ca losses by absorbing dietary Ca and mobilizing bone at rates sufficient to support plasma Ca at normal levels. It can be prevented by preparing the pregnant cow for upregulated bone mobilization and enteric Ca absorption. Infield practice, this is done by feeding her a relatively low-Ca diet (100 g/day); parenteral treatment with 1,25-$(OH)_2$-D_3 is also effective.

Responses to Treatment

Supplementation and food fortification has proven to be safe and effective in normalizing circulating levels of 25-OH-D_3 and correcting associated physiological defects. Vitamin D is routinely included in the vitamin–mineral supplements of formulated foods for livestock and pets. A meta-analysis of 15 studies found serum 25-OH-D responses of humans to vitamin D-fortified foods varied according to dose level, latitude, and baseline status; but that, on average, a 1 μg/day (40 IU/day) of vitamin D from fortified foods increased serum 25-OH-D level by an average of 1.2 nM.[189]

Correction of vitamin D deficiency may be impossible to achieve without the use of large-dose supplements. A meta-analysis of randomized trials noted the inconsistency

189. Black, L., Seamans, K.M., Cashman, K.C., et al., 2012. J. Nutr. 142, 1102–1108.

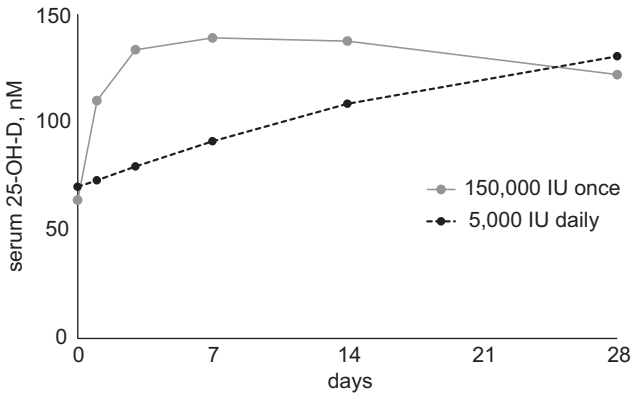


FIGURE 7.25 Comparison of daily versus monthly administration of vitamin D_3 to healthy, nonpregnant, nonlactating, women. *After Meekins, M.E., Oberhelman, S.S., Lee, B.R., et al., 2014. Eur. J. Clin. Nutr. 68, 632–634.*

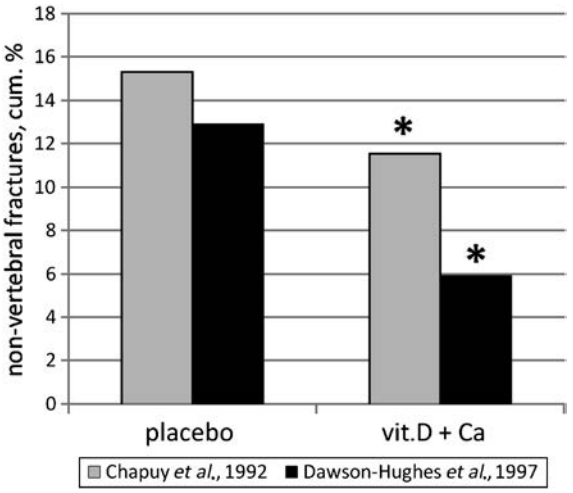


FIGURE 7.26 Prevention of fractures by vitamin D supplementation in two cohorts of older adults *(Chapuy, M.C., Arlo, M.E., Duboeuf, F., et al., 1992. N. Engl. J. Med. 327, 1637–1642.)*: 2790 women, 84 ± 6 years, receiving 1200 mg Ca and 800 IU D_3 daily; *(Dawson-Hughes, B., Harris, S.S., Krall, E.A., et al., 1997. N. Engl. J. Med. 337, 670–676.)*: 389 men and 213 women, 72 ± 5 years, receiving 500 mg Ca and 700 IU D_3 daily). *Significantly different from respective placebo level, $p < .05$.

of effects of vitamin D supplementation on infectious disease; it has been suggested that immunomodulatory efficacy may require higher serum 25-OH-D_3 levels than presently considered adequate. A study with T2D subjects in Finland showed that one-third or more did not respond to low-level (40 or 80 µg/day) D_3 supplements determined by expression of VDR-related genes.[190] Singh and Bonham[191] used responses of some 1300 subjects to develop an algorithm for vitamin D replacement dosing:

> D_3 dose, IU/day = [(8.52 – desired change in 25-OH-D_3 level, ng/mL) + (0.074 x age, year) – (0.20 x BMI) + (1.74 x albumin level, g/dL) – (0.62 x baseline 25-OH-D_3 level, ng/mL)]/–0.002

Such estimates indicate that the DRI levels of vitamin D intake are grossly inadequate for correcting low-vitamin D status; such conditions require does >2000 IU/day. Doses of 1000 IU have been found effective in reducing bone loss at the hip in postmenopausal women;[192] a meta-analysis of 23 randomized intervention trials in older subjects vitamin D-adequate subjects showed no effects of lower doses on bone mineral content at most sites.[193] A single dose of 150,000 IU D_3 and a lower daily dose of 5000 IU D_3 showed comparable plasma 25-OH-D_3 levels by 28 days, but the high bolus dose supported greater plasma 25-OH-D_3 levels for most of that period (Fig. 7.25). The synthetic vitamin D analogue, 1α,25-(OH)2-2β-(3-hydroxypropyloxyl)-D_3 (eldecalcitol), has been shown to be effective in normalizing plasma 25-OH-D_3 and reducing the incidence of new vertebral fractures in older subjects.[194]

Vitamin D_3 supplementation to correct low/deficient vitamin D status has been found to reduce risks to several outcomes: falling and fractures, including those unrelated to falls (Fig. 7.26), peritonitis and improve survival in renal patients on peritoneal dialysis, and to improve antibacterial immunity in HIV+ patients, to reduce disease activity in subjects with Crohn's disease, and to improve winter-related atopic dermatitis in children. Correction of low-vitamin D status has been found to improve seizure control in epilepsy[195] and to stabilize Parkinson's disease in individuals with the *Fok*I TT or CT genotypes of VDR.[196]

10. VITAMIN D IN HEALTH AND DISEASE

Immune Dysfunction

Clinical studies have shown that oral and topical applications of either 1,25-$(OH)_2$-D_3 or the synthetic analogue 1α,25-$(OH)_2$-D_3[197] can be effective in managing psoriasis,[198] a Th1-mediated, hyperproliferative disease in which cathelicidin converts otherwise inert self-DNA and -RNA into autoimmune stimuli. Vitamin D treatment

190. Saka, N., Neme, A., Ryynänen, J., et al., 2015. J. Steroid Biochem. Mol. Biol. 148, 275–282.
191. Sing, G., Bonham, A.J., 2014. J. Am. Board Fam. Med. 27, 495–509.
192. MacDonald, H.M., Wood, A.D., Aucott, L.S., et al., 2013. J. Bone Miner. Res. 28, 2202–2213.
193. Reid, I.R., Bolland, M.J., Greay, A., 2014. Lancet 383, 146–155.
194. Noguchi, Y., Kawate, H., Nomura, M., et al., 2013. Clin. Interv. Aging 8, 1313–1321.
195. Holló, A., Clemens, Z., Kamondi, A., et al., 2012. Epilepys Behav. 24, 131–133.
196. Suzuki, M., Yoshioka, M., Hashimoto, M., et al., 2013. Am. J. Clin. Nutr. 97, 1004–1013.
197. Also called **calcipotriol**.
198. Results of a clinical series showed that topical application of 1,25-$(OH)_2$-vitamin D_3 caused complete clearing of lesions in 60% of patients, with an additional 30% of patients showing significant decreases in scale, plaque thickness, and erythema.

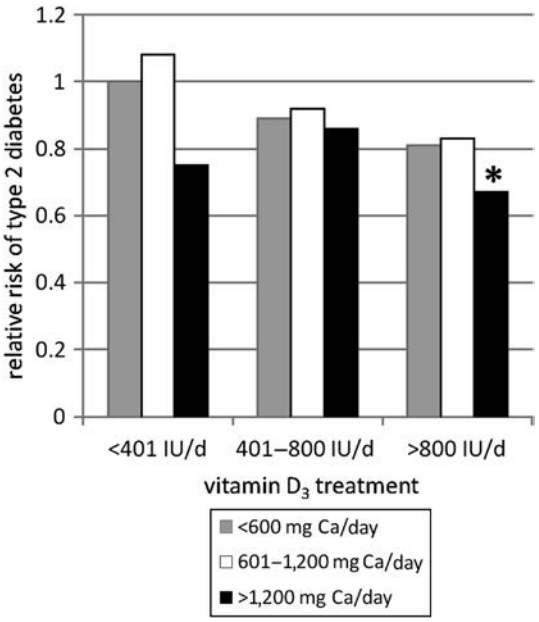


FIGURE 7.27 Reduction of type 2 diabetes risk associated with vitamin D and Ca supplement use by in 83,779 women in the Nurses' Health Study. * indicates significant difference ($p < .05$) from the low vitamin D, low Ca group. *After Pittas, A.G., Dawson-Hughes, B., Lit, T., et al., 2006. Diabetes Care 29, 650–656.*

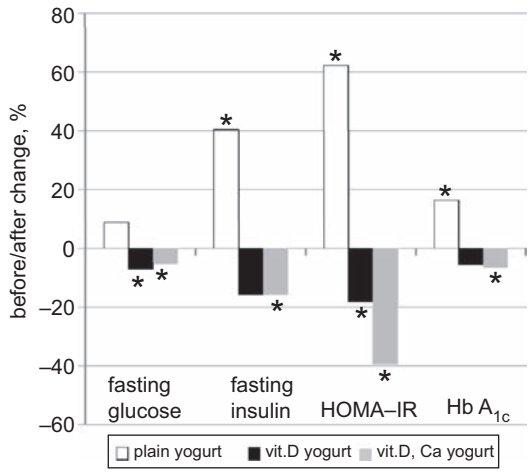


FIGURE 7.28 Effects of 12-week, yogurt-based supplementation with vitamin D (500 IU/d) and Ca (150 mg/day) on parameters of glycemic control in type 2 diabetes patients (fasting serum glucose >126 mg/dL), adults (n = 30 per treatment group); * indicates significant difference ($p < .05$) from the respective baseline value. *After Nikooyeh, B., Neyestani, T.R., Farvid, M., et al., 2011. Am. J. Clin. Nutr. 93, 764–771.*

has also been found to reduce rejection of allografts of heart, kidney, liver, pancreatic islets, small intestine, and skin in animals given high, nonhypercalcemic doses in both the presence and absence of the immunosuppressive drug cyclosporin A. These effects involve the suppression of antibody-presenting cells (particularly dendritic cells and T cells that play major roles in immunological graft rejection) and enhancing numbers of suppressive T ($CD4^+$, $CD25^+$) cells.

Obesity and Type 2 Diabetes

The efficacy of vitamin D in preventing T2D may depend on an individual's degree of adiposity, as the relationship between vitamin D status and T2D risk is strongest for overweight/obese individuals.[199] While a few clinical trials have found weight loss to improve 25-OH-D_3 levels and D_3 supplements to improve insulin sensitivity, such effects have not been observed in most trials, nor has vitamin D supplementation been found to affect body fat accumulation or weight loss. A 20-year follow-up of the Nurses' Health Study cohort found T2D risk to be one-third less for women reporting the use of vitamin D and Ca supplements (Fig. 7.27). An intervention with D_3 in Iran, a country with high reported prevalences of low-vitamin D status, metabolic syndrome, and T2D, found improvement in glycemic control in T2D patients, as indicated by reductions in fasting glucose level and insulin resistance (Fig. 7.29).[200]

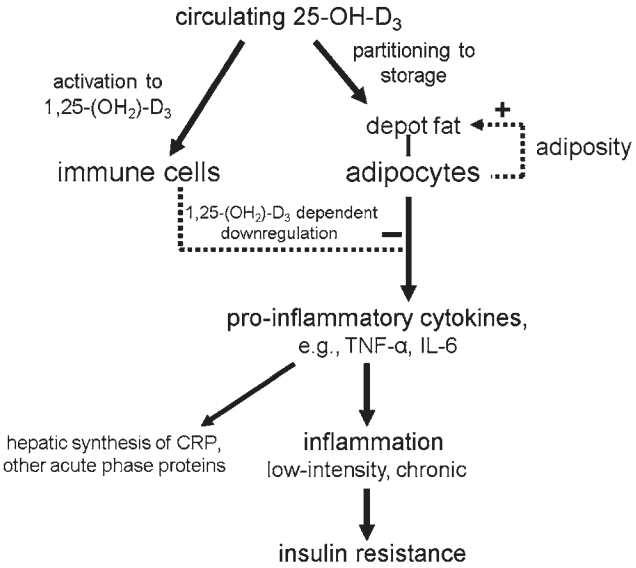


FIGURE 7.29 Hypothetical relationships of vitamin D status and adiposity in affecting insulin resistance. CRP, C-reactive protein; *IL-6*, interleukin-6; *TNF-*, tumor necrosis factor.

Cardiovascular Health

Epidemiological studies have shown that low-circulating 25-OH-D_3 levels as well as factors known to affect vitamin

199. Isaia, G., Giorgino, R., Adami, S., et al., 2001. Diabetes 24, 1496–1503.

200. Insulin resistance was measured by HOMA-IR, the homeostasis model assessment of insulin resistance, i.e., the products of the fasting serum concentrations of insulin and glucose.

D status (latitude, altitude, season) are associated with the prevalence of coronary risk factors and CVD mortality. The 2001–2006 NHANES data showed Americans of low-vitamin D status (serum 25-OH-D_3 <21 ng/mL) had serum homocysteine levels that were inversely related to their 25-OH-D_3 levels.[201] Vitamin D is known to suppress several mechanisms of cardiovascular pathogenesis: proliferation of vascular smooth muscle, vascular calcification, and production of proinflammatory cytokines. Intervention trials have not yielded conclusive results. Most have found vitamin D supplements to have little, if any, effects on blood pressure or other risk factors.

Anticarcinogenesis

Vitamin D was proposed to protect against colon cancer nearly 75 years ago, based on epidemiologic associations with sunlight exposure. Many subsequent studies in Europe and the United States have found positive associations between latitude; sun exposure; and risks of cancers of the prostate, colon, and breast. Residents of the northern United States have nearly a twofold higher risk of total cancer mortality than those of the southern States. The Nurses' Health Study showed a significant inverse linear association between plasma 25-OH-D_3 and colon cancer risk.[202] Risk factors for cancers of the prostate, colon, and breast are related to vitamin D status: dark skin color, northern latitude residence, and increasing age. Highest risks are seen for densely pigmented individuals who also tend to have lower circulating levels of 25-OH-D_3. A meta-analysis found carriers of the VDR *Bsm1 B* allele to have 6–7% less total cancer.[203] Randomized trials and prospective follow-up studies have found risk reductions of 17–60% per 25 nM increase in 25-OH-D_3 level.[204] A 4-year clinical trial found that a high dose (1000 IU [25 mg]/day) of vitamin D_3 significantly increased the protective effect of supplemental Ca against all-cause cancer (Fig. 6.30).

Anticarcinogenic effects of 1,25-$(OH)_2$-D_3 have been demonstrated in more than a dozen animal models (Table 7.21). These have shown vitamin D to inhibit cancer cell growth, angiogenesis, and metastasis. These effects appear to involve VDRs, which have been identified in many tumors and malignant cell types. VDR activation is known to signal the expression of many genes, including genes involved in differentiation; apoptosis; and inhibiting proliferation, invasiveness, angiogenesis, and metastatic potential. Ablation of VDR in mice increases tissue proliferation and apoptosis, enhances oxidative DNA damage, increases inflammation, and increases activation of oncogenes and loss of tumor-suppressor genes.

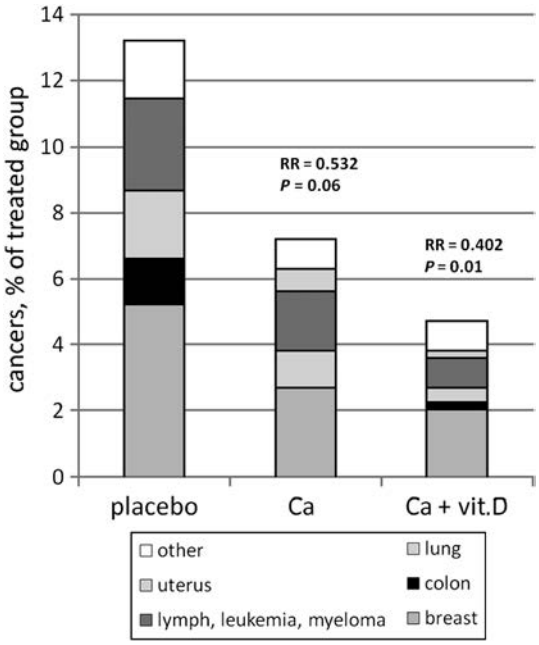


FIGURE 7.30 Four-year cancer incidence in 1179 postmenopausal women randomized to treatment with a placebo, Ca (1400–1500 mg/day) alone or with vitamin D_3 (1100 IU [27.5 mg]/day). *After Lappe, J.M., Travers-Gustafson, D., Davies, K.M., et al., 2007. Am. J. Clin. Nutr. 85, 1586–1591.*

Colorectal Cancer

Mortality to colorectal cancer is significantly higher in the northern and northeasters United States than in the southwest, Hawaii, and Florida. Observational studies have been inconsistent in linking this phenomenon to vitamin D status; however, a meta-analysis has shown significant reduction in colorectal risk associated with serum 25-0H-D_3 levels >20 ng/mL or intakes of at least 1000–2000 IU/day.[205] *In vitro* studies have demonstrated that 1,25-$(OH)_2$-D_3 can both attenuate the growth of rapidly dividing colonic tumor cells and reverse colonocytes from a malignant to a normal phenotype. These effects appear to depend on the activation of VDR, which represses signaling through β-catenin (a mediator of the Wnt pathway) and induces apoptosis. VDR expression appears to decline during the progression of colon cancer; this has been linked to upregulation of transcriptional repressors, which bind to the VDR promotor, and to a shift toward catabolism of both 25-OH-D_3 and 1,25-$(OH)_2$-D_3. These effects appear to involve the suppression of inflammation by the vitamin D system;

201. Amer, M., Qayyum, R., 2014. J. Endocrinol. Metab. 99, 633–638.
202. Feskanich, D., Ma, J., Fuchs, C.S., et al., 2004. Cancer Epidemiol. Biomarkers 13, 1502–1508.
203. Raimondi, S., Johansson, H., Maisonneuve, P., et al., 2009. Carcinogenesis 30, 1170–1180.
204. Pilz, S., Tomaschitz, A., Obermayer-Pietsch, B., et al., 2009. Anticancer Res. 29, 3699–3704.
205. Gorham, E.D., Garland, C.F., Garland, F.C., et al., 2007. Am. J. Prev. Med. 32, 210.

TABLE 7.21 Animal Tumor Models in Which Anticarinogenic Activity of 1,25-$(OH)_2$-D_3 Has Been Demonstrated

Species	Site	Mode of Induction
Mouse	Skin	Oral treatment: Dimethybenzanthracine, phorbol ester
Mouse (athymic)	Adenocarcinoma	Implantation: CAC-8 cells
	Kaposi sarcoma	Implantation: KS YH-1 cells
	Melanoma	Implantation: human melanoma cells
	Osteosarcoma	Implantaton: LNCaP cells
	Retinoblastoma	Implantation: malignant cells
Mouse, APCmin	Colon	(Spontaneous)
Rat	Mammary	Oral treatment: N-methylnitrosourea, dimethylhydrazine
	Colon	Implantation: human colon cancer cells
		Oral treatment: dimethylhydrazine
	Leydig	Implantation: Leydig tumor cells
	Prostate	Implantation: Dunning LyLu cells
	Walker carcinoma	Implantation: Walker carcinoma cells

supplementation of mice with D_3 and 25-OH-D_3 reduced the development of colon tumors induced by inflammatory stimuli by 50%.[206] High VDR expression has been associated with a more favorable prognosis for colorectal cancer patients.[207] VDR can also bind the secondary bile acid, lithocholic acid, a potent enteric carcinogen. Single nucleotide polymorphisms in the VDR gene have been associated with differences in colon cancer risk. Carriers of *Cdx-2* AA of *FokI* TT showed twice the risk of colorectal cancer compared to other genotypes; those with the *Cdx-2-FokI* A-T, *FokI TaqI* T-G, or *Cdx-2-FokI-TaqI* A-T-G haplotypes showed two- to three fold risks of colon cancer compared to other haplotypes.[208]

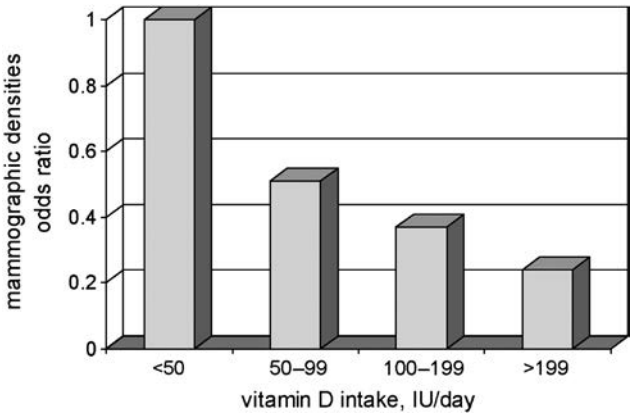


FIGURE 7.31 Inverse relationship of frequency of mammographic breast density and vitamin D status in a cohort of 543 women aged 40–60 years undergoing screening mammographies. *After Berube, S., Diorio, C., Verhoek-Oftedahl, W., et al., 2004. Cancer Epidemiol. Biomarkers Prev. 13, 1466–1472.*

Relatively lower risks of colon (and breast and prostate) cancer have been observed among groups with high consumption of soy foods. It is possible that this may relate to the effects of phytoestrogenic soy isoflavones in upregulating colonocyte production of 1,25-$(OH)_2$-D_3, as genistein has been found to increase expression of the colonic 1-hydroxylase (CYP27B1) while markedly decreasing expression of the 24-hydroxylase (CYP24A1).

Breast Cancer

Studies have shown low-serum 1,25-$(OH)_2$-D_3 levels to be associated with increased risk of breast cancer (Fig. 7.31). Most breast cancer cells express VDR and are growth impaired by physiological concentrations of 25-OH-D_3 or 1,25-$(OH)_2$-D_3. Studies with cultured breast cancer cells have shown that liganded VDR functions in arresting growth, inducing differentiation and apoptosis, and inducing the expression of factors involved in the regulation of cell proliferation, thus, blocking mitogenic signaling including estrogen-driven proliferation.[209] A pooled analysis of two observational studies showed a negative association of vitamin D status and breast cancer risk, with individuals with serum 25-OH-D_3 levels averaging 52 ng/mL having half the risk of those with serum 25-OH-D_3 levels <13 ng/mL and about 60% of the risk of those with serum levels around 20 ng/mL.[210] A meta-analysis found breast cancer risk is less for individuals of the VDR *Fok1 FF* genotype than for those of the ff genotype.[211] Several studies shown breast cancer incidence and mortality to be inversely correlated

206. Murillo, G., Nagpal, N., Tiwari, N., et al., 2010. J. Steroid Biochem. Mol. Biol. 121, 403–407.
207. Evans, S.R.T., Nolla, J., Hanfelt, J., et al., 1998. Clin. Cancer Res. 4, 1591–1595.
208. Ochs-Baalcom, H.M., Cicek, M.S., Thompson, C.L., 2008. Carcinogenesis 24, 1788–1793.
209. e.g., Cyclins C and D1, Kip1, WAF1, c-Fos, C-Myc, c-JUN, and members of the TNF-β family.
210. Garland, C.F., Gorham, E.D., Mohr, S.B., et al., 2007. J. Steroid Biochem. Mol. Biol. 103, 708.
211. Tang, C., Chen, N., Wu, M., et al., 2009. Breast Cancer Res. Treat. 30, 1170.

with serum 25-OH-D_3 level. It has been estimated that, in the United States and Canada, increasing year-round serum 25-OH-D_3 levels to 40–60 ng/mL (100–150 nM) would prevent some 58,000 new cases of breast cancer each year and cut breast cancer mortality in half.[212]

Prostate Cancer

Vitamin D metabolites promote differentiation and inhibit proliferation, invasiveness, and metastasis in prostate cells. These effects are thought to result from VDR activation. High VDR expression has been associated with reduced risk of initiation and progression of prostate cancer in younger men.[213] Studies with rat and human prostate cells have shown 1,25-$(OH)_2$-D_3 to act via VDR synergistically with androgen signaling to upregulate the expression of some 250 genes that are not regulated by either factor alone. Among these genomic changes are differentiation of stem cells to androgen receptor-positive luminal epithelial cells and augmentation of tumor-suppressive micro-RNAs (miRNAs). A meta-analysis found prostate cancer risk is less for carriers of the VDR *Bsm1 B* allele than those without that allele.[214] Several studies shown the incidences of benign prostatic hyperplasia and prostate cancer incidence and mortality to be inversely correlated with serum 25-OH-D_3 level. An open-label trial in South Carolina found daily supplementation with 4000 IU D_3 for a year to reduce Gleason scores of half of men diagnosed with early-stage, low-risk prostate cancer.[215] It has been estimated that, in the United States and Canada, increasing year-round serum 25-OH-D_3 levels to 40–60 ng/mL (100–150 nM) would prevent some 49,000 new cases of breast cancer each year and cut breast cancer mortality in half.

Skin Cancer

Vitamin D functions in maintaining ordered proliferation of keratinocytes by inhibiting β-catenin signaling and also by protecting them from UV-induced DNA damage by upregulating p53, inhibiting stress-activated kinases, and suppressing nitric oxide production. These effects depend on VDR, as its ablation in the mouse results in increased sensitivity of skin cells to tumorigenesis induced chemically or by UV light. That it is the unbound VDR that is involved in antitumorigenesis is indicated by finding that ablation of *CYP27B1* does enhance skin carcinogenesis. Some, but not all, epidemiological studies have found inverse correlations of 25-OH-D_3 level and risk to melanoma but no clear relationship with nonmelanoma skin cancer.[216]

Other Cancers

That vitamin D may have a role in protecting against leukemia is suggested by the finding that 1,25-$(OH)_2$-D_3 treatment can suppress cell division and induce differentiation in human leukemic cells. This effect involves downregulating the expression of the protooncogene c-*myc*. Case–control studies have provide strong evidence of relationships of vitamin D status and risk to non-Hodgkin lymphoma. A meta-analysis found individuals of the VDR *Fok1* TT genotype to have lower risk of skin cancer (including malignant melanoma) than those of the other genotypes.[217] Vitamin D intake was found to be inversely related to lung cancer risk in never-smoking, premenopausal women in the Women's Health Initiative. Evidence is inconsistent for a protective role of vitamin D in ovarian cancer; a long-term, case–control studies have patients with higher prediagnostic serum 25-OH-D levels to have greater risks of surviving ovarian cancer compared to patients of lower vitamin D status.

11. VITAMIN D TOXICITY

Excessive intakes of vitamin D are associated with increases in circulating levels of 25-OH-D_3, the critical metabolite in vitamin D intoxication. This is especially true for D_3, high intakes of which produces greater serum 25-OH-D_3 levels than comparable intakes of D_2, making D_3 10–20 times more toxic than D_2. The basis of toxicity appears to be the ability of 25-OH-D_3 at high levels to compete successfully for VDR binding, bypassing the regulation of the 1-hydroxylase to induce transcriptional responses normally signaled only by 1,25-$(OH)_2$-D_3. Therefore, risk to hypervitaminosis D is increased under conditions in which the normal feedback regulation of the renal 25-OH-D_3-1-hydroxylase is compromised, e.g., chronic inflammation.[218] Recommended upper limits of intake are shown (Table 7.22).

Hypervitaminosis D is characterized by **hypercalcemia** resulting from increased enteric absorption and bone resorption of Ca, with attendant decreases in serum PTH and glomerular filtration. Bone mobilization also results in increased serum levels of zinc from that reserve. Vitamin D-intoxicated individuals show different signs including anorexia, vomiting, headache, drowsiness, diarrhea, and polyuria (Table 7.23). Chronically elevated serum Ca and phosphorus levels ultimately result in **calcinosis**, i.e., the deposition of Ca and P in soft tissues, especially heart and kidney but also the vascular and respiratory systems and

212. Garland, C.F., Gorham, E.G., Mohr, S.B., et al., 2009. Ann. Epidemiol. 19, 468–483.
213. Ahonen, M.H., Tenkanen, L., Teppo, L., et al., 2000. Cancer Causes Control 11, 847–852.
214. Berndt, S.I., Dodson, J.L., Huang, W.Y., et al., 2006. J. Urol. 175, 1613.
215. Hollis, B.W., Marshall, D.T., Savage, S.J., et al., 2013. J. Steroid Biochem. Mol. Biol 136, 233–237.
216. e.g., Actinic keratoses, basal cell carcinomas.
217. Mocellin, S., Nitti, D., 2008. Cancer 113, 2398–2405.
218. Other conditions include tuberculosis and sarcoidosis.

TABLE 7.22 Recommended Upper Tolerable Vitamin D Intakes (UL)

Age–Sex	UL[a], µg/day
0–11 months	25–38
1–3 years	63
4–8 years	75
9–70 + years	100
Pregnancy	100
Lactation	100

[a]*Food and Nutrition Board, 2010. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.*

TABLE 7.23 Signs and Symptoms of Hypervitaminosis D

Anorexia
Gastrointestinal distress, nausea, vomiting
Headache
Muscular weakness, lameness
Polyuria, polydypsia
Nervousness
Hypercalcemia
Calcinosis

practically all other tissues.[219] Therefore, risk of hypervitaminosis D is not only dependent on exposure to vitamin D but also on concomitant intakes of Ca and phosphorus. That serum 25-OH-D_3 level may have a biphasic relationship with adverse outcomes is suggested by the findings of a prospective study of 6000 older women that showed both frailty and mortality to be lower for subjects with baseline 25-OH-D_3 levels of 20–29 ng/mL (50–72.5 nM) compared to subjects with baseline levels below or above that range.[220]

The IOM[221] suggested that serum 25-OH-D_3 levels greater than 100 nM may be associated with increased risk to all-cause mortality, but a careful examination of the primary data does not support that conclusion. A systematic review of clinical trial results found no evidence for adverse effects for vitamin D_3 doses as high as 10,000 IU/day, and no consistent and reproducible effects, including hypercalcemia, at doses five times that amount.[222] A few cases of hypervitaminosis D have been documented among consumers of milk that, through processing errors, was sporadically fortified with very high levels of the vitamin.[223] Vitamin D_3 has been found safe for pregnant and lactating women and their children at oral doses of 100,000 IU/day.[224] There are no documented cases of hypervitaminosis D due to excessive sunlight exposure.

Hypercalcemia can occur with combined supplementation with vitamin D and Ca. A 7-year study of some 36,000 postmenopausal women found a 17% increase (compared to controls) in renal stones in subjects given a daily supplement of 1000 mg Ca plus 400 IU (10 mg) vitamin D_3.[225] That effect is more likely related to total Ca intake, estimated at 2000 mg/day, as that vitamin D dose would be expected to increase serum 25-OH-D_3 levels by only c.7 nM, i.e., not to levels approaching those (>600 nM) found to produce hypercalcemia. Other clinical intervention trials have used similar vitamin D_3 doses without adverse effects; the few that have observed calciuria have used high D_3 doses (e.g., 2000 IU [50 mg]) in combination with Ca (≥500 mg). A naturally occurring calcinosis in grazing livestock was traced to the consumption of water-soluble glycosides of 1,25-$(OH)_2$-D_3 present in some plants.[226] These compounds appear to be deglycosylated metabolically to yield 1,25-$(OH)_2$-D3, which is 100 times more toxic than the dominant circulating metabolite 25-OH-D_3.

Vitamin D hypersensitivity has been proposed as the basis for Williams–Beuren syndrome, a rare condition of hypercalcemia and Ca hyperabsorption in humans. The syndrome is manifest in infancy; it is characterized by failure to thrive with mental handicap and long-term morbidity. Patients have been found to have normal circulating levels of 25-OH-D_3, but they appear to have exaggerated responses to oral doses of vitamin D_3 and one report presented elevated serum levels of 1,25-$(OH)_2$-D_3 in patients.

219. The condition idiopathic infantile hypercalcemia, formerly thought to be due to hypervitaminosis D, appears to be a multifactorial disease with genetic as well as dietary components.

220. Ensrud, K.E., Ewing, S.K., Fredman, L., et al., 2010. J. Clin. Endocrinol. Metab. 95, 5266–5273.

221. Institute of Medicine, 2011. Dietary Reference Intakes: Calcium, Vitamin D. National Academy Press, Washington, DC, 1115 pp.

222. Hathcock, J. N., Shao, A., Vieth, R., et al., 2007. Am. J. Clin. Nutr. 85, 6–18.

223. Eight cases were described; each consumed a local dairy's milk, which varied in vitamin D content (some contained as much as 245,840–IU of D_3 per liter) (Jacobus, C.H., Holick, M.F., Shao, Q., et al., 1992. N. Engl. J. Med. 326, 1173–1177). US regulations stipulate that milk is to contain 400–IU/qt "within limits of good manufacturing practice"; however, a survey found milk and infant formula preparations rarely contained the amounts of vitamin D stated on the label, owing to both under- and overfortification.

224. Goodenday, L.S., Gordon, G.S., 1971. Ann. Intern. Med. 75, 807–808.

225. Jackson, M.D., Lacroix, A.Z., Gass, M., et al., 2006. N. Engl. J. Med. 354, 669–683.

226. D_3 glycosides have been identified in several species in the families Solanaceae (*Solanum glaucophyllum*, *Solanum malacoxylon*, *Solanum torvum*, *Solanum verbascifolium, Cestrum diurnum*, and *Nierembergia veitchii*).

The availability of synthetic 1α-OH-D_3 in recent years has meant that it can be used at very low doses to treat vitamin D-dependent or -resistant osteopathies.[227] This has reduced the risks of hypervitaminosis that attend the use of the massive doses of D_3 that are needed to provide effective therapy in such cases.

12. CASE STUDIES

Instructions

Review each of the following case reports, paying special attention to the diagnostic indicators on which the respective treatments were based. Then answer the questions that follow.

Case 1

When the patient was first evaluated at the National Institutes of Health, he was a thin, short, bowlegged, 20-year-old male. His height at that time was 159 cm (below the first percentile) and he weighed 52 kg. In addition to his dwarfism, he showed a varus deformity of both knees, and he walked with a waddling gait. Radiographs showed diffusely decreased bone density, subperiosteal resorption, and a **pseudofracture**[228] of the left ischiopubic ramus.[229]

Parameter	Patient	Normal Range
Serum Ca	8.0 mg/dL	8.5–10.5 mg/dL
Serum phosphorus	2.2 mg/dL	3.5–4.5 mg/dL
Serum alkaline phosphatase	152 U/mL	<77 U/mL
Urine chromatography	Generalized aminoaciduria	

The patient's history revealed that he had been a normal, full-term infant weighing 3.2 kg. He had been breast-fed and had been given supplementary vitamin D. At 20 months, however, he failed to walk unsupported and was diagnosed as having active rickets, as revealed by genu varum, irregular cupped metaphyses, and widened growth plates,[230] with reductions of both Ca and phosphorus in his blood. The rickets did not respond to oral doses of ergocalciferol (normally effective in treating nutritional rickets), but healing was observed radiographically after intramuscular administration of 1,500,000 IU (37.5 mg) of vitamin D_2 weekly for 5 months. The patient continued to receive vitamin D in the form of cod liver oil, 5000–20,000 IU (125–500 mg) per day. At 4 years of age, corrective surgery was performed for deformities of the tibias and femurs. At age 14, the patient's height was in the 15th percentile. Additional surgery was performed, after which vitamin D therapy was stopped and, over the next 2 years, weakness and severe bone pain became evident. At age 19, bilateral femoral osteotomies[231] were performed again. As an outpatient at the NIH Clinical Center, the patient received oral ergocalciferol, 50,000 IU daily, for the next 6 years and experienced remission of pain and weakness and normalization of serum Ca and phosphorus levels. His height reached 161 cm (63.3 in.), i.e., still below the first percentile. At 27 years of age, his radiographs showed improved density of the skeletal cortices and healing of the pseudofractures, but the patient still showed the clinical stigmata[232] of rickets.

Parameter	Patient	Normal Range
Serum PTH (parathyroid hormone)	0.31 ng/mL	<0.22 ng/mL
Urine cAMP	6 nmol/dL	2.3 ± 1.2 nmol/dL
^{47}Ca absorption	19%	33–43%
Plasma 25-OH-D_3	25 ng/mL	10–40 ng/mL
Plasma 1,25-$(OH)_2$-D_3	213 pg/mL	20–60 pg/mL
Plasma 24,25-$(OH)_2$-D_3	1.0 ng/mL	0.8–3 ng/mL

Two hundred micrograms of 25-OH-D_3 was then given orally daily for 2 weeks. Calcium retention improved, urinary cAMP fell, and plasma phosphorus and Ca rose, each to the normal level. Vitamin D_3 maintenance doses (about 40,000 IU, i.e., 1 mg/day) were given periodically to prevent recurrent osteomalacia.

Case 2

This patient was a sister of the patient described in Case 1. She was first evaluated at the NIH when she was 18 years old. She was a thin female dwarf (147 cm tall, below the first percentile) weighing 44.8 kg. She walked with a waddling gait and had mild bilateral varus deformities of the knees. **Chvostek's sign**[233] was present bilaterally. Analyses of her serum showed 7.0 mg of Ca and 3.0 mg of phosphorus per deciliter and alkaline phosphatase at 110 U/ml. Skeletal radiographs showed delayed ossification of several

227. These diseases include hypoparathyroidism, genetic or acquired hypophosphatemic osteomalacias, renal osteodystrophy, vitamin D-dependent rickets, and osteomalacia associated with liver disease and enteric malabsorption.
228. i.e., New bone detected radiographically as thickening of the periosteum at the site of an injury to the bone.
229. A narrow process of the pelvis.
230. Failure of mineralization of the growing ends of long bones.
231. Surgical correction of bone shape.
232. Residual abnormalities.
233. Facial spasm induced by a slight tap over the facial nerve.

epiphyses and a pseudofracture in the left tibia. Her plasma 25-OH-D was 44 ng/mL, 1,25-$(OH)_2$-D_3 was 280 pg/ml, and 24,25-$(OH)_2$-D_3 was 2.5 ng/mL.

Her history showed that she had been a normal, full-term infant who weighed 3.8 kg at birth. At 5 months of age, she showed radiographic features of rickets. During infancy and childhood, she received vitamin D as cod liver oil, in doses of 2000–10,000 IU/day. She began to walk at 9 months and developed slight varus deformity of both legs. Her rate of growth was at the fifth percentile until the vitamin D was discontinued when she was 11 years old. Within 3 years, her height fell below the first percentile. From ages 15 to 16, the bowing of her legs progressed moderately. When she was 18 years old, at the time of her first admission to the NIH Clinical Center, she was treated with 200 μg of 25-OH-D_3 per day for 2 weeks. During this time, her Ca retention improved, and her serum Ca and P increased. Studies showed that 500 μg 20 (IU) of D_3 per day was required to maintain her plasma Ca in the normal range. At this dose, her 25-OH-D_3 was 141 ng/ml, 1,25-$(OH)_2$-D_3 was 640 pg/mL, and 24,25-$(OH)_2$-D_3 was 3.6 ng/mL (above normal). When she was 24 years old, i.e., 6 years after her first admission to the center, she was readmitted for studies of the effectiveness of oral 1,25-$(OH)_2$-D_3 with a supplement of 800 mg of Ca per day. Serum Ca remained below normal on doses of 2–10 μg of 1,25-$(OH)_2$-D_3 per day. Only when the dose was increased to 14–17 μg of 1,25-$(OH)_2$-D_3 per day did her plasma Ca reach the normal range. PTH remained elevated at 0.40 ng/mL. At these high doses of 1,25-$(OH)_2$-D_3, her plasma 25-OH-D_3 was 26 ng/mL, and her 1,25-$(OH)_2$-D_3 was 400 pg/mL. While on 1,25-$(OH)_2$-D_3, her osteomalacia improved, and serum Ca and P entered normal ranges.

Case Questions

1. What are the common clinical features (physical and biochemical observations, response to treatment, etc.) of these two cases?
2. What can you infer about the nature of vitamin D metabolism in these siblings?
3. Propose a hypothesis to explain these cases of vitamin D-resistant rickets. How might you test this hypothesis?

13. STUDY QUESTIONS AND EXERCISES

1. Construct a flow diagram showing the metabolism of vitamin D to its physiologically active and excretory forms.
2. Construct a "decision tree" for the diagnosis of vitamin D deficiency in a human or animal. How can deficiencies of vitamin D and Ca be distinguished?
3. How does the concept of solubility relate to vitamin D utilization? What features of the chemical structure of vitamin D relate to its utilization?
4. Relate the concept of organ function to the concept of vitamin D utilization/status.
5. Discuss the concept of homeostasis, using vitamin D as an example.
6. Using an evidence-based approach, discuss whether physiological conditions in addition to those caused by vitamin D deficiency should be considered in establishing the RDA for vitamin D.

RECOMMENDED READING

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Chapter 8

Vitamin E

Chapter Outline

Anchoring Concepts

1. Vitamin E is the generic descriptor for all tocopherol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol.
2. The E vitamers are hydrophobic and, thus, are insoluble in aqueous environments (e.g., plasma, interstitial fluids, cytosol).
3. By virtue of the phenolic hydrogen on the C-6 ring hydroxyl group, the E vitamers have antioxidant activities.
4. Deficiencies of vitamin E have a wide variety of clinical manifestations in different species.

Vitamin E is a focal point for two broad topics, namely, biological antioxidants and lipid peroxidation damage. Vitamin E is related by its reactions to other biological antioxidants and reducing compounds that stabilize polyunsaturated lipids and minimize lipid peroxidation damage. In vivo lipid peroxidation has been identified as a basic deteriorative reaction in cellular mechanisms of aging processes, in some phases of atherosclerosis, in chlorinated hydrocarbon hepatotoxicity, in ethanol-induced liver injury, and in oxygen toxicity. These processes may be indicative of a universal disease, the chemical-deteriorative effects of which might be slowed by the use of increased amounts of antioxidants.

A. L. Tappel[1]

1. Aloys (Al) Tappel is a leader in the field of oxidative damage and antioxidant and was the first to suggest a synergistic relationship of vitamins E and C. His productive career was spent on the faculty of the Department of Food Science and Technology of the University of California at Davis.

LEARNING OBJECTIVES

1. To understand the various sources of vitamin E
2. To understand the means of enteric absorption, transport, and cellular uptake of vitamin E
3. To understand the metabolic functions of E vitamers
4. To understand the interrelationships of vitamin E and other nutrients
5. To understand the physiological implications of high doses of vitamin E

VOCABULARY

Abetalipoproteinemia
Antioxidant
Apolipoprotein E (apoE)
Ataxia
Ataxia with vitamin E deficiency
α-Carboxyethylhydroxychroman (α-CEHC)
5′-α-Carboxymethylbutylhydroxychroman (5′-α-CMBHC)
Catalase
Conjugated diene
Cysteine
Cytochrome *P*-450
Encephalomalacia
Ethane
Exudative diathesis
Familial isolated vitamin E deficiency
Foam cells
Free radicals
Free-radical theory of aging
Glutathione (GSH)
Glutathione peroxidases

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00008-3

Glutathione reductase
Hemolysis
Hemolytic anemia
High-density lipoproteins (HDLs)
Hydroperoxide
Hydroxyl radical (HO·)
Hydrogen peroxide (H_2O_2)
Intraventricular hemorrhage
Ischemia–reperfusion injury
Lipid peroxidation
Lipofuscin
Lipoprotein lipase
Lipoproteins
Liver necrosis
Low-density lipoproteins (LDLs)
Malonyldialdehyde (MDA)
Mitochondrial hormesis
Mulberry heart disease
Myopathy
5-Nitro-tocopherol
Oxidative stress
Oxidized LDLs
Pentane
Peroxide
Peroxyl radical (ROO·)
Phospholipid transfer protein (PLTP)
Polyunsaturated fatty acids (PUFAs)
Prooxidant
Reactive oxygen species (ROS)
Redox tone
Resorption–gestation syndrome
Respiratory burst
Scavenger receptors
Selenium
Simon metabolites
Steatorrhea
Superoxide dismutases
Superoxide radical, $O_2\cdot^-$
Thiobarbituric acid (TBA)
Tocol
Tocopherol-associated protein (TAP)
Tocopherol ω-hydrolases
Tocopherols
α-Tocopherol
α-Tocopherol transfer protein (α-TTP)
β-Tocopherol
γ-Tocopherol
δ-Tocopherol
α-Tocopheronic acid
α-Tocopheronolactone
α-Tocopheroxyl radical
α-Tocopheryl hydroquinone
α-Tocopheryl phosphate
α-Tocopheryl polyethylene glycol-succinate
α-Tocopheryl quinone
α-Tocopheryl succinate
Tocotrienols
Very low density lipoprotein (VLDL)
White muscle disease

1. SIGNIFICANCE OF VITAMIN E

Vitamin E has a fundamental role in the normal metabolism of all cells. Therefore, its deficiency can affect several different organ systems. Its function is related to those of several other nutrients and endogenous factors that, collectively, comprise a multicomponent system that provides protection against the potentially damaging effects of reactive species of oxygen formed during metabolism or that are encountered in the environment. Both the need for vitamin E and the manifestations of its deficiency can be affected by antioxidant nutrients such as **selenium** and vitamin C and by exposure to **prooxidant** factors such as **polyunsaturated fatty acids** (**PUFAs**), air pollution, and ultraviolet (UV) light. Recent evidence indicates that vitamin E may also have nonantioxidant functions in regulating gene expression and cell signaling.

Unlike other vitamins, vitamin E is not only essentially nontoxic but also appears to be beneficial at dose levels appreciably greater than those required to prevent clinical signs of deficiency. Most notably, supranutritional levels of the vitamin have been useful in reducing the oxidation of **low-density lipoproteins** (**LDLs**) and, thus, reducing the risk of atherosclerosis. Although vitamin E is present in most plants, only plant oils are rich sources, and most people consume less than recommended levels.[2] Its low regular intake, ubiquitous and complex nature of its biological function, its demonstrated safety, and its apparent usefulness in combating a variety of **oxidative stress** disorders have generated enormous interest in this vitamin among the basic and clinical science communities and the lay public.

2. PROPERTIES OF VITAMIN E

Vitamin E Structure

The term vitamin E describes **tocopherols** and **tocotrienols**, both of which are isoprenoid side chain derivatives of 6-chromanol that show the biological activity of α-tocopherol. The tocopherols have side chains comprising three fully saturated isopentyl units; the most important of these is **α-tocopherol**. The tocotrienols have side chains containing three double bonds. For these compounds to have

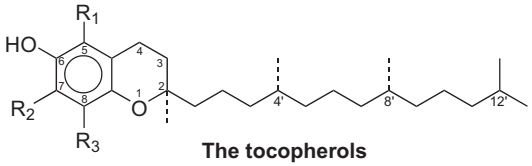
2. Maras, J.E., Bermudez, O.I., Qiao, N., et al., 2004. J. Am. Diet. Assoc. 104, 567–575.

TABLE 8.1 Chromanol Head Group Substituents of Major E Vitamers

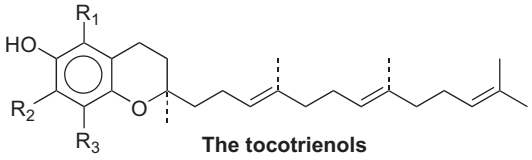
Vitamer	R_1	R_2	R_3
α-Tocopherol/α-tocotrienol	CH_3	CH_3	CH_3
β-Tocopherol/β-tocotrienol	CH_3	H	CH_3
γ-Tocopherol/γ-tocotrienol	H	CH_3	CH_3
δ-Tocopherol/δ-tocotrienol	H	H	CH_3
Tocol/tocotrienol	H	H	H

biological vitamin E activity, the obligate structural feature is a free hydroxyl or ester linkage on C-6 of the chromanol nucleus. Hence, the E vitamers are named according to the position and number of methyl groups on their chromanol nuclei (Table 8.1).

Chemical structures of the vitamin E group.



The tocopherols



The tocotrienols

Because the tocopherol side chain contains two chiral centers (C-4′, C-8′) in addition to the one at the point of its attachment to the nucleus (C-2), eight stereoisomers are possible. However, only one stereoisomer occurs naturally: the *R,R,R*-form. The chemical synthesis of vitamin E produces mixtures of other stereoisomers, depending on the starting materials.[3] Although the use of phytol from natural sources (with the *R*-configuration at both the four- and 8-carbons) yields a product racemic at only the 2-position, commercial synthesis of vitamin E now uses primarily a fully synthetic side chain, which yields a mixtures of all eight possible stereoisomers, i.e., 2*RS*, 4′*RS*, and 8′*RS* compounds. This mixture is designated with the prefix all-*rac*- (e.g., all-*rac*-α-tocopherol, all-*rac*-α-tocopheryl acetate). The acetate esters of vitamin E are used in human nutritional supplements and animal feeding, whereas the unesterified (i.e., free alcohol) forms are used as antioxidants in foods and pharmaceuticals. Other forms (e.g., α-tocopheryl hydrogensuccinate, α-tocopheryl polyethylene glycol-succinate) are also used in multivitamin preparations.

Vitamin E Chemistry

The tocopherols are light yellow oils at room temperature. They are insoluble in water, but are readily soluble in nonpolar solvents. Tocopherols and their acetates have absorption maxima in the range of 280–300 nm (α-tocopherol, 292 nm); however, their extinction coefficients are not great (70–91). Their fluorescence is significant (excitation, 294 nm; emission, 330 nm), particularly in polar solvents (e.g., diethyl ether or alcohols); this property has analytical utility. Being monoethers of a hydroquinone with a phenolic hydrogen (in the hydroxyl group at position C-6 in the chromanol nucleus), with the ability to accommodate an unpaired electron within the resonance structure of the ring (undergoing transition to a semistable chromanoxyl radical before being converted to tocopheryl quinone), they are good quenchers of free radicals and thus serve as antioxidants.

Vitamin E Stability

The properties that make tocopherols effective antioxidants also render them unstable under aerobic conditions. They are easily oxidized and can be destroyed by peroxides, ozone, and permanganate in a process catalyzed by light and accelerated by PUFAs and metal salts. They are resistant to acids; under anaerobic conditions, they are stable to bases. Tocopheryl esters, by virtue of the blocking of the C-6 hydroxyl group, are very stable in air; therefore, they are the forms of choice as food/feed supplements. Because tocopherol is liberated by the saponification of its esters, extraction and isolation of vitamin E requires the use of protective antioxidants (e.g., propyl gallate, ascorbic acid), metal chelators, inert gas environments, and subdued light.

Vitamin E Biopotency

The E vitamers vary in biopotency, depending on the positions and numbers of their nucleus methyl groups, which determine antioxidant activity, and the conformation of their side chains, which determine distribution and retention in tissues. The vitamer with greatest biopotency is the trimethylated (α) tocopherol (Table 8.2).

Expressing Vitamin E Activity

Vitamin E activity is shown to different degrees by several side chain isomers and methylated analogs of tocopherol and tocotrienol (Table 8.2), the epimeric configuration at the

3. Through the early 1970s, the commercial synthesis of vitamin E used, as the source of the side chain, isophytol isolated from natural sources (with the *R*-configuration at both the 4- and 8-carbons). Tocopherols so produced were racemic at only the C-2 position. The mixture, 2*RS*-α-tocopherol, was called D,L-α-tocopherol; its acetate ester was the form of commerce and was adopted as the international standard on which the biological activities of other forms of the vitamin are still based.

TABLE 8.2 Relative Biopotencies of Vitamin E–Active Compounds

Trivial Designation	Systematic Name	Biopotency (IU/mg)[a]
R,R,R-α-tocopherol[b]	2*R*,4′*R*,8′*R*-5,7,8-Trimethyltocol	1.49
R,R,R-α-tocopheryl acetate	2*R*,4′*R*,8′*R*-5,7,8-Trimethyltocyl acetate	1.36
All-*rac*-α-tocopherol[c]	2*RS*,4′*RS*,8′*RS*-5,7,8-Trimethyltocol	1.1
All-*rac*-α-tocopheryl acetate	2*RS*,4′*RS*,8′*RS*-5,7,8-Trimethyltocylacetate	1.0
R,R,R-β-tocopherol	2*R*,4′*R*,8′*R*-5,8-Dimethyltocol	0.12
R,R,R-γ-tocopherol	2*R*,4′*R*,8′*R*-5,7-Dimethyltocol	0.05
R-α-tocotrienol	*trans*-2*R*-5,7,8-Trimethyltocotrienol	0.32
R-β-tocotrienol	*trans*-2*R*-5,8-Dimethyltocotrienol	0.05
R-γ-tocotrienol	*trans*-2*R*-5,7-Dimethyltocotrienol	—

[a]*Based chiefly on rat gestation–resorption bioassay data.*
[b]*Formerly called* D-α-*tocopherol.*
[c]*Formerly called* DL-α-*tocopherol, this form remains the international standard despite the fact that it has not been produced commercially for many years.*

TABLE 8.3 Standards for Potency of Major Vitamers E

Vitamer	mg/IU	α-Tocopherol Equivalents (mg)
All-*rac*-α-tocopherol	0.91	0.74
All-*rac*-α-tocopheryl acetate	1	0.67
R,R,R-α-tocopherol	0.67	1
R,R,R-α-tocopheryl acetate	0.74	0.91

2-position being important in determining biological activity. Therefore, the use of an international standard facilitated the referencing of these various sources of vitamin E activity, reflecting differences in their absorption, transport, retention and metabolism, and their intrinsic biopotency. The original preparation "D,L-α-tocopheryl acetate"[4] that served as the international standard has not existed for more than 30 years; R,R,R-α-tocopherol is now used as the international standard (Tables 8.3 and 8.4).[5]

Some of the E vitamers commonly found in foods (**β-** and **γ-tocopherol**, the **tocotrienols**) have little biological activity. The most biopotent vitamer, i.e., the vitamer of greatest interest in nutrition, is α-tocopherol, which occurs naturally as the *RRR* stereoisomer [(*RRR*)-α-tocopherol].

4. Through the 1970s, the standard was called D,L-α-tocopheryl acetate; now it would be called (2*RS*)-α-tocopheryl acetate. Because of uncertainty about the proportions of the two diastereoisomers in that mixture, once the supply was exhausted it was impossible to replace it.

5. This system distinguishes only the methylated analogs and not the particular diastereoisomers possible for each.

3. SOURCES OF VITAMIN E

Distribution in Foods

Vitamin E is synthesized only by photosynthetic organisms – plants, algae, and some cyanobacteria – where it is thought to function as a protective antioxidant in germination and cold adaptation. All higher plants appear to contain **α-tocopherol** in their leaves and other green parts. Because α-tocopherol is contained mainly in the chloroplasts of plant cells (whereas, the β-, γ-, and δ-vitamers are usually found outside of these particles), green plants tend to contain more vitamin E than yellow plants. The richest food sources are plant oils: wheat germ, sunflower, and safflower oils are rich sources of α-(*RRR*)-tocopherol; corn and soybean oils contain mostly γ-(*RRR*)-tocopherol. Some plant tissues, notably bran and germ fractions[6], can also contain tocotrienols, often in esterified form, unlike the tocopherols which exist only as free alcohols. Animal tissues tend to contain low amounts of α-tocopherol, the highest levels occurring in fatty tissues. These levels vary according to the dietary intake of the vitamin.[7] Because vitamin E occurs naturally in fats and oils, reductions in fat intake can be expected also to reduce vitamin E intake. An amphipathic

6. Palm oil and rice bran have high concentrations of tocotrienols; other natural sources include coconut oil, cocoa butter, soybeans, barley, and wheat germ.

7. Muscle from beef fed high levels of vitamin E (e.g., 1300 IU/day) before slaughter can yield vitamin E in excess of 16 nmol/g; this level is effective in reducing postmortem oxidation reactions, thus delaying the onset of meat discoloration because of hemoglobin oxidation and the development of oxidative rancidity.

TABLE 8.4 Relative Biopotencies (%) of Tocopherols and Tocotrienols by Different Bioassays

Vitamer	Prevention of Fetal Resorption (Rat)	Prevention of Hemolysis (Rat)	Prevention of Myopathy (Chick)	Therapy for Myopathy (Rat)
α-Tocopherol	100	100	100	100
β-Tocopherol	25–40	15–27	12	—
γ-Tocopherol	1–11	3–20	5	11
δ-Tocopherol	1	0.3–2	—	—
α-Tocotrienol	28	17–25	—	28
β-Tocotrienol	5	1–5	—	—

metabolite, **α-tocopheryl phosphate**[8], has also been identified at trace levels in foods and animal tissues.

The important sources of vitamin E in human diets and animal feeds are vegetable oils and, to lesser extents, seeds and cereal grains (Table 8.5). The dominant dietary form (70% of tocopherols in American diets) is γ-tocopherol (Tables 8.6 and 8.7). Wheat germ oil is the richest natural source, containing 0.9–1.3 mg of α-tocopherol per gram, i.e., about 60% of its total tocopherols. The seeds and grains from which these oils are derived also contain appreciable amounts of vitamin E. Plants also synthesize tocotrienols. The richest food sources are rice bran oil, in which tocotrienols comprise most of the E vitamers; and palm oil, in which tocotrienols comprise 70% of total E vitamers. Cereals contain small amounts of tocotrienols. Accordingly, cereals in general and wheat germ in particular are good sources of the vitamin. Foods that are formulated with vegetable oils (e.g., margarine, baked products) tend to vary greatly in vitamin E content because of differences in the types of oils used and the thermal stabilities of the E vitamers present.[9] α-Tocopherol is used in dietary supplements.[10] Regardless of the form consumed, α-tocopherol is the main form found in tissues.

The processing of foods and feedstuffs can remove substantial amounts of vitamin E. Vitamin E losses can occur as a result of exposure to peroxidizing lipids formed during the development of oxidative rancidity of fats and to other oxidizing conditions such as drying in the presence of sunlight and air, the addition of organic acids,[11] irradiation, and canning. Milling and refining can reduce the vitamin E content by removal of tocopherol-rich bran and germ fractions and because of the use of bleaching agents (e.g., hypochlorous acid) to improve the baking characteristics of the flour. Some foods (e.g., milk and milk products) also show marked seasonal fluctuations in vitamin E content related to variations in vitamin E intake of the host (e.g., vitamin E intake is greatest when fresh forage is consumed). The many potential sources of vitamin E loss mean that the vitamin E contents of foods and feedstuffs vary considerably.[12]

High–Vitamin E Animal Foods

High-level vitamin E supplementation (e.g., α-tocopheryl acetate fed at levels 10- to 50-fold standard practice) of the diets of poultry, swine, and beef have been found to be effective in increasing the α-tocopherol contents of many tissues. High levels of the vitamin in edible tissues serve to inhibit postmortem oxidative production of off-flavors (oxidative rancidity of lipids) and color (hemoglobin oxidation), which reduce drip loss and increase the effective shelf life of the retail cuts of meat. Studies show that the incorporation of α-tocopherol into muscle is linearly related to α-tocopherol intake to about 220 IU/day for pork and 600 IU/day for beef at which each approached an apparent asymptote of c. 5 μg/g tissue, which corresponded to peroxidizability minima.[13] α-Tocopheryl acetate is often used to protect hens from aflatoxin contaminants of feedstuffs; the α-tocopherol content of eggs has also been increased using high-level vitamin E supplements to laying hen diets. The addition of c. 270 IU α-tocopheryl acetate per kilogram of feed increased egg α-tocopherol threefold, to about 18 mg per egg.[14]

8. Water-soluble and resistant to both acid and alkaline hydrolysis, this metabolite has been missed by traditional methods of vitamin E analysis.
9. Tocotrienols tend to be less stable to high temperatures than tocopherols; baking tends to destroy them selectively.
10. Water-dispersible formulations have been developed for treating lipid-malabsorbing patients.
11. The addition of 1% propionic acid (as an antifungal agent) to fresh grain can destroy up to 90% of its vitamin E.
12. For example, refining losses in edible plant oils are typically 10–40%, but can sometimes be much greater.
13. Sales, J., Koukolová, V., 2011. J. Anim. Sci. 89, 2836–2848.
14. Sujatha, T., Narahari, D., 2011. J. Food Sci. Technol. 48, 494–497.

TABLE 8.5 Significant Food Sources of Vitamin E (≥2 mg/100 g)

Food	Vitamin E (mg/100 g)
Fats and Oils	
Wheat germ oil	149.4
Sunflower oil	41.1
Rice bran oil	32.3
Canola oil	17.5
Palm oil	15.9
Peanut oil	15.7
Olive oil	14.4
Corn oil	14.3
Mayonnaise	11.8
Soybean oil	8.2
Magarine, hard type	3.1
Chicken fat, beef tallow	2.7
Egg yolk	2.3
Butter	2.3
Nuts and Seeds	
Sunflower kernels, dried	35.2
Almonds, dry roasted, unblanched	23.9
Filberts (hazelnuts), dry roasted	15.3
Peanuts, dry roasted	4.9
Pistachio nuts, dry roasted	2.2
Walnuts, dried	2.1
Fish	
Swordfish, dry heat cooked	2.4
Shrimp, moist heat cooked	2.2
Vegetables and Fruits	
Dandelion greens, raw	3.4
Turnip greens, boiled	2.1
Avocados, raw	2.1
Spinach, raw	2.0
Other	
Chilli powder	38.1
Curry powder	25.2
Wheat germ	16.0

From USDA National Nutrient Database for Standard Reference, Release 28. http://www.ars.usda.gov/ba/bhnrc/ndl.

4. ABSORPTION OF VITAMIN E

The primary site of absorption appears to be the medial small intestine. Esterified forms of the vitamin E are hydrolyzed, probably by a mucosal esterase; the predominant forms absorbed are free alcohols. Most studies have shown no appreciable differences in the efficiency of absorption of the acetate ester and free alcohol forms, nor differences in the absorption of the various tocopherol and tocotrienol vitamers. It is clear, however, that regardless of the form absorbed, higher intakes lead to higher amounts of absorption but lower absorption efficiencies (i.e., fractional absorption). At nutritionally important intakes, variable (generally, 20–70%[15]) absorption efficiencies have been reported, with large portions of ingested vitamin E appearing in the feces.

Micelle-Dependent Diffusion

Similar to other hydrophobic substances, vitamin E appears to be absorbed by nonsaturable passive diffusion dependent on micellar solubilization and, hence, the presence of bile salts and pancreatic juice. It is clear that the enteric absorption of vitamin E is dependent on the adequate absorption of lipids, the process requires the presence of fat in the lumen of the gut, and the secretion of pancreatic esterases for the release of free fatty acids from dietary triglycerides, bile acids for the formation of mixed micelles, and esterases for the hydrolytic cleavage of tocopheryl esters when those forms are consumed. Individuals unable to produce pancreatic juice or bile (e.g., patients with biliary obstruction, cholestatic liver disease, pancreatitis, cystic fibrosis, short bowel syndrome) show impaired absorption of vitamin E, and other fat-soluble substances dependent on micelle-facilitated diffusion for their uptake. The micelle-dependent absorption of vitamin E would imply a need for dietary fat to facilitate the process; need for lipid would explain reports of vitamin E in dietary supplements not being well absorbed unless taken with a meal.[16] Studies with radiolabeled α-tocopherol have shown its enteric absorption in humans to be impaired by dietary fat levels less than c. 10% (i.e., 21% of total calories) (Fig. 8.1);[17] however, absorption of that vitamer by the rat was not impaired by feeding a diet containing fat as only 1.6% of total calories. It has been suggested that children can adequately absorb the fat-soluble vitamins with fat intakes as low as 5 g per day. Furthermore, tocopherols can interact with

15. The enteric absorption of γ-tocopherol appears to be only 85% of that of α-tocopherol.
16. Leonard, S.W., Good, C.G., Gugger, T.E., et al., 2004. Am. J. Clin. Nutr. 79, 86–92.
17. Bruno, R.S., Leonard, S.W., Park, S.I., et al., 2006. Am. J. Clin. Nutr. 83, 299–304.

TABLE 8.6 E Vitamers in Fats and Oils

	Tocopherols (%)			Tocotrienols (%)			
Item	**α**	**γ**	**δ**	**α**	**β**	**γ**	**δ**
Animal Fats							
Lard	>90	<5		<5			
Butter	>90	<10					
Tallow	>90	<10					
Plant Oils							
Soybean	4–18	58–69					
Cotton	51–67	33–49					
Maize	11–24	76–89					
Coconut	14–67		<17	<14	<3	<53	<17
Peanut	48–61	39–52					
Palm	28–50		<9	16–19	4	34–39	<9
Safflower	80–94	6–20					
Olive	65–85				15–35		

From Chow, C.K., 1985. World Rev. Nutr. Diet. 45, 133–166.

TABLE 8.7 E Vitamers in Grains and Oil Seeds

	Tocopherols (%)				Tocotrienols (%)	
Item	**α**	**β**	**γ**	**δ**	**α**	**γ**
Grains						
Maize	6–15		29–55		5–10	34–77
Oats	4–8	<1			10–22	
Milo	4–7		14–17		<1	
Barley	8–10	1–2	3–4		23–28	3
Wheat	8–12	4–6			2–3	
Oil Seeds						
Soybean	1–3		3–33	2–6		trace
Cotton seed	1–18		5–18			1–2

From Cort, W.M., Vicente, T.S., Waysek, E.H., et al., 1983. J. Agric. Food Chem. 31, 1330–1333.

PUFAs in the intestinal lumen; this can result in absorption being stimulated by medium-chain triglycerides and inhibited by linoleic acid.

Role of Mucosal Receptors

Evidence has been presented for roles of cholesterol and lipid transporters in the uptake of α-tocopherol by enterocytes. Several have been suggested: the scavenger receptor class B type I (SR-BI),[18] CD36,[19] NPC1L1,[20]

18. Mice lacking SR-BI show marked reductions in the amounts of α-tocopherol in plasma (particularly, in the HDL fraction) and tissues (Mardones, P., Strobel, P., Miranda, S., et al., 2002. J. Nutr. 132, 443–449).
19. Cluster determinant 36, also called fatty acid translocase.
20. Niemann-Pick C1–like 1; mutations cause a lysosomal disorder, Niemann-Pick type C disease characterized by massive intracellular accumulation of cholesterol and other lipids.

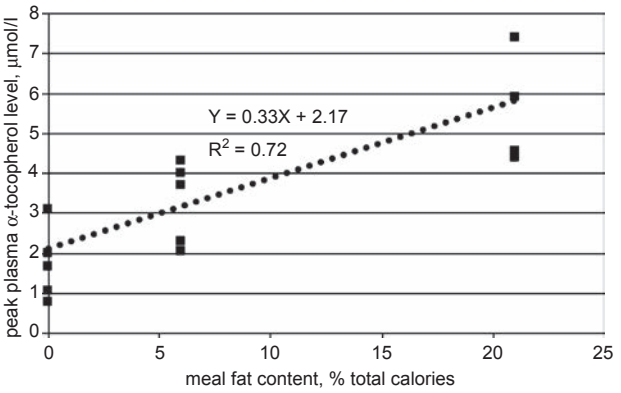


FIGURE 8.1 Effect of the fat level of a meal on the absorption of deuterium-labeled α-tocopherol from that meal by healthy adults. *After Bruno, R.S., Leonard, S.W. Park, S.I., et al., 2006. Am. J. Clin. Nutr. 83, 299–304.*

and ABCA1.[21] ABCA1 has been found to be involved in the export of tocopherols from enterocytes into the lymphatic circulation.[22] Its interaction with α-tocopherol transfer protein (α-TTP) promotes preferential trafficking of α-tocopherol over non–α-vitamers. The inhibition of α-tocopherol enteric absorption by carotenoids, green tea catechins, and γ-tocopherol may involve competitive binding to these receptors.

Uptake Into Lymphatic Circulation

Absorbed vitamin E, similar to other hydrophobic substances, enters the lymphatic circulation[23] in association with nascent triglyceride-rich chylomicra. Studies with radiolabeled compounds have showed preferential lymphatic uptake of α-tocotrienol over α-tocopherol and γ- and δ-tocotrienols. Within the enterocytes, vitamin E combines with other lipids and apolipoproteins to form chylomicra, which are released into the lymphatics in mammals or the portal circulation in birds and reptiles. Thus, the kinetics of vitamin E absorption are biphasic, reflecting the initial uptake of the vitamin by existing chylomicra followed by a lag phase because of the assembly of new chylomicra.

5. TRANSPORT OF VITAMIN E

Being virtually insoluble in aqueous environments, vitamin E is dependent on carriers for its transport to the tissues. Unlike vitamins A and D, vitamin E does not have a specific carrier protein in the plasma. Instead, it is rapidly transferred from chylomicra to plasma lipoproteins to which it binds nonspecifically.

21. ATP-binding cassette A1.
22. Oram, J.F., Vaughan, A.M., Stocker, R., 2001. J. Biol. Chem. 276, 39,898–39,902.
23. That is, the portal circulation in birds, fishes and reptiles.

Role of Chylomicra

Vitamin E is transported to the liver by triglyceride-rich chylomicra. The vitamin appears to partition directly into the plasma membranes of parenchymal cells. It may also be taken up with circulating lipoproteins to which the vitamin can transfer as chylomicra are metabolized (Fig. 8.2).

Roles of Lipoproteins

Vitamin E is transported from the liver to peripheral tissues by **VLDLs** synthesized by parenchymal cells. The concentration of vitamin E in plasma is linearly related to the intake of the vitamin up to about 200 mg/day (Fig. 8.3). The diminishing response above that level reflects uptake by the liver for the selective incorporation of vitamin E into nascent VLDLs. Although the majority of the triglyceride-rich VLDL remnants are returned to the liver, some are converted by lipoprotein lipase to **LDLs**. It appears that, during this process, vitamin E also transfers spontaneously to apolipoprotein B (apoB)–containing lipoproteins including the VLDLs, LDLs, and **high-density lipoproteins** (**HDLs**). Therefore, plasma tocopherols are distributed among these three lipoprotein classes, with the more abundant LDL and HDL classes comprising the major carriers of vitamin E. As each class of lipoproteins derives its tocopherols ultimately from chylomicra, differences in their α-tocopherol transport comprise the major source of interindividual variation in response to ingested vitamin E. These kinetics are altered by hypercholesterolemia and hypertriglyceridemia in which tocopherol uptake into and turnover in the plasma is reduced. Tocopherol metabolism is also related to **apolipoprotein E (apoE)**, which affects the hepatic binding and catabolism of several classes of lipoproteins. ApoE genotype in the mouse has been found to affect genes encoding for proteins involved in α-tocopherol transport and catabolism; the apoE4 genotype was associated with lower tissue retention of α-tocopherol apparently because of increased retention of the vitamer by LDLs.[24] These differences are accompanied by 15–60% reductions in the levels of mRNA for factors involved in vitamin E binding and transport (SR-BI, LDL receptor, LDL receptor–related protein, ABCA1, the multidrug-resistant transporter) and a more than doubling of the mRNA for CYP3A4, which is involved in tocopherol side chain metabolism.[25] In humans, the apoE4 genotype[26] has been

24. Huebbe, P., Lodge, J.K., G. Rimbach, 2010. Mol. Nutr. Food Res. 54, 623–630.
25. Huebbe, P., Jofre-Monseny, L, Rimbach, G., 2009. IUBMB Life 61, 453–456.
26. The apoE4 genotype is an important genetic risk factor for age-dependent chronic diseases, including cardiovascular disease and Alzheimer's disease.

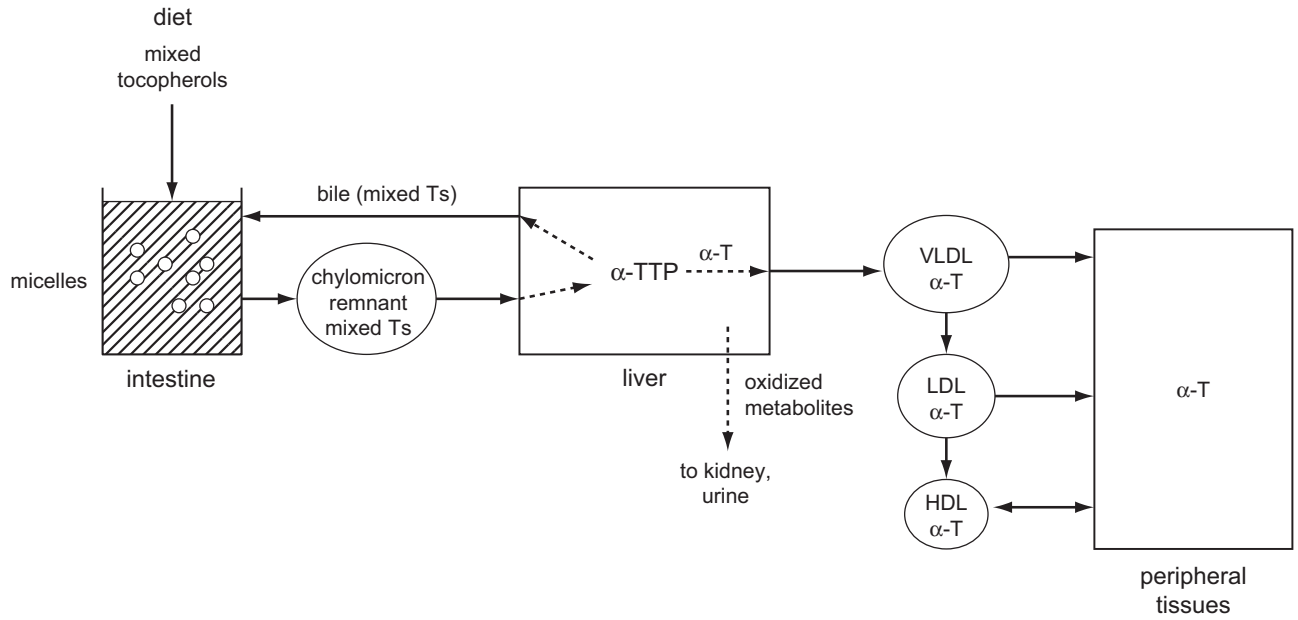


FIGURE 8.2 Absorption and transport of vitamin E. Abbreviations: α-T, α-tocopherol: mixed Ts, mixed tocopherols; **α**-TTP, **α**-tocopherol transfer protein.

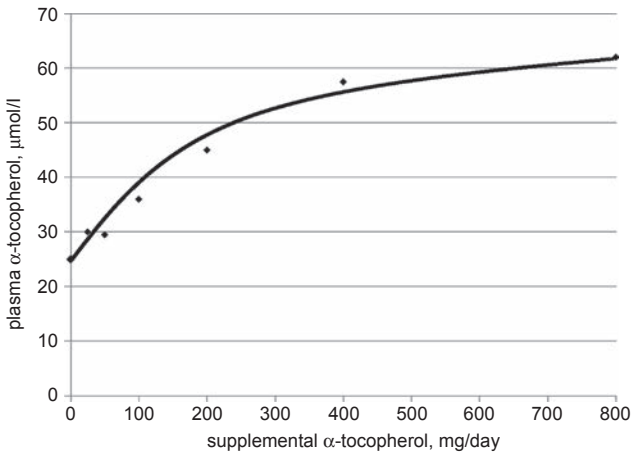


FIGURE 8.3 Plasma response to supplemental α-tocopherol. *After Princen, H.M., van Duyvenvoorde, W. Buytenhek, R., et al., 1995. Arterioscler. Throm. Vasc. Biol. 15, 325–333.*

TABLE 8.8 Relationship of apo E Genotype and Plasma Tocopherol Levels in Children[a]

Genotype	n	α-Tocopherol (μM)[b]	γ-Tocopherol (μM)[b]
E2/2	6	26.5[a] (23.8–29.2)	3.10[a] (2.27–4.22)
E3/2	89	20.8[b] (20.1–21.5)	1.90[b] (1.75–2.07)
E3/3	660	21.3[b] (21.1–21.6)	2.06[a,b] (2.00–2.12)
E4/3	150	21.4[b] (20.9–21.9)	2.05[a,b] (1.92–2.18)
E4/2	8	21.7[a,b] (19.4–23.9)	1.81[a,b] (1.39–2.36)
E4/4	13	19.0[b] (17.2–20.8)	1.84[a,b] (1.49–2.27)

p < .05.
[a]*Ortega, H., Casellia, P., Gomez-Coronado, D., et al., 2005. Am. J. Clin. Nutr. 81, 624–632.*
[b]*Means (95% CI); means with common superscripts are not significantly different,* p > .05.

associated with lower plasma vitamin E concentrations (Table 8.8). Tocopherol exchanges rapidly between the lipoproteins, mediated by the **phospholipid transfer protein** (**PLTP**)[27] and between lipoproteins and erythrocytes, which contain 15–25% of the vitamin E in plasma. Thus, the concentration of vitamin E is correlated with the number of erythrocytes in blood.[28] A fourth of total erythrocyte vitamin E turns over every hour. Postprandial levels of tocopherols exceed those of tocotrienols; this reflects the more rapid metabolic degradation of the latter.

27. Rats, horses, and chicks transport 70–80% of plasma α-tocopherol with HDLs, 18–22% with LDLs, and <8% with VLDLs. Human females, too, transport α-tocopherol preferentially with HDLs; but males transfer most (65%) with LDLs, only 24% with HDLs and 8% with VLDLs.

28. Patients with abetalipoproteinemia are notable exceptions; they may show normal erythrocyte tocopherol concentrations even though their serum tocopherols levels are undetectable.

Cellular Uptake

The cellular uptake of vitamin E appears to occur in the same ways that other lipids are transferred between lipoproteins and cells:

- **Lipase-mediated lipid transfer**. Uptake of α-tocopherol from the amphipathic lipoprotein outer layer is mediated in a directional way by PLTP[29] and lipoprotein lipase-mediated exchange from chylomicra. This route is thought to be important in the uptake of vitamin E by cells that express lipase (adipose, muscle, brain) and particularly important in the transport of α-tocopherol across the blood–brain barrier into the central nervous system.[30]
- **Receptor-mediated endocytosis of lipoproteins**. Evidence suggests that the binding of lipoproteins to specific cell surface receptors must occur to allow the vitamin E to enter cells either by diffusion or with the bulk entrance of lipoprotein-bound lipids. That LDL receptor–deficient cells cannot take up LDL-bound vitamin E at normal rates suggest the involvement of those receptors. Such deficiencies do not necessarily reduce tissue tocopherol levels; but studies in animal models show apoE genotype to be a determinant of both circulating and tissue tocopherol levels.[31] There is also evidence for receptor-mediated uptake of α-tocopherol without uptake of the apolipoprotein, as described for the cellular uptake of cholesterol from HDLs. This appears to involve the scavenger receptors SR-BI[32] and CD36. Polymorphisms of CD36 have been associated with differences in plasma α-tocopherol concentration.[33]
- **Membrane lipid transporter–mediated uptake**. ABCA1, a transporter of cholesterol and other lipids, has been shown to be involved in the export of tocopherols from cells. Its interaction with α-TTP promotes preferential trafficking of α-tocopherol over non–α-vitamers.

Role of the α-Tocopherol Transfer Protein

Although all E vitamers are taken up by the liver, only α-tocopherol is released into the circulation. This is because of the function of a specific tocopherol-binding protein, the **α-tocopherol transfer protein** (**α-TTP**). Originally described in rat liver cytosol where it was found to facilitate the transfer of α-tocopherol between microsomes and mitochondria, α-TTP has been identified in liver, brain, spleen, lung, kidney, uterus, and placenta. It is highly conserved: the rat and human liver proteins show 94% sequence homology, and homologies to the interphotoreceptor retinol-binding protein (IRBP), cellular retinal-binding protein (CRALBP), and a PLTP.

This 32-kDa protein binds α-tocopherol with high affinity. It binds *RRR*-α-tocopherol ninefold more avidly than it does the SRR stereoisomer.[34] It can also bind tocotrienols, although with only 12% of the affinity it shows for α-tocopherol, apparently because of the difficulty of the unsaturated phytyl side chains to fit within the ligand-binding pocket. That pocket comprises an N-terminal helical domain and a C-terminal domain; the latter contains a fold that readily accommodates α-tocopherol and four water molecules, two of which are hydrogen bonded to the hydroxyl group on the chroman ring. Binding affinity for tocopherols is also determined by the degree of methylation of the chroman ring, which determines the extent of van der Waals contacts with the pocket. The relative affinities are as follows: α-tocopherol (100) > β-tocopherol (38) > γ-tocopherol (19) > α-tocotrienol (8.5) > δ-tocopherol (5).

The liganded α-TTP acts as a chaperone for α-tocopherol, taking up the vitamer from endocytic vesicles in which it binds to phospholipid membranes and moving it through the cytoplasm to transport vesicles that travel to the plasma membrane such that the vitamer is ultimately secreted complexed to lipoprotein particles in the circulation.[35] The uptake phase of this process is thought to involve the SR-BI; the discharge phase is thought to involve ABCA1, which interacts with α-TTP to release the ligand from its binding pocket and transfer it to apoA1 and HDL. The selectivity of α-TTP for α-tocopherol contributes to the differences in tissue retention and biopotency of these vitamers,[36] explaining the fact that, whereas γ-tocopherol is the dominant dietary form of vitamin E, α-tocopherol constitutes 90% of body vitamin E. Animal models that do not express α-TTP absorb α-tocopherol normally, showing normal levels in chylomicra, but fail to release the vitamer from the liver,

29. Mice lacking PLTP show high plasma levels of α-tocopherol in apoB-containing lipoproteins (Jiang, X.C., Tall, A.R., Qin, S., et al., 2002. J. Biol. Chem. 277, 31,850–31,856).
30. Mice lacking lipoprotein lipase show low brain α-tocopherol levels (although no associated pathologies have been reported; Goti. D., Balazs, Z., Panzenboeck, U., et al., 2002. J. Biol. Chem. 277:28,537–28,544).
31. apoE4 mice show lower tissue α-tocopherol levels than apoE3 mice (Huebbe, P., Lodge, J.K., Ribach, G., 2010. Mol. Nutr. Food Res. 54, 623–630).
32. Mice lacking SR-BI show marked reductions in the amounts of α-tocopherol in plasma (particularly, in the HDL fraction) and tissues (Mardones, P., Stobel, P., Miranda, S., et al., 2002. J. Nutr. 132, 443–449).
33. Lecompte, S., de Edelenyi, F.S., Goumide, L., et al., Am. J. Clin. Nutr. 93, 644–651.
34. The preferential incorporation of the *RRR*-α-isomer into milk by the lactating sow (Lauridson, C., Engel, H., Jensen, S.K., et al., 2002. J. Nutr. 132, 1258–1264) suggests the presence of α-TTP in the mammary gland.
35. Qian, J., Morley, S., Wilson, K., et al., 2005. J. Lipid Res. 46, 2072–2082; Qian, J. Altkinson, J., Manor, D., 2006. Biochem. 45, 8236–8242; Negris, Y., Meydani, M., Zingg, J.M., et al., 2007. Biochim. Biophys. Res. Commun. 359, 348–353.
36. Neither LDL receptor nor lipoprotein lipase mechanisms of vitamin E uptake by cells discriminate between these stereoisomers; yet, α-tocopherol predominates in plasma because of its preferential incorporation into nascent VLDLs, whereas the form often predominating in foods, γ-tocopherol, is left behind only to be more rapidly excreted.

accumulating hepatic stores at the expense of α-tocopherol in peripheral tissues.[37]

The expression of α-TTP occurs predominantly in the liver in adults; but studies in the Zebrafish show that it plays an essential role in embryogenesis. Studies with immortalized human hepatocytes have shown that the α-TTP messenger RNA is increased in response to oxidative stress, hypoxia, agonists of the nuclear receptors retinoid X receptor and peroxisome proliferator-activated receptor alpha, and increased cAMP levels mediated by the cAMP response element-binding transcription factor.[38] Allelic variants in the human α-TTP gene have been associated with differences in circulating α-tocopherol levels.[39] More serious outcomes have been identified in subjects with other α-TTP gene defects:

- **Familial isolated vitamin E** deficiencyhas been identified in a group of Americans with sporadic vitamin E deficiency, with poor incorporation of *RRR*-α-tocopherol into their VLDLs and an inability to discriminate between the *RRR* and *SRR* vitamers. These patients have exceedingly low circulating tocopherol concentrations unless maintained on high-level vitamin E supplements (e.g., 1 g/day). If untreated, they experience progressive peripheral neuropathy (characterized by pathology of the large-caliber axons of sensory neurons) and **ataxia**.
- **Deletion of the terminal 10% of the α-TTP peptide chain** has been identified in several highly consanguineous Tunisian families whose members show low serum tocopherol levels and ataxia both responsive to high-level vitamin E supplements.[40]
- A missense mutation that inserts histidine in place of glutamine at position 101 of the **α-TTP peptide chain has been identified in** Japanese subjects, whose α-TTP has only 11% of the transfer activity of the wild-type protein. Heterozygous individuals show no clinical signs, but have circulating tocopherol levels 25% lower than those of normal subjects.

Other Tocopherol-Binding Proteins. Tocopherols bind other hydrophobic ligand-binding proteins that share the *cis*-retinal binding motif[41] of α-TTP. These proteins include the cellular retinoic acid–binding protein **CRALBP** and the interphotoreceptor retinol–binding protein **IRBP** (*see* Chapter 6), apparently in the same site as retinol, which readily displaces the tocopherol. A related, tocopherol-associated protein (TAP) has been identified;[42] it appears to have the same amino acid sequence as the previously described supernatant protein factor and may be the same protein. TAP has been found in most tissues, with greatest concentrations in liver, brain, and prostate. That TAP may be a transcription factor is suggested by the finding that liganded TAP translocates from the cytosol to the nucleus and activated gene transcription.[43] TAP appears to have a role in regulating cell growth: its knock-down enhanced prostate cell growth, whereas its overexpression suppressed growth of prostate cancer cells.[44] Evidence also suggests that TAP can serve as a tumor suppressor.[45] TAP mRNA has been found to be negatively associated with tumor stage in breast cancer and, hence, a prospective biomarker of the less aggressive breast carcinoma.

Tissue E Vitamers

Tocopherols. The preferential uptake of α-tocopherol results in that vitamer predominating in tissues. Those contents vary among tissues and tend to be related to vitamin E intake, showing no deposition or saturation thresholds (Table 8.9). In fact, increased intake of α-tocopherol displaces non–α-vitamers in tissues (Fig. 8.4). Neural tissues exhibit very efficient retention, i.e., very low apparent turnover rates, of the vitamin.[46] Kinetic studies indicate that tissues have two pools of the vitamin: a *labile*, rapidly turning over pool; and a *fixed*, slowly turning over pool. The labile pools predominate in such tissues as plasma and liver, as the tocopherol contents of those tissues are depleted rapidly under conditions of vitamin E deprivation. Non–*RRR*-α-tocopherols are quickly removed from the plasma. In humans, *RRR*-α-tocopherol remains in plasma nearly four times longer than *SRR*-α-tocopherol (apparent half-lives: 13 h versus 48 h), and three times longer than *RRR*-γ-tocopherol.[47]

Tocotrienols. Tocotrienols can occur in tissues, but in much lower amounts than tocopherols. How they are taken up is not clear. Although tocotrienols can be bound by α-TTP, that binding is much weaker than that of the tocopherols and evidence indicates that they are taken up by other means. Genetic deletion of α-TTP in the mouse has been found to produce classical signs of vitamin E deficiency (midgestational fetal deaths) in α-tocopherol–fed dams,

37. Leonard, S.W., Terasawa, Y., Farese, Jr., R.V., et al., 2002. Am. J. Clin. Nutr. 75, 555–560.
38. Ulatowski, L., Dreussi, C., Noy, N., 2012. Free Radic. Biol. Med. 53, 2318–2326.
39. Wright, M.E., Peters, U., Gunter, M.J., et al., 2011. Cancer Res. 69, 1429–1438.
40. Previously called **Friedreich ataxia,** this condition is now called **ataxia with vitamin E deficiency**.
41. CRAL_TRIO.
42. Stocker, A., Zimmer, S., Spycher, S.E., et al., 1999. IUBMB Life 48, 49–55.
43. Yamauchi, J., Iwamoto, T., Kida, S., 2001. Biochem. Biophys. Res. Commun. 285, 295–299.
44. Ni, J., Wen, X., Yao, J., et al., 2005. Cancer Res. 65, 9807–9816.
45. Wang, X., Ring, B.Z., Seitz, R.S., 2015. BMC Clin. Pathol. 15, 21–31.
46. For example, weanling rats from vitamin E–adequate dams do not show neurologic signs of vitamin E deficiency for as long as 7 weeks when fed a vitamin E–free diet.
47. Traber. M.G., Ramakrishnan, R., Kayden, H.J., 1994. Proc. Natl. Acad. Sci. U.S.A. 91, 10,005–10,008; Leonard, S.W., Paterson, E., Atkinson, J.E., et al., 2005. Free Radic. Biol. Med. 38, 857–866.

TABLE 8.9 Concentrations of α-Tocopherol in Human Tissues

	α-Tocopherol	
Tissue	**Tissue (μg/g)**	**Lipid (μg/g)**
Plasma	9.5	1.4
Erythrocytes	2.3	0.5
Platelets	30	1.3
Adipose	150	0.2
Kidney	7	0.3
Liver	13	0.3
Muscle	19	0.4
Ovary	11	0.6
Uterus	9	0.7
Testis	40	1.0
Heart	20	0.7
Adrenal	132	0.7
Hypophysis	40	1.2

From Machlin, L. J., 1984. Handbook of Vitamins. Machlin, L., (Ed.). Marcel Dekker, New York, p. 99.

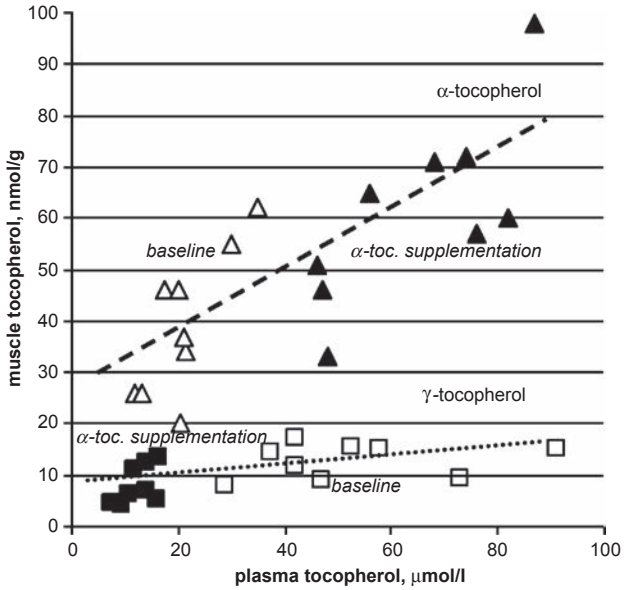


FIGURE 8.4 Correlation of α-tocopherol and γ-tocopherol contents of muscle (*m.* gastrocnemius) and plasma, respectively. Note opposite effects of supplemental α-tocopherol (800 IU/day for 30 days): increased α-tocopherol and reduced γ-tocopherol. *After: Meydani, M., Fielding, R.A., Cannon, J.G., et al., 1997. Nutr. Biochem. 8, 74–81.*

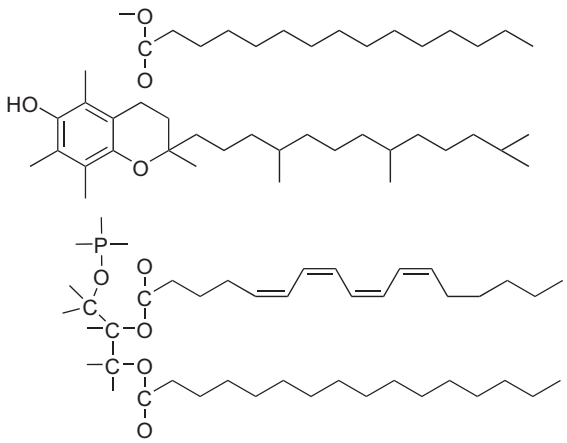


FIGURE 8.5 Proposed interdigitation of tocopherols and polyunsaturated fatty acids in biological membranes.

which are prevented by feeding α-tocotrienol.[48] Such findings show that tocotrienols can be transported to peripheral tissues by α-TTP–independent means.

Membrane Vitamin E. In most nonadipose cells, vitamin E (mostly α-tocopherol) is localized almost exclusively in membranes. The highest concentrations are found in the Golgi membranes and lysosomes, where the ratio of vitamin E:phospholipids is approximately 1:65. Other subcellular membranes contain an order of magnitude less vitamin E. It is thought that the vitamins may reside in intimate contact with PUFAs by virtue of their complementary three-dimensional structures (Fig. 8.5). Fluorescence techniques have revealed that vitamin E partitions into membranes where its weak surface-active properties orient it at the interface between the aqueous phase and hydrophobic domain with its phenoxy group being hydrogen bonded to the carbonyl group of the fatty acid ester in the phospholipid bilayer. Dynamic spectroscopic studies have shown that α-tocopherol (and α-tocotrienol) so oriented can rotate about its long axis perpendicular to the plane of the membrane and can diffuse laterally among leaflets of the phospholipid bilayer.

Although vitamin E is the major antioxidant in membranes, research has focused on how the vitamin functions effectively given the relatively enormous quantities of polyunsaturated lipids also present in membranes. This may be because of the vitamin clustering in membrane locations of greatest need, which α-tocopherol has been found to do by forming complexes with membrane lysolipids, particularly choline lysophosphatides.[49] Such complex formation results in a nonrandom distribution of vitamin E in the phospholipid membrane bilayer, instead of associations with structures analogous to "lipid rafts," i.e., highly disordered,

48. Jishage, K., Arita, M., Igarishi, K., et al., 2001. J. Biol. Chem. 276, 1669–1672.

49. A lysophosphatide results from the partial hydrolysis of a phospholipid (e.g., phosphatidylcholine), which removes one of the fatty acid moieties. Such hydrolysis is catalyzed by phospholipase A_2.

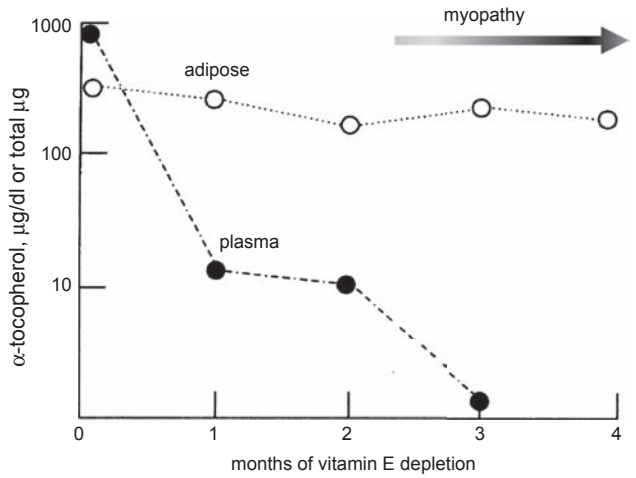


FIGURE 8.6 Retention of α-tocopherol in guinea pig adipose tissue during vitamin E depletion. *Machlin, L.J., Keating, J., Nelson, J., et al., 1979. J. Nutr. 109, 105–109.*

PUFA-rich microdomains depleted of cholesterol and sphingomyelin. Because hydrolytic products are known to have destabilizing effects on membranes, the formation of such complexes of α-tocopherol (but not other isomers) has been shown to stabilize membranes. It has been proposed that, by affecting protein–lipid and/or protein–protein interactions, vitamin E can affect embedded signal transduction pathways.

Adipose tissue. Some 90% of vitamin E in the body is contained in adipose tissue where it resides mostly in the bulk lipids. This constitutes a fixed pool from which the vitamin is slowly mobilized, thus, having long-term physiological significance (Fig. 8.6). After a change in α-tocopherol intake, adipose tocopherols may not reach a new steady state for two or more years; adipose vitamin E levels can be nearly normal even in animals showing clinical signs of vitamin E deficiency. That adipose comprises a sink for vitamin E is indicated by the fact that circulating tocopherols are inversely related to body fat mass and that people on weight-loss programs do not lose vitamin E from their adipose tissues. However, circulating tocopherol levels have been found to increase significantly (10–20%) during intensive exercise, and it has been suggested that vitamin E may be mobilized from its fixed pools by way of the lipolysis induced under such conditions.

6. METABOLISM OF VITAMIN E

Most α-tocopherol is transported to the tissues without metabolic transformation; subsequent metabolism involves head-group and side chain oxidation.[50] The selective accumulation of RRR-α-tocopherol in tissues is the result of discrimination among the various tocopherols consumed. This is effected in two ways: selective retention of α-tocopherol via α-TTP binding and selective metabolism of non–α-vitamers.

Oxidation of the chroman ring. The chromanol hydroxyl group renders tocopherols and tocotrienols capable of undergoing both one- and two-electron oxidations. α-Tocopherol is, thus, converted to α-tocopheryl quinone and (5,6- or 2,3-)-epoxy-α-tocopherylquinones, respectively (Fig. 8.7), thus, enabling it to scavenge free radicals such as peroxynitrate and lipid peroxyl radicals.

Redox Cycling. Oxidation of the chromanol ring is the basis of the in vivo antioxidant function of the vitamin. It involves oxidation primarily to tocopherylquinone, which proceeds through the semistable tocopheroxyl radical intermediate. A significant portion of vitamin E may be recycled in vivo by reduction of tocopheroxyl radical back to tocopherol. This hypothesis is supported by several findings: the very low turnover of α-tocopherol, the slow rate of its depletion in vitamin E–deprived animals, and the relatively low molar ratio of vitamin E to PUFA (about 1:850) in most biological membranes. Several mechanisms have been proposed for the in vivo reduction of tocopheroxyl by various intracellular reductants. *In vitro* studies have demonstrated that this can occur in liposomes by ascorbic acid, in microsomal suspensions by NAD(P)H, and in mitochondrial suspensions by NADH and succinate, with the latter two systems showing synergism with reduced glutathione (GSH) or ubiquinones. Indeed, a membrane-bound tocopheroxyl reductase activity has been suggested. To constitute a physiologically significant pathway in vivo, such a multicomponent system may be expected to link the major reactants, which are compartmentalized within the cell (e.g., ascorbic acid in the cytosol and tocopheroxyl in the membrane). Thus, it is possible that the recycling of tocopherol may be coupled to the shuttle of electrons between one or more donors in the soluble phase of the cell and the radical intermediate in the membrane, resulting in the reduction of the latter.

According to this model, tocopherols and tocotrienols are retained through recycling until the reducing systems in both aqueous and membrane domains become rate limiting, whereupon lipid peroxidation and protein oxidation would increase. Although the monovalent oxidation of tocopherol to the tocopheroxyl radical is reversible (at least in vitro), further oxidation of the radical intermediate is unidirectional. Because tocopherylquinone lacks vitamin E activity, its production represents loss of the vitamin from the system. It can be reduced to α-tocopherylhydroquinone, which can be conjugated with glucuronic acid and secreted in the bile, thus making excretion with the feces the major route of elimination of the vitamin. Under conditions of intakes of nutritional

50. Excretion of the nonmetabolized α-tocopherol occurs only at high doses (e.g., >50 mg), which apparently exceeds the binding capacity of α-TTP.

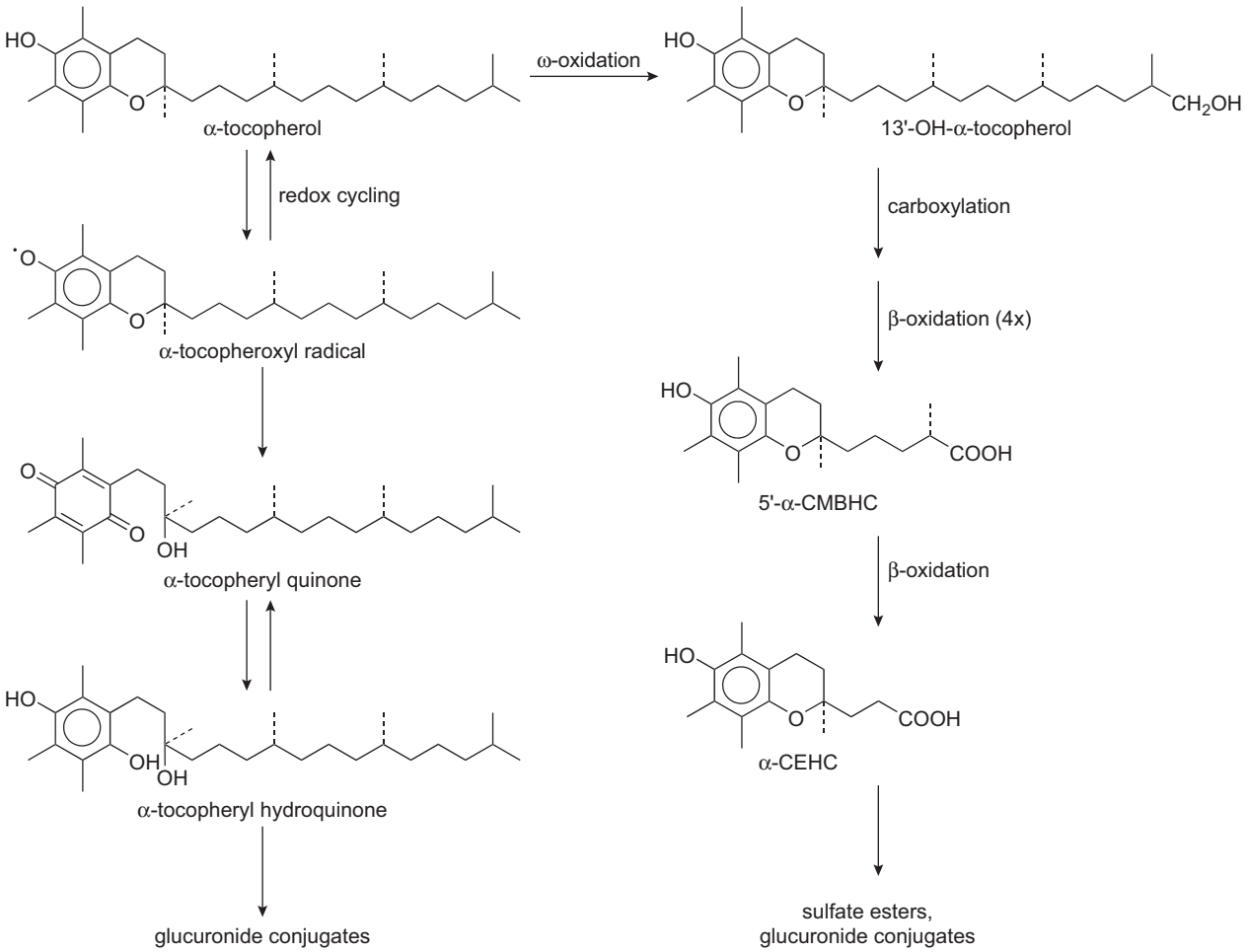


FIGURE 8.7 Vitamin E metabolism (shown with α-tocopherol). *5′-α-CMBHC*, 5′-α-carboxymethylbutylhydroxychroman; *α-CEHC*, α-carboxyethylhydroxychroman.

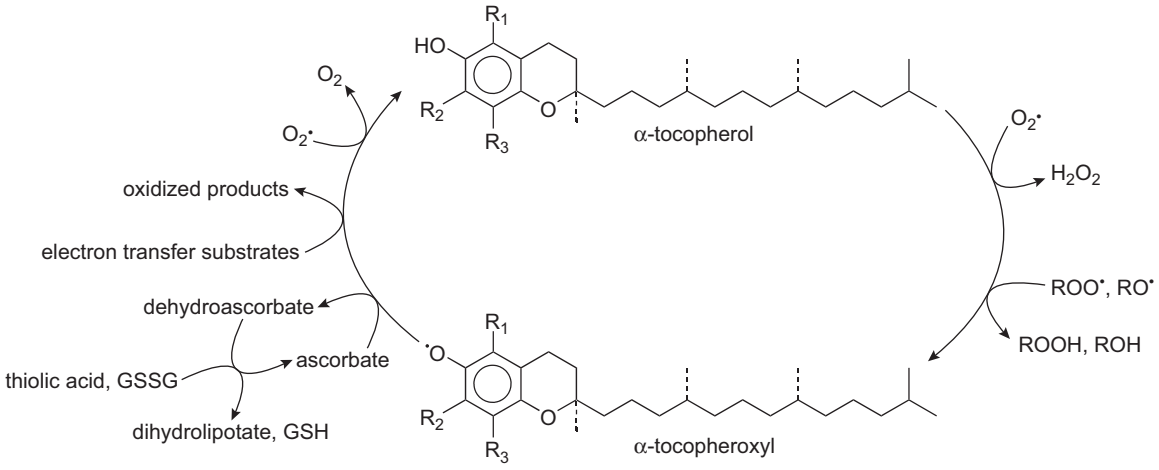


FIGURE 8.8 The vitamin E redox cycle.

levels of vitamin E, less than 1% of the absorbed vitamin is excreted with the urine.

ω-Oxidation of the phytyl side chain. Vitamin E is catabolized to water-soluble metabolites by a cytochrome *P*-450–mediated process initiated by a hydroxylation of a terminal methyl group of the phytyl side chain.[51] This hepatic ω-hydroxylation step, catalyzed by a microsomal cytochrome *P*-450 isoform (CYP4F2 in humans and CYP4F14 in the mouse) also involved in leucotriene ω-hydroxylation, is followed by dehydrogenation to the 13′-chromanol and subsequent truncation of the phytyl side chain through the removal of two- and three-carbon fragments (Fig. 8.7). That other ω-hydrolases exist in extrahepatic tissues is indicated by the fact that genetic deletion of CYP4F14 in the mouse reduced α-tocopherol metabolism by only 70–90%.[52] The products of the side chain oxidation include are excreted in the urine, often as glucuronyl conjugates; they include the folloiwng:[53]

5′(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)-2-methylpentanoic acid (α-CMBHC)
3-(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)propanoic acid (α-CEHC)

It has been suggested that these and perhaps other long-chain chromanol metabolites may be more than excretory products; i.e., that they may be metabolic effectors. Support for that hypothesis comes from findings that such long-chain hydroxy- and carboxy-chromanols reduced the uptake of oxidized LDLs by macrophages and induced CD36, the major scavenger receptor for oxidized LDLs in human macrophages.[54]

This pathway catabolizes non–α-tocopherols more extensively than it does α-tocopherol, resulting in much faster turnover of those vitamers.[55] It does, however, appear to be upregulated by high doses of α-tocopherol, suggesting that it is also important in clearing that vitamer. Accordingly, high maternal vitamin E intakes during pregnancy have been found to increase α-CEHC levels in the fetal circulation. At high intakes, α-tocopherol is also excreted in the feces.

TABLE 8.10 Plasma Tocopherols in Smokers and Nonsmokers

Metabolite	Nonsmokers (n = 19)	Smokers (n = 15)
α-Tocopherol (μM)	16.0 ± 4.0	15.9 ± 5.0
γ-Tocopherol (μM)	1.76 ± 0.98	1.70 ± 0.69
5-Nitro-γ-tocopherol (nM)	4.03 ± 3.10	8.02 ± 3.33*

*$p < .05$.
From Leonard, S.W., Bruno, R.S., Paterson, E., et al., 2003. Free Radic. Biol. Med. 12, 1560–1567.

Other metabolism. The detection of small amounts of **α-tocopheryl phosphate** in tissues of vitamin E–fed animals suggests that the vitamin can be phosphorylated. The metabolic significance of this metabolite is unclear and a kinase has not been identified. Although the metabolic role of α-tocopheryl phosphate is not clear, evidence suggests that it may serve as an active lipid mediator of signal transduction and gene expression.[56] That vitamin E can also be nitrated in vivo is indicated by the occurrence of **5-nitro-γ-tocopherol** in the plasma of cigarette smokers (Table 8.10), presumably because of the high amounts of reactive nitrogen species and the stimulatory effects of cigarette smoke on inflammatory responses. This reaction may be the basis for the enhanced turnover of tocopherols and reduced production of carboxyethylchromanyl metabolites in smokers.

7. METABOLIC FUNCTIONS OF VITAMIN E

Vitamin E as a Biological Antioxidant

The primary nutritional role of vitamin E is as a biological antioxidant. An **antioxidant** is an agent that inhibits oxidation and, thus, prevents such oxidation reactions as the conversion of PUFAs to fatty hydroperoxides, the conversion of free or protein-bound sulfhydryls to disulfides, etc. In reducing **free radicals**, it protects against the potentially deleterious reactions of such highly reactive oxidizing species, thus having functional importance in maintaining membrane integrity in all cells of the body.

Production of Free Radicals and Reactive Oxygen Species (ROS). Free radicals (X·) are produced in cells either by homolytic cleavage of a covalent bond, as in the formation of a C-centered free radical of a PUFA or by a univalent electron transfer reaction. It has been estimated that as much as 5% of inhaled molecular oxygen (O_2) is

51. Sontag, T.J., Parker, R.S., 2007. J. Lipid Res. 48, 1090–1098.
52. Bardowell, S.A., Duan, F., Manor, D., et al., 2012. J. Biol. Chem. 287, 260,077–26,086.
53. **Tocopheronic acid** and **tocopheronolactone**, referred to as **Simon metabolites** after E. J. Simon who described them in the urine of rabbits and humans, were thought to be urinary metabolites of vitamin E. It now appears that they were artifacts resulting from β-oxidation of the vitamin during isolation.
54. Wallert, M., Mosig, S., Rennert, K., et al., 2014. Free Radic. Biol. Med. 68, 42–51.
55. For this reason, the ω-oxidation pathway would appear to contribute, along with the binding specificity of α-TTP, to what has been called the "α-tocopherol phenotype", i.e., the dominance of α-tocopherol in tissues. That *Drosophila*, which appear to lack α-TTP (Parker, R.S., McCormick, C.C., 2005. Biochem. Biophys. Res. Commun. 338, 1537–1541), also show the "α-tocopherol phenotype" suggests the primacy of the ω-oxidation pathway in this regard.
56. Zingg, J.M., Meydani, M., Azzi, A., 2010. Mol. Nutr. Food Res. 54, 679–692.

metabolized to yield the so-called reactive oxygen species, i.e., the one- and two-electron reduction products superoxide radical $O_2\cdot^-$ and H_2O_2, respectively. There appear to be three sources of ROS:

- **Normal oxidative metabolism**. The mitochondrial electron transport chain, which involves a flow of electrons from NADH and succinate through a series of electron carriers to cytochrome oxidase reducing oxygen to water, leaks a small amount of electrons that reduce O_2 to $O_2\cdot^-$.
- **Microsomal cytochrome *P*-450 activity**. Several xenobiotic agents are metabolized by the microsomal electron transport chain to radical species (e.g., the herbicide paraquat is converted to an N-centered radical anion) that can react with O_2 to produce $O_2\cdot^-$.
- **Respiratory burst of stimulated phagocytes**. Macrophages produce $O_2\cdot^-$ and H_2O_2 during phagocytosis.

Physiological roles of ROS. ROS play key roles in immune function. On encountering or ingesting a bacterium or other foreign particle, activated[57] neutrophils and macrophages produce large amounts of $O_2\cdot$ and H_2O_2 in a process referred to as the "respiratory burst." This involves myeloperoxidase, which catalyzes the H_2O_2-dependent oxidation of halide ions yielding such powerful oxidizing agents as hypochlorous acid and xanthine oxidase, which catalyzes the reaction of xanthine or hypoxanthine with O_2 to generate uric acid. These reactions are important in killing pathogens, but they can also be deleterious to immune cells themselves. If not controlled, they can contribute to the pathogenesis of disease.

Metabolically produced ROS appear to have essential metabolic functions as signaling molecules for the adaptation of skeletal muscle to accommodate the stresses presented by exercise training or periods of disuse. This signaling involves redox-sensitive kinases, phosphatases, and nuclear factor κB (NF-κB), which affect the rate of mitochondrial biogenesis, and the induction of genes related to insulin sensitivity [peroxisome proliferator-activated receptor coactivator (PGC)-1α/β, PPARγ] and ROS defense [superoxide dismutase 1/2 (SOD1/2), glutathione peroxidase (GPX1)]. This system of adaptive responses to oxidative stress facilitates the ultimate development of long-term resistance to that stress (Fig. 8.9). That this system, which has been called **mitochondrial hormesis**,[58] can be impaired by high-level antioxidant treatment was demonstrated by the finding that supplements of vitamins E and C (400 IU α-tocopheryl acetate + two 500-mg doses of ascorbic acid per day) blocked the upregulation of muscle glucose uptake otherwise induced by exercise.[59] This finding raises several questions: Is this effect because of vitamin E, vitamin C, or the combination? What antioxidant dose is required for such effects? What level of redox tone will be beneficial?

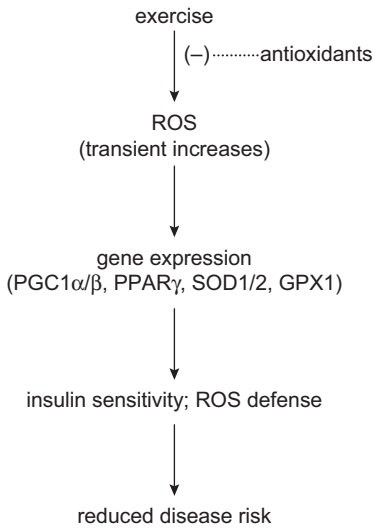


FIGURE 8.9 Mitochondrial hormesis: ROS signaling of insulin sensitivity. *After Ristow, M., Zarse, K., Oberbach, A., et al., 2009. Proc. Natl. Acad. Sci. USA 106, 8665–8670.*

Adverse effects of ROS. In the presence of transition metal ions (particularly, Fe^{2+} or Cu^+), $O_2\cdot^-$ and H_2O_2 can react to yield a very highly reactive free-radical species[60], **hydroxyl radical** (**HO·**) ($O_2\cdot^- + H_2O_2 + Fe^{2+} \rightarrow O_2 + HO\cdot + HO^- + Fe^{3+}$). These divalent metals can also catalyze the decomposition of H_2O_2 or fatty acyl **hydroperoxide** (ROOH) produced by lipid peroxidation to yield the oxygen-centered radical, RO· or HO·, respectively (**ROOH/HOOH** + $Fe^{2+} \rightarrow$ **RO·/HO·** + $HO^- + Fe^{3+}$). Molecular targets of HO· include:

- **DNA** – to cause oxidative base damage[61]
- **Proteins** – to cause production of carbonyls and other amino acid oxidation products[62]
- **Lipids** – to cause oxidation of PUFAs of membrane phospholipids, the formation of lipid peroxidation products (e.g., MDA, isoprostanes, pentane, ethane), and resulting in membrane dysfunction

Of these, PUFA-containing membrane lipids are particularly susceptible to attack by virtue of their 1,4-pentadiene

57. Various cytokines, such as tumor necrosis factor and interferon-γ, can activate phagocytic cells to increase their $O_2\cdot$ generation.

58. Hormesis is the term for a generally favorable biological response to low exposures to stressors/toxins.

59. Ristow, M., Zarse, K., Oberbach, A., et al., 2009. Proc. Natl. Acad. Sci. U.S.A. 106, 8665–8870.

60. Otherwise, neither $O_2\cdot^-$ nor H_2O_2 is highly reactive, and $O_2\cdot^-$ is cleared rapidly (its half-life is c. 1 s).

61. Base damage products such as 8-hydroxydeoxyguanosine (8OHdG), resulting from DNA repair processes, are excreted in the urine. Smokers typically show elevated 8OHdG excretion.

62. For example, methionine sulfoxide, 2-ketohistidine, hydroxylation of tyrosine to DOPA, formylkynurenine, *o*-tyrosine, and protein peroxides.

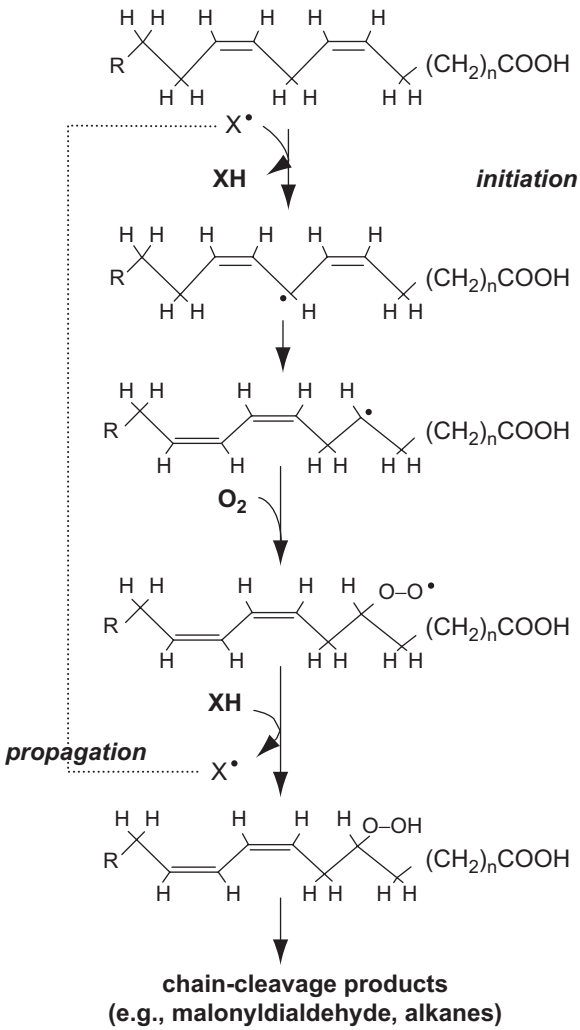


FIGURE 8.10 The self-propagating nature of lipid peroxidation.

systems, which allow abstraction of a complete hydrogen atom (i.e., with its electron) from one of the —CH_2— groups in the carbon chain with the consequent generation of a C-centered free radical (—C—) (Fig. 8.10). This initiation of lipid peroxidation can be accomplished by HO· and, possibly, HOO· (but *not* by H_2O_2 or $O_2\cdot^-$). The C-centered radical, being unstable, undergoes molecular rearrangement to form a conjugated diene, which is susceptible to attack by O_2 to yield a peroxyl radical (ROO·). Peroxyl radicals are capable of abstracting a hydrogen atom from other PUFAs and, thus, propagating a chain reaction that can continue until the membrane PUFAs are completely oxidized to hydroperoxides (ROOH).

Fatty acyl hydroperoxides are degraded in the presence of transition metals (Cu^{2+}, Fe^{2+}) and heme and heme proteins (cytochromes, hemoglobin, myoglobin) to release radicals that can continue the chain reaction of lipid peroxidation,[63] also yielding other chain-cleavage products: **MDA**,[64] **pentane**, and **ethane**.[65] This pattern of oxidative degradation of membrane phospholipid PUFAs is believed to disrupt membrane function. Cellular oxidant injury can also occur without significant lipid peroxidation, by oxidative damage to critical macromolecules (DNA and proteins) and decompartmentalization of Ca^{2+}.[66]

> In vivo lipid peroxidation has been surprisingly difficult to demonstrate. Little or no evidence of lipid peroxides or their decomposition products have been found in tissues. Although expired breath contains volatile alkanes likely from the decomposition of fatty acyl hydroperoxides, it is difficult to exclude their possible production by gut or skin microbes.

Scavenging Free Radicals. Because of the reactivity of the phenolic hydrogen on its C-6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, vitamin E can terminate chain reactions among PUFAs in membranes. This free-radical scavenging property involves donation of the phenolic hydrogen to a fatty acyl free radical (or $O_2\cdot^-$) to prevent the attack of that species on other PUFAs. Tocopherols have great reactivities toward peroxyl and phenoxyl radicals, but can also quench mutagenic electrophiles such as reactive nitrogen oxide species (NO_x). The antioxidant activities of the E vitamers relate to the leaving ability of the phenolic hydrogen; when assessed in vitro, α-tocopherol has the greatest antioxidant activity,[67] followed by the β- and γ-vitamers, which are greater than δ-vitamer.[68] The tocotrienols can also scavenge peroxyl radical. In contrast, NO_x is trapped more effectively by γ-tocopherol than by the α-vitamer.

63. Therefore, a single radical can initiate a chain reaction that may self-propagate repeatedly.

64. Although MDA is a minor product of lipid peroxidation, it has received a great deal of attention because of the ease of measuring it colorimetrically using 3-thiobarbituric acid (TBA), which has been widely used to assess lipid peroxidation. The TBA test faces limitations: much of the MDA it detects may not have been present in the original sample, as lipid peroxides can decompose to MDA during the heating stage of the test; the reaction can also be affected by the presence of iron salts.

65. Pentane and ethane are produced from the oxidative breakdown of ω-6 and ω-3 fatty acids, respectively. Both are excreted across the lungs and can be detected in the breath of vitamin E–deficient subjects.

66. For example, pulmonary injury by the bipyridylium herbicide paraquat involves lipid peroxidation only as a late-stage event.

67. The chemical antioxidant activity of α-tocopherol is about 200-fold that of the commonly used food antioxidant butylated hydroxytoluene.

68. The biological activities of the E vitamers are functions of both their intrinsic chemical antioxidant activities and their efficiencies of absorption and retention. Thus, γ-tocopherol has only 6–16% of the biological activity of the α-vitamer. An exception to this relationship occurs in the case of sesame seed lignans, which potentiate the biopotency of γ-tocopherol. Therefore, sesame oil, which contains only the γ-vitamer, has a biopotency equivalent to that of α-tocopherol. The potentiating factor is believed to be a lignan phenol, sesamolin, to which antiaging properties have been attributed.

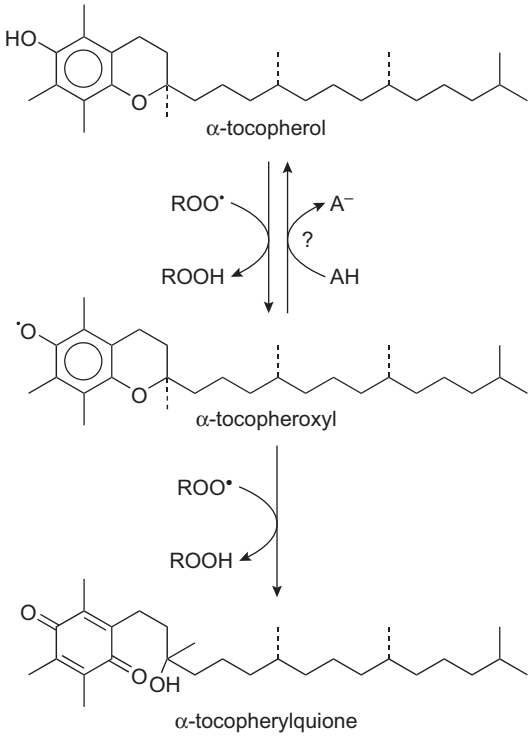


FIGURE 8.11 Oxidation of tocopherols by reaction with peroxyl radicals.

In scavenging free radicals, tocopherols and tocotrienols undergo oxidation of their respective alcohol forms to semistable radical intermediates, **tocopheroxyl** (or chromanoxyl) **radicals** (Figs. 8.7 and 8.8). Unlike free radicals formed from PUFAs, the tocopheroxyl radical is relatively unreactive, thus stopping the destructive propagative cycle of lipid peroxidation. In fact, tocopheroxyl is sufficiently stable to react with a second peroxyl radical to form inactive, nonradical products including **tocopherylquinone**[69]. It is, therefore, referred to as a **chain-breaking antioxidant**. Because α-tocopherol can compete for peroxyl radicals much faster than PUFAs, small amounts of the vitamin are able to effect the antioxidant protection of relatively large amounts of PUFAs (Fig. 8.11). **Tocotrienols** are thought to have more potent antioxidant protective potential than tocopherols, as their unsaturated side chains facilitate their more efficient penetration into tissues containing saturated fatty layers, e.g., brain, liver. Factors that increase the production of ROS (e.g., xenobiotic metabolism, ionizing radiation, exposure to prooxidants such as O_3 and NO_2) can increase the metabolic demand for antioxidant protection.

The Antioxidant Defense System. Because it is distributed in membranes, vitamin E serves as a lipid-soluble biological antioxidant with high specificity for loci of potential lipid peroxidation. Its co-transport with polyunsaturated

69. Evidence indicates that **tocopherylquinones** can induce apoptosis in cancer cells.

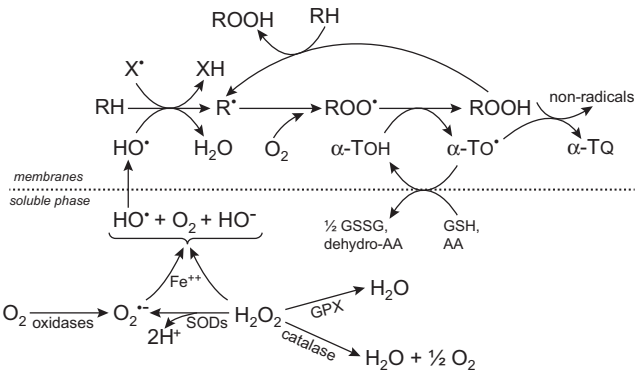


FIGURE 8.12 The cellular antioxidant defense system. *Note:* (a) removal of H_2O_2 to prevent production of ROS; (b) quenching of free radicals by oxidation of α-tocopherol; and (c) regeneration of α-tocopherol by soluble reductants such as reduced glutatione or ascorbic acid. *GSH*, reduced glutathione; *GSSG*, oxidized glutathione; *AA*, ascorbic acid; *α-TOH*, α-tocopherol; *α-TO*, α-tocopheryl radical; *α-TQ*, α-tocopherylquinone; *GPX*, glutathione peroxidase; *SOD*, superoxide dismutase.

lipids ensures protection of the latter from free-radical attack; circulating tocopherol levels tend to correlate with those of total lipids and cholesterol.[70] However, vitamin E is one of several factors in an antioxidant defense system that protects cells from the damaging effects of oxidative stress (Fig. 8.12). That system includes:

- **Membrane antioxidants** – mostly tocopherols, but also ubiquinones and carotenoids.
- **Soluble antioxidants** – NADPH, NADH, ascorbic acid, GSH and other thiols, uric acid, thioredoxin, bilirubin, polyphenols, and several metal-binding proteins (copper: ceruloplasmin, metallothionein, and albumin; iron: transferrin, ferritin, and myoglobin).
- **Antioxidant enzymes –** SODs,[71] GPXs,[72] thioredoxin reductase,[73] and catalase.[74]

70. Therefore, high plasma vitamin E levels occur in hyperlipidemic conditions (hypothyroidism, diabetes, and hypercholesterolemia), whereas low plasma vitamin E levels occur in conditions involving low plasma lipids (abetalipoproteinemia, protein malnutrition, and cystic fibrosis).

71. The SODs are metalloenzymes. The mitochondrial SOD contains manganese at its active center, whereas the cytosolic SOD contains both copper and zinc as essential cofactors. Although not found in animals, an iron-centered SOD has been identified in blue-green algae.

72. The GPXs contain selenium (Se) at their active centers and depend on adequate Se status for their synthesis. There are four isoforms; each uses reducing equivalents from GSH to reduce H_2O_2 to water, or fatty acyl hydroperoxides to the corresponding fatty alcohols. One isoform is found in membranes and has specificity for esterified hydroperoxides; the others are soluble and have specificities for nonesterified hydroperoxide substrates including H_2O_2. The activities of these enzymes depend on the flavoenzyme **glutathione reductase** to regenerate GSH from its oxidized form (GSSG).

73. Thioredoxin reductase is also a selenoprotein; there are three isoforms.

74. Catalase has an iron redox center. Because its distribution is almost exclusively limited to the peroxisomes/lysosomes, it is not considered of prime importance in antioxidant protection in the cytosol.

In this multicomponent system, vitamin E scavenges radicals within the membrane, where it blocks the initiation and interrupts the propagation of lipid peroxidation. The group of metalloenzymes collectively blocks the initiation of peroxidation from within the soluble phase of the cell: SODs convert $O_2 \cdot^-$ to H_2O_2; catalase and GPXs each further reduce H_2O_2. The aggregate effect of this enzymatic system is to clear $O_2 \cdot^-$ by reducing it fully to H_2O, thus preventing the generation of other, more highly ROS [e.g., HO· and singlet oxygen ($^1O_2$)]. The GPXs can also reduce fatty acyl hydroperoxides to the corresponding fatty alcohols, thus serving to interrupt the propagation of lipid peroxidation.

Some components of this system are endogenous (e.g., NADPH, NADH, and, for most species, ascorbic acid), whereas other components must be obtained, at least in part, from the external chemical environment. The diversity of this system implies the ability to benefit from various antioxidants and other key factors obtained from dietary sources in variable amounts. That the components of the defense system function cooperatively is evidenced by the nutritional "sparing" observed particularly for vitamin E and Se in the etiologies of several deficiency diseases (e.g., **exudative diathesis** in chicks, **liver necrosis** in rats, and **white muscle disease** in lambs and calves). In those species, nutritional deprivation of either vitamin E or Se alone is usually asymptomatic; deficiencies of both nutrients are required to produce disease.

The components of this system respond to changes in cellular redox state, increasing antioxidant protection during cell differentiation. Several lines of evidence indicate that ROS gradients and cellular redox state influence gene expression. Oxidizing conditions have been found to affect cellular ion distribution, expression of chromatin-controlling proteins, and the cytoskeleton and nuclear matrix, which, in turn, affect chromatic configuration and pre-mRNA processing. Thus, it appears that the well-functioning antioxidant defense system serves to maintain low, optimal levels of ROS in cells such that the beneficial effects of prooxidizing conditions are realized and their deleterious effects are minimized. This concept has been called healthy "**redox tone.**"

Because most ROS are produced endogenously by mitochondria, which process 99% of the oxygen utilized by the cell, exercise-related increases in oxidative metabolism[75] are thought to increase needs for vitamin E. Indeed, exercise has been found to increase ROS production, which contributes to fatigue, and lipid peroxidation; both are reduced by vitamin E supplementation. However, low levels of ROS in skeletal muscle are necessary for normal force production,[76] and myocytes respond to oxidative stress by upregulating a variety of proteins involved in the maintenance of cellular integrity. Hence, regular exercise increases the activities of enzymes involved in antioxidant protection (GPXs, mangano-superoxide dismutase, γ-glutamyl synthase, catalase), DNA repair, and cytoprotection (e.g., heat-shock factor-1). This adaptation relies on ROS signaling protein phosphorylation and the binding of redox-regulated transcription factors, NF-κB, and AP-1. ROS also signal mitochondrial biogenesis by stimulating the expression of several proteins including PGC-1α, nuclear factor (erythroid-derived) (NRF)-1, and mitochondrial transcription factor A (mtTFA). This signaling can be blunted by antioxidants. Therefore, trained athletes do not show increases in oxidative stress after accustomed vigorous exercise activities; for them, supplemental antioxidants can prevent metabolic adaptation to training.[77] Studies with humans and animal models have yielded inconsistent results concerning the effects of vitamin E supplementation on exercise performance.

Prooxidant Potential of Vitamin E

α-Tocopherol can also promote lipid peroxidation in LDLs in the absence of other antioxidants (e.g., ascorbic acid, coenzyme Q_{10}, urate). Under such conditions, the single-electron oxidation of tocopherol converts it to the tocopheroxyl radical, which moves into the particle's core where it can abstract hydrogen from a cholesteryl-PUFA ester to yield a peroxyl radical. The presence of secondary antioxidants is needed to prevent LDL oxidation. Otherwise, vitamin E becomes a **chain-transfer agent** to propagate lipid peroxidation in the lipid core. Accordingly, a very high dose (1050 mg/day) of α-tocopherol has been found to increase susceptibility to peroxidation.[78]

Nonantioxidant Functions of Vitamin E

The recognition in the early 1990's that vitamin E could inhibit cell proliferation and protein kinase C (PKC) activity suggested that vitamin E may function in vivo in ways that are unrelated to its function as a biological antioxidant. Subsequent research has demonstrated antiproliferative, proapoptotic, anti-inflammatory, an antiangiogenic effects of tocopherols and tocotrienols that do not appear to involve their antioxidant functions.

Enzyme Regulation. For several enzymes, α-tocopherol appears to participate in complex membrane-based recruitment processes affecting function: inhibition of PKC, NADPH oxidase, phospholipase A_2, protein kinase B/Akt, 5-lipoxygenase, cyclooxygenase A_2, and 3-hydroxymethyl-3-glutaryl-coenzyme A (HMG-CoA) reductase; activation

75. O_2 utilization increases 10- to 15-fold during exercise.
76. Reid, M.B., 2001. J. Appl. Physiol. 90, 724–731.
77. Venditte, P., Napolitano, G., Barone, D., et al., 2014. Free Radic. Res. 48, 1179–1189.
78. Brown, K.M., Morrice, P.C., Duthie, G.G., 1977. Am. J. Clin. Nutr. 65, 496–502.

TABLE 8.11 Vitamin E Target Genes

Function	Gene Product
Tocopherol uptake, metabolism	α-TTP, CYP3A, CYP4F2, HMG-CoA reductase, CRABP-II
Lipid uptake	SrbI, CD36, SR-AI/II, LDL-R, PPARγ
Cholesterol/steroid synthesis	HMG-CoA-r, HMG-CoS, 7DHC, IPδ1, FPPS, 5αR1
Antioxidant defence	γGCS
Cell adhesion	E-selectin, L-selectin, ICAM-1, VCAM-1, integrins, MAC1
Cell growth	Connective tissue growth factor
Extracellular matrix	Tmp1, collagen α1(1), MMP1, MMP9, connective tissue growth factor, glycoprotein IIb
Inflammation	Il-2, Il-4, IL-1-β, TGF-β
Clotting	Christmas factor
Cytoarchitecture	Tmp2, Myh1, Tnni2, Acta1, Krt15
Cell cycle regulation	Cyclin D1, cyclin E1, Bcl12-L1, p27, CD95
Apoptosis	CD95 L, Bcl2-L1
Other functions	Leptin, β-secretase

of protein phosphatase 2A, diacylglycerol kinase, and HMG-CoA reductase. Tocotrienols have been found to impart a variety of effects on cell functions: inhibition of NF-κB, transforming growth factor β (TGF-β), tumor necrosis factor α, IL1β, and P38 signaling; activation of caspases; and downregulation of Bcl2, cyclin D, c-Src, and the Raf/Erk pathway.[79] The effects of tocotrienols would appear to be related to the fact that, because they are inefficiently removed from the liver, they can stimulate stress responses including induction of detoxification and antioxidant genes. These effects appear to be greatest for the undermethylated forms (γ- and δ-) and in hypoxic cells, e.g., tumor cells.

Gene Expression. Vitamin E also appears to participate in transcriptional regulation (Table 8.11). The transcriptional effects of α-tocopherol imply the existence of nuclear receptors for tocopherol and corresponding DNA-responsive elements. One group of nuclear receptors, the pregname X receptor has been found to bind vitamin E; however, the metabolic significance of such binding is presently unclear. Rats deprived of vitamin E show altered patterns of gene expression. Studies have revealed a large number of genes being downregulated, although a number of transport-related genes that were upregulated in liver were downregulated in the cerebral cortex. α -Tocopherol also appears to affect gene expression posttranscriptionally by affecting the expression of small, noncoding RNA (miRNA);[80] this has been demonstrated for miRNA-122 and miRNA-125b.[81] Therefore, the emerging picture is one of α-tocopherol involvement in the regulation of a variety of cellular processes, while inducing its own transport and catabolism, both of which are significantly affected by oxidant/antioxidant status and apoE genotype.

Physiological Functions

Inflammation and Immunity. Vitamin E is essential for optimal function of the immune system. Studies with animals have found that optimization of certain immune parameters requires intakes of at least an order of magnitude greater than those required to prevent clinical signs of deficiency. In humans, supranutritional doses (up to 800 mg α-tocopherol per day) have been found to restore responses to DTH, increase induction of IL-2, and reduce lymphocyte levels of the proinflammatory lipid mediator prostaglandin E_2 (PGE_2) (Table 8.12). Doses as low as 50 mg/day have been associated with reduced incidence of the common cold.[82] Studies with animal models have found vitamin E supplementation to reduce joint swelling, and randomized controlled trials with patients having rheumatoid arthritis (RA) have shown high-level supplementation with the vitamin (100–600 IU/day) to relieve pain and inflammation. Studies in animal models have found tocotrienols to be effective in increasing macrophage production of IL-6, IL-10, IL-1β, and PGE_2, particularly in older animals.[83]

Vitamin E can affect the pathogenesis of several viral infections in which oxidative stress has been implicated. Deprivation of the vitamin increases susceptibility of the mouse to cardiophilic RNA viruses, particularly when animals consume diets containing high amounts of PUFAs, e.g., fish oil (Fig. 8.13). Protection appears to involve suppression of oxidative stress, which provides an environment in which the virus can mutate to more highly virulent forms.[84] This phenomenon has been shown for several RNA viruses including hepatitis, influenza, and AIDS.

Neurologic Function. Vitamin E is essential for neurologic function. It is conserved by neural tissues, to which it is redistributed from other tissues under conditions of deficiency. Neurons are susceptible to deleterious effects of oxidative stress. They contain large amounts of PUFAs and

79. Ahsan, H., Ahad, A., Iqbal, J., et al., 2014. Nutr. Metab. 11, 52–74.

80. Micro-RNAs bind at the mRNA 3′-untranslated region to inhibit translation. Several miRNAs have been identified; each is believed to be capable of binding a 100 different target mRNAs, allowing posttranscriptional silencing of many different genes.

81. Rimbach, G., Moehring, J., Huebbe, P., et al., 2010. Molecules 15, 1746–1761.

82. Hemila, H, Kaprio, J., Albanes, D., et al., 2002. Epidemiol. 13, 32–37.

83. Ren, Z., Pae, M., Dao, M.C., et al., 2010. J. Nutr. 140, 1335–1341.

84. Beck, M.A., Levander, O.A., 1998. Ann. Rev. Nutr. 18, 93–116.

TABLE 8.12 Enhancement of Immune Responses by Vitamin E Supplementation of Healthy Adults

Treatment	Days of Treatment	Vit E in PMNs (nmol)[a]	DTH index (mm)[b]	PMN Proliferation ($\times 10^3$ cpm)[c]	IL-2 Production (kU/L)[d]
Placebo	0	0.14 ± 0.04	16.5 ± 2.2	24.48 ± 2.73	31.8 ± 8.3
	30	0.19 ± 0.03	16.9 ± 2.1	21.95 ± 2.90	37.5 ± 12.5
Vitamin E[e]	0	0.12 ± 0.02	14.2 ± 2.9	20.55 ± 1.93	35.6 ± 9.1
	30	0.39 ± 0.05[f]	18.9 ± 3.5[f]	23.77 ± 2.99[f]	49.6 ± 12.6[f]

[a]*Polymorphonucleocyte α-tocopherol content.*
[b]*Delayed-type hypersensitivity skin test.*
[c]*Concanavalin A–induced proliferation of PMNs.*
[d]*Concanavalin A–induced production of interleukin 2 by PMNs.*
[e]*A total of 800 IU all-rac-α-tocopheryl acetate per day.*
[f]$p < .05$.
From Meydani, S.N., Barklund, M.P., Kiu, S., et al., 1990. Am. J. Clin. Nutr. 52, 557–563.

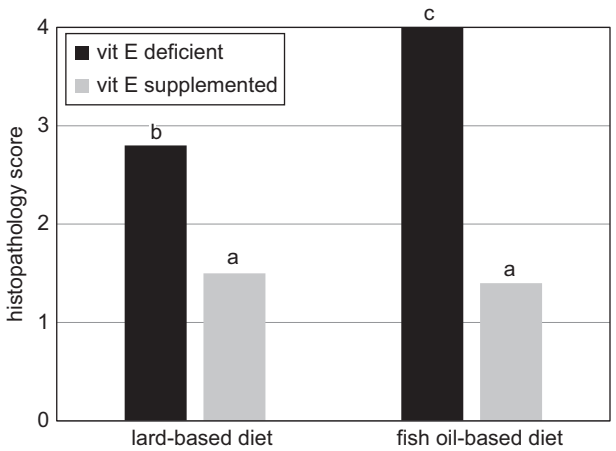


FIGURE 8.13 Cardioprotective effect of α-tocopherol in the mouse: reduction of cardiac damage from Coxsackievirus B3. Means with same superscripts are not significantly different ($p > .05$). *After Beck, M.A., Kolbeck, P.C., Rohr, L.H., et al., 1994. J. Nutr. 124, 345–358.*

iron, but lack extensive antioxidant defense systems. They are terminally differentiated and cannot replicate when damaged. They generate ROS when exposed to redox-cycling drugs (which can cause Parkinson-like neural damage in animal models) or metabolizing dopamine.[85] Exposure to hyperbaric oxygen can cause seizures. Epidemiological studies have found high vitamin E intake to be associated with reduced risks to Alzheimer disease[86] and Parkinson disease.[87]

85. By monoamine oxidase B.
86. Alzheimer disease is the world's most prevalent neurodegenerative disease, affecting an estimated 20–30 million, including almost half of people over the age of 85 years. It is characterized by memory dysfunction, loss of lexical access, temporal and spatial disorientation, and impaired judgment.
87. Parkinson disease is characterized by progressive loss of postural stability, with slowness of movement and tremor.

Cardiovascular Health. Vitamin E serves as an antioxidant in LDLs, which may protect against atherosclerosis.[88] Being rich in both cholesterol and PUFA (Table 8.13), LDLs are susceptible to peroxidation by ROS. Oxidized LDLs stimulate the recruitment, in the subendothelial space of the vessel wall, of monocyte–macrophages that can take up the oxidized particles via scavenger receptors[89] to form the lipid-containing foam cells found in the early stages of atherogenesis. Evidence indicates that vitamin E can also reduce the adherence and aggregation of platelets, to retard the progression of a fatty streak and cell proliferation to advanced lesions (Fig. 8.14).

8. BIOMARKERS OF VITAMIN E STATUS

Plasma α-tocopherol concentration is the most useful biomarker of vitamin E status, particularly at limiting levels. It is directly related to α-tocopherol intake up to about 200 mg/day, but plasma concentrations are inconsistently related at greater vitamin E intakes. As vitamin E is membrane protective, plasma tocopherol levels are inversely related to susceptibility of erythrocytes to oxidative hemolysis. This relationship makes the plasma α-tocopherol level

88. Atherosclerosis is the progressive, focal accumulation of acellular, lipid-containing plaques in the intima of arteries. It is a specific type of arteriosclerosis, although the terms are often used interchangeably. Arteriosclerosis refers to the thickening and loss of elasticity of arteries because of infiltration of the intima by fats and calcific plaques, reducing blood flow to the organs served by affected vessels and leading to such symptoms as angina, cerebrovascular insufficiency, and intermittent claudication.
89. Monocyte–macrophages have few LDL receptors, which are downregulated. Therefore, when incubated with nonoxidized LDLs, they do not form foam cells, as the accumulation of cholesterol further reduces LDL receptor activity. On the other hand, these cells have "**scavenger receptors**" specific for modified LDLs. It is thought that LDL lipid peroxidation products may react with amino acid side chains of apoB to form epitopes that have affinities for the scavenger receptor.

TABLE 8.13 Lipid and Antioxidant Contents of Human Low-Density Lipoproteins

Component	Moles per Mole LDL
Total phospholipids	700 ± 122
Fatty Acids	
Free	26
Total	2700
Triglycerides	170 ± 78
Cholesterol	
Free	600 ± 44
Esters	1600 ± 119
Total	2200
Antioxidants	
α-Tocopherol	6.52
γ-Tocopherol	1.43
Ubiquinonol-10	0.33
β-Carotene	0.27
Lycopene	0.21
Cryptoxanthin	0.13
α-Carotene	0.11

From Keaney, J.F., Frei, B., 1994. Natural Antioxidants in Human Health and Disease. Frei, B. (Ed.). Academic Press, San Diego, p. 306–307.

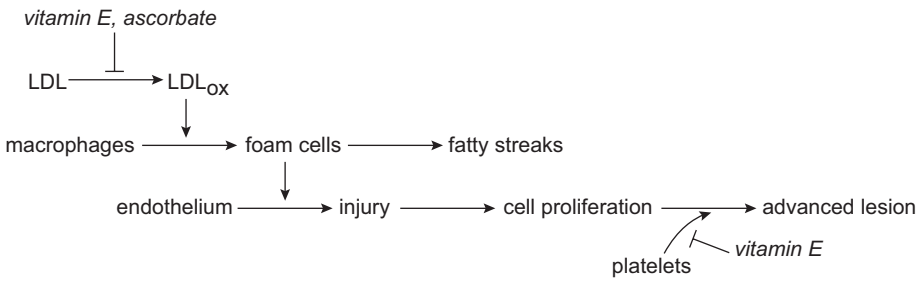


FIGURE 8.14 Model for prevention of atherogenesis by vitamin E.

useful as a parameter of vitamin E status. In individuals, values ≥0.5 mg/dL (≥12 μM) are associated with protection against hemolysis in vitro and are taken to indicate nutritional adequacy The NHANES 1999–2000 data showed plasma α-tocopherol concentrations to average 30 μM (1.3 mg/dL), with some 2% in the deficient range.[90] Plasma concentrations of γ-tocopherol tend to be 10–20% of the level of α-tocopherol. Maternal tocopherol levels increase during pregnancy, but fetal levels remain low, suggesting a barrier to transplacental movement of the vitamin. Infants' serum tocopherol levels are approximately 25% of those of their mothers. They increase to adequate levels within a few weeks after birth, except in infants with impaired abilities to utilize lipids (e.g., premature infants, infants with biliary atresia); they show very low circulating levels of vitamin E (Table 8.14). An analysis of the NHANES 2003–2006 data found plasma tocopherol to be unaffected by inflammation status, but to increase 3–4% during short-term fasting and to be some 20% greater for patients with renal dysfunction compared to matched controls.[91]

90. Ford, E.S., Schleicher, R.L., Mokdad, A.H., et al., 2006. Am. J. Clin. Nutr. 84, 375–383.

91. Haynes, B.M.H., Pfeiffer, C.M., Sternberg, M.R., et al., 2013. J. Nutr. 143, 1001S–1010S.

TABLE 8.14 Serum α-Tocopherol Concentrations in Humans

Group	α-Tocopherol (mg/dL)[a]
Healthy adults	0.85 ± 0.03
Postpartum mothers	1.33 ± 0.40
Infants	
Full term, at delivery	0.22 ± 0.10
Premature, at delivery	0.23 ± 0.10
Premature, at 1 month	0.13 ± 0.05
2 months, breast-fed	0.71 ± 0.25
2 months, bottle-fed	0.33 ± 0.15
5 months	0.42 ± 0.20
2 years	0.58 ± 0.20
Children, 2–12 years	0.72 ± 0.02
Cystic fibrotics, 1–19 years	0.15 ± 0.15
Biliary atresia, 3–15 months	0.10 ± 0.10

[a]*Mean ± SD.*
From Gordon, H.H., Nitowsky, H.M., Tildon, J.T., 1958. Pediatrics 21, 673–681.

Urinary metabolites of α-tocopherol offer potential as biomarkers of vitamin E status. These include α-CMBHC, α-CEHC, and, perhaps, α-tocopheronolactone,[92] and their glucuronyl conjugates.

A novel approach to developing new biomarkers of vitamin E status was taken by West et al.[93] They used a proteomic approach to identify an "α-tocopherome", i.e., a group of proteins expressed in relation to plasma α-tocopherol concentration. Of nearly a 1000 proteins quantifiable in plasma from Nepali children, they identified 6 that explained 71% of the variability in plasma α-tocopherol concentration.[94]

Plasma concentrations of F_2-isoprostanes,[95] which are formed to nonenzymic oxidation of PUFAs, have utility as biomarkers of oxidative stress. Although they are elevated in individuals of low vitamin E status, particularly those also overweight [body mass index (BMI) 25–29.9] or obese (BMI ≥ 30), they are not informative regarding vitamin E status per se. In humans, plasma F_2-isoprostane levels respond to high-dose vitamin E supplementation over a period of weeks (Fig. 8.15). With daily α-tocopherol doses of 800 IU/day, responses were seen only in subjects with relatively high baseline plasma F_2-isoprostane concentrations (>50 μg/mL).[96]

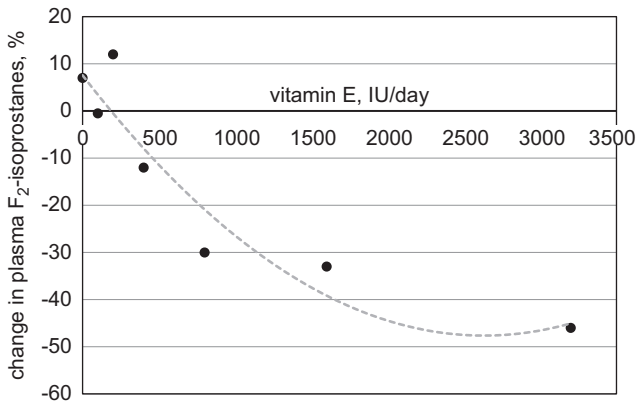


FIGURE 8.15 Reduction in plasma F_2-isoprostane concentration in response to supplemental α-tocopherol. *After Roberts, L.J., Oates, J.A., Linton, M.F., et al., 2007. Free Radic. Biol. Med. 43, 1388–1393.*

9. VITAMIN E DEFICIENCY

Vitamin E deficiency can have primary (privational) and secondary (nonprivational) causes.

- **primary causes** involve inadequate vitamin E supply
  - dietary patterns that fail to provide vitamin E in adequate amounts
- **secondary causes** relate to impaired absorption, metabolism, or metabolic function of the vitamin
  - **Lipid malabsorption** including those resulting in loss of pancreatic exocrine function (e.g., pancreatitis, pancreatic tumor, nutritional pancreatic atrophy in severe selenium deficiency), those involving a lumenal deficiency of bile (e.g., biliary stasis because of mycotoxicosis, biliary atresia), those caused by defects in lipoprotein metabolism (e.g., **abetalipoproteinemia**[97]), and those typical in prematurity (Table 8.16).
  - **High PUFA intake** increases need for vitamin E. Animals fed high-PUFA diets require more vitamin E than those fed low-PUFA diets. It has been estimated that vitamin E needs increase by 0.18–0.60 mg

92. Although it has been identified in urine samples, it is not clear whether α-tocopheronolactone may be an artifact formed from α-CEHC in sample preparation.
93. West, Jr., K.P., Cole, R.N., Shrestha, S., et al., 2015. J. Nutr. 145, 2645–2656.
94. apoC-III, apoB, pyruvate kinase (muscle), forkhead box 04, unc5 homolog C, regulator of G-protein signaling 8.
95. F_2-isoprostanes are prostaglandin-like compounds formed in vivo from the free radical–catalyzed peroxidation of essential fatty acids, primarily arachidonic acid.
96. Block, G., Jensen, C.D., Morrow, J.D., et al., 2008. Free Radic. Biol. Med. 45, 377–384.
97. Humans with this rare hereditary disorder are unable to produce apoB, an essential component of chylomicra, VLDLs, and LDLs. The absence of these particles from the serum prevents the absorption of vitamin E because of the inability to transport it into the lymphatics. These patients show generalized lipid malabsorption with **steatorrhea** (i.e., excess fat in feces) and have undetectable serum vitamin E levels.

TABLE 8.15 Recommended Vitamin E Intakes

US		FAO/WHO		
Age-Sex	RDA[a] (mg/day)	Age-Sex		RNI[b] (μg/day)
0–6 months	4	0–6 months		2.7
7–11 months	5	7–11 months		2.7
1–3 years	6	1–3 years		5
4–8 years	7	7–9 years		7
9–13 years	11	10–18 years	Females	7.5
14–70+ years	15		Males	10
		19–65+	Females	7.5
			Males	10
Pregnancy	15	Pregnancy		–
Lactation	19	Lactation		–

[a]*Recommended Dietary Intakes; Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, 506 pp.*
[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*

α-tocopherol per gram of PUFA consumed; the upper end of that range is frequently cited as a guideline for estimating vitamin E needs.[98]

- **Deficiency of Se**, which spares the need for vitamin E in antioxidant defense. Animals fed low-Se diets generally require more vitamin E than those fed the same diets supplemented with an available source of Se.

Vitamin E needs can be affected by status with respect to other nutrients involved in the cellular antioxidant defense system (sulfur-containing amino acids;[99] copper, zinc, and/or manganese;[100] and riboflavin[101]), and by intake of synthetic antioxidants[102] (e.g., butylated hydroxytoluene,[103] BHA,[104] and DPPD[105]) and, possibly, by vitamin C.[106]

98. There is no consensus among experts in the field as to the quantitation of this obviously important relationship.
99. **Cysteine**, which can be synthesized via transsulfuration from methionine, is needed for the synthesis of glutathione, the substrate for the Se-dependent GPX.
100. These are essential cofactors of the superoxide dismutases.
101. Riboflavin is required for the synthesis of FAD, the coenzyme for glutathione reductase, which is required for regeneration of GSH.
102. Although vitamin E can be replaced by a variety of antioxidants, it should be noted that the effective levels of other antioxidants are considerably greater (two orders of magnitude) than those of α-tocopherol.
103. Butylated hydroxytoluene.
104. Butylated hydroxyanisole.
105. *N,N′*-Diphenyl-*p*-phenylenediamine.
106. The sparing effect of vitamin C is thought to involve its functioning in the reductive recycling of tocopherol.

Recommended intakes of vitamin E have been established (Table 8.15).

Groups at Risk of Vitamin E Deficiency.
Individuals with
very low fat intakes
low intakes of plant oils and nuts
lipid malabsorption syndromes and
dyslipidemias

Vitamin E Deficiency Signs in Humans

Vitamin E deficiency is not common in adults. It manifests clinically as hemolytic anemia, i.e., normochromic anemia with reticulocytosis after long periods of depletion of body stores. This was demonstrated in the now-classical vitamin E depletion study by Max Horwitt at Elgin State Hospital.[107] This demonstrated that significant erythrocyte fragility, as evidenced by increased oxidative (H_2O_2-inducible) hemolosis, did not occur when plasma α-tocopherol concentrations exceeded ~0.5 mg/dL and that depletion to such levels required nearly a year of consumption of a low–vitamin E diet (Fig. 8.16).[108]

107. Horwitt (1908–2000) was for many years the Director of the L.B. Mendel Research Laboratory at Elgin State Hospital, Elgin, IL, where he conducted a series of now-classic studies with volunteers. These included some of the first studies of the nutritional aspects of aging, and studies of requirements for thiamin, riboflavin, tryptophan-niacin, and vitamin E.
108. Horwitt, M.K., Harvey, C.C., Duncan, G.D., et al., 1956. Am. J. Clin. Nutr. 4, 408–419.

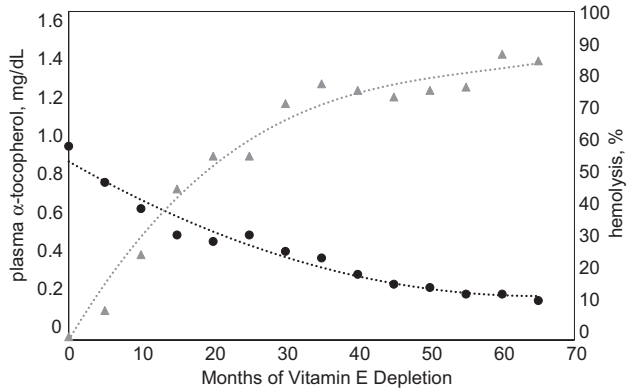


FIGURE 8.16 Relationship of vitamin E depletion and H_2O_2-induced hemolysis in Elgin Project volunteers. *After Horwitt, M.K., 1993. Max K. Horwitt: His Life and Science, M.K. Horwitt, St. Louis, p. 89.*

Chronic deficiency of vitamin E has been described in individuals with variant α-TTPs. These vary from moderate reductions in circulating tocopherol levels to untreatable progressive ataxia, depending on the degree of α-TTPs functionality.[109] Chronic deficiency can produce cerebellar and spinal cord damage manifest as ataxia, impaired reflexes, and impaired proprioreception. Subclinical deficiency can be detected as increased susceptibility to oxidative hemolysis in vitro. In pregnant women, this increases the risk for miscarriage,[110] apparently because of cellular anoxia resulting from primary lesions of the vascular system. In premature infants, vitamin E deficiency is manifest as membrane intraventricular hemorrhage and edema. Recommended intakes of vitamin E have been established (Table 8.15).

Vitamin E Deficiency Signs in Animals

The clinical manifestations of vitamin E deficiency vary considerably between species. In general, however, the targets are the neuromuscular,[111] vascular, and reproductive systems. The various signs of vitamin E deficiency are believed to be manifestations of membrane dysfunction resulting from the oxidative degradation of polyunsaturated membrane phospholipids and/or the disruption of other critical cellular processes.[112] Some signs (e.g., **encephalomalacia** in the chick) (Fig. 8.17) appear to involve local cellular anoxia resulting from primary lesions of the vascular system. Others, such as the nutritional myopathies (Fig. 8.18) appear to involve the lack to protection from oxidative stress. It has also been proposed that some effects (e.g., impaired immune cell functions) may involve loss of control of the oxidative metabolism of arachidonic acid in its conversion to leukotrienes; vitamin E is known to inhibit the 5´-lipoxygenase in that pathway.

Vitamin E deficiency has been shown in experimental animals (and children) to compromise both humoral and cell-mediated immunity. Deficient individuals have PMNs with impaired phagocytic abilities, suppressed oxidative burst and bactericidal activities, and decreased chemotactic responses. They can also show generally suppressed lymphocyte production, impaired T-cell functions, and decreased antibody production. Vitamin E deprivation has been found in animals to increase susceptibility to viral infections and to enhance the virulence of cardiophilic viruses passed through antioxidant-deficient hosts.[113] Vitamin E supplements are used to reduce the risk of mastitis in dairy cows and protect against aflatoxins in poultry diets. These effects are thought to involve loss of redox tone and appear to involve impaired cellular membrane fluidity and enhance PGE_2 production by the host, creating an environment in which viral mutation rates increase.

10. VITAMIN E IN HEALTH AND DISEASE

Oxidative stress plays a role in several conditions that have been associated with relatively low vitamin E. For such conditions, protection would be expected from increasing vitamin E intake. Supranutritional intakes[114] of vitamin E have been found beneficial under some of these conditions.

Cardiovascular Disease

Observational studies have consistently demonstrated benefits of vitamin E on cardiovascular disease risk.[115] Seven of the nine major cohort studies conducted to date (and involving nearly a quarter-million subjects) found inverse associations of vitamin E intake and cardiovascular disease incidence or associated mortality. Two large cohort studies found beneficial effects of high vitamin E intakes achieved through the use of dietary supplements for at least 2 years' duration (Table 8.17).[116] The results

109. Gotoda, T., Arita, M., Arai, H., et al., 1995. N. Engl. J. Med. 333, 1313–1318.

110. Shamin, A.A., Schulze, K., Merrill, R.D., et al., 2015. Am. J. Clin. Nutr. 101, 294–301.

111. Skeletal myopathies of vitamin E–deficient animals entail lesions predominantly involving type I fibers.

112. It is interesting to note a situation in which vitamin E deficiency would appear advantageous: the efficacy of the antimalarial drug derived from Chinese traditional medicine, *qinghaosu* (artemisinin), is enhanced by deprivation of vitamin E. The drug, an endoperoxide, is thought to act against the plasmodial parasite by generating free radicals in vivo. Thus, depriving the patient of vitamin E appears to limit the parasite's access to the protective antioxidant.

113. This involved an increase in viral mutation rate (Levander, O.A., Beck, M.A., 1997. Biol. Trace Elem. Res. 56, 5–21) and was also prevented by selenium.

114. That is, intakes substantially greater than required to prevent signs of nutritional deficiency.

115. *See* review: Cordero, Z., Drogen, D., Weikert, C., et al., 2010. Crit. Rev. Food Sci. Nutr. 50, 420–440.

116. Stampfer, M., Hennekens, C.H., Manson, J.E., et al., 1993. N. Engl. J. Med. 328, 1444–1450; Rimm, E.B., Stampfer, M.J., Ascherio, A., et al., 1993. N. Engl. J. Med. 328, 1450–1456.

TABLE 8.16 Signs of Vitamin E Deficiency

Organ System	Sign	Responds to		
		Vitamin E	Selenium	Antioxidants
General	Loss of appetite	+	+	+
	Reduced growth	+	+	+
Dermatologic	None			
Muscular	Myopathies			
	Striated muscles[a]	+	+	
	Cardiac muscle[b]	+	+	
	Smooth muscle[c]	+		
Skeletal	None			
Vital organs	Liver necrosis[d]	+	+	
	Renal degeneration[d]	+		+
Nervous system	Encephalomalacia[e]	+		+
	Areflexia, ataxia[f]	+		
Reproduction	Fetal death[g]	+	+	+
	Testicular degeneration[h]	+	+	
Ocular	Cataract[i]	+		
	Retinopathy[j]	+?		
Vascular	Anemia[j,k]	+		
	RBC hemolysis[l]	+		
	Exudative diathesis[e]	+	+	
	Intraventricular hemorrhage[j]	+		

[a] *Nutritional muscular dystrophies (white muscle diseases) of chicks, rats, guinea pigs, rabbits, dogs, monkeys, minks, sheep, goats, and calves.*
[b] ***Mulberry heart disease*** *(congested heart failure) of pigs.*
[c] *Gizzard* ***myopathy*** *of turkeys and ducks.*
[d] *In rats, mice, and pigs.*
[e] *In chicks.*
[f] *In humans with abetalipoproteinemia.*
[g] *In rats, cattle, and sheep.*
[h] *In chickens, rats, rabbits, hamsters, dogs, pigs, and monkeys.*
[i] *Reported only in rats.*
[j] *Low–vitamin E status is suspected in this condition in premature human infants.*
[k] *In monkeys, pigs, and humans.*
[l] *In chicks, rats, rabbits, and humans.*

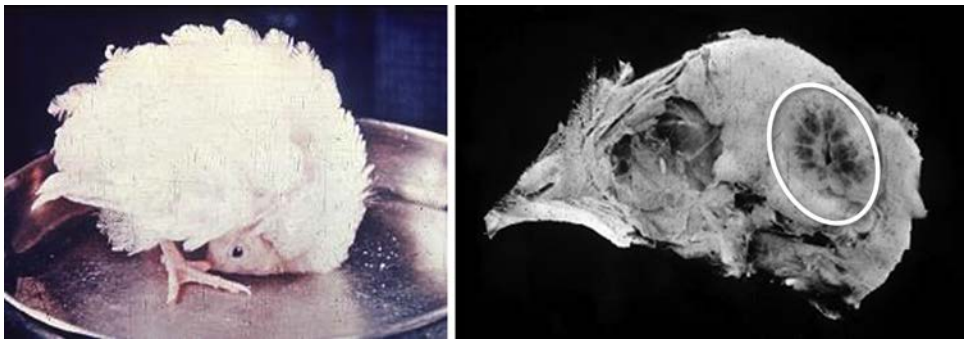


FIGURE 8.17 Encephalomalacia in a vitamin E–deficient chick. Chick is unable to maintain normal posture, tends to fall over, and cannot eat; postmortem examination shows cerebellar hemorrhage.

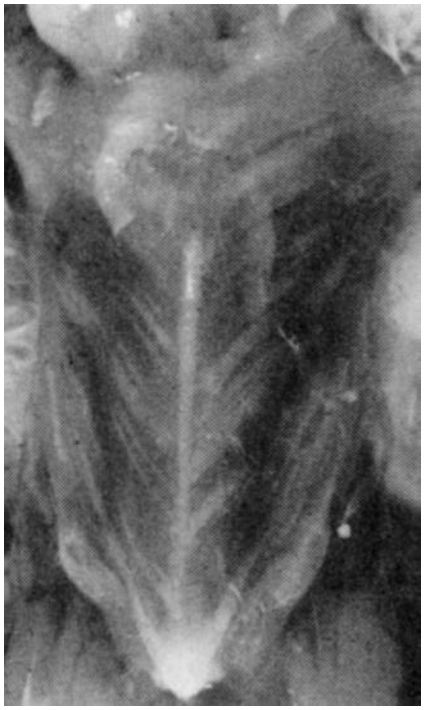


FIGURE 8.18 Nutritional muscular dystrophy in a chick deficient in vitamin E, selenium, and cysteine. Note: white striated breast muscle (*m. pectorales*) because of Zenker-type hyaline degeneration of muscle fibers. This condition, frequently called "white muscle disease", also occurs in vitamin E– and selenium-deficient lambs and calves.

of case–control and cohort studies have, however, been mixed. This is not surprising, given the many sources of variation in such studies: inherent errors in estimating vitamin E intake, variability in cardiovascular risk factors, variability in vitamin E utilization and baseline status, oxidative degradation of tocopherols during sample handling and storage, etc.

That vitamin E may protect against cardiovascular disease would appear likely. It can protect LDLs from oxidative damage, which is thought to be a factor in the etiology of atherosclerosis. Enrichment of LDLs with vitamin E, the predominant antioxidant occurring naturally in those particles, increases the lag phase of their oxidation in vitro, indicating increased resistance to oxidation (Table 8.18). Tocotrienols have been found to produce significant (28%) reductions in circulating triglyceride levels by reducing the upstream regulators of lipid homeostasis genes (Fig. 8.19).[117] When given intravenously, they have been found to inhibit acute platelet-mediated thrombus formation and collagen and ADP-induced platelet aggregation.[118]

The majority of randomized clinical trials have not found vitamin E supplementation to reduce cardiovascular risk. A meta-analysis of randomized trials found vitamin E supplementation to be associated with a 10% reduction in ischemic stroke, but a 22% increase in hemorrhagic stroke.[119] As the intervention agent in most studies has been α-tocopheryl acetate, some have asked whether non–α-vitamers, particularly γ-tocopherol and the tocotrienols, may be effective. It is more likely that beneficial effects may have been missed by not considering disease subtypes or sensitive subgroups, e.g., individuals with polymorphisms with specific genes involved in cellular antioxidant protection. A recent randomized trial found that adults with type 2 diabetes (T2D) showed reduced cardiovascular disease risk in response to supplemental vitamin E *only* if they also had a particular haptoglobin polymorphism, i.e., the *Hp2-2* genotype (Table 8.19).[120,121] Genetic polymorphisms affecting circulating α-tocopherol levels may also affect responsiveness to vitamin E, i.e., those involved in vitamin E transport/retention (α-TTP, TAPs, apoE and A, SR-BI and CD36 scavenger receptors, LDL receptor, PLTP, microsomal triglyceride transfer protein, lipoprotein lipase, and ATP-binding cassette transporter); vitamin E–mediated gene expression (pregnane X receptor); vitamin E metabolism (CYP3A and CYP4F2); and antioxidant metabolism (dehydroascorbate reductase, sodium-coupled ascorbic acid transporters).

Neurodegenerative Disorders. Epidemiological studies have found vitamin E intake to be inversely associated with risks for cognitive impairment[122] and to Alzheimer disease and Parkinson disease, the etiologies of which are thought to involve oxidative stress. Most randomized clinical trials have found vitamin E supplementation to yield inconsistent results with respect to those diseases and amyotrophic lateral sclerosis (ALS),[123] which is thought to

117. Zaiden, N., Yap, W.N., Ong, S., et al., 2010. J. Atheroscler. Thromb. 17, 1019–1032.

118. Qureshi, A.A., Karpen, C.W., Qureshi, N., et al., 2011. Lipids Health Dis. 10, 58–71.

119. For example, Shűrks, M., Glynn, R.J., Rist, P.M., et al., 2010. Br. J. Med. 341, c5702) found supplemental vitamin E not to affect stroke risk, but to reduce risk of ischemic stroke by 10% and increase risk of hemorrhagic stroke by 22%.

120. Milman, U., Blum, S., Shapira, C., et al., 2008. Arterioscler. Thromb. Vasc. Biol. 28, 341–348.

121. Haptoglobin (Hp) complexes with hemoglobin (Hb) to shield its iron center, thus reducing its prooxidative effect. The complex is recognized by the CD163 scavenger receptor and is, thus, cleared from the plasma endocytotically into the reticuloendothelial system. There are two common alleles at the Hp locus, 1 and 2, and functional differences between the Hp1 and Hp2 proteins. Individuals homozygous for Hp2 (*Hp2-2*) have the polymorphism that forms cyclic polymers instead of the linear ones formed by the *Hp1-1* and *Hp1-2* genotypes. *Hp2-2* polymorphisms form complex Hb with 10-fold greater affinity than the linear Hp polymorphisms, bind less avidly CD163, and are, therefore, less efficiently cleared from the plasma. Individuals with the *Hp2-2* phenotype would be expected to have greater needs for the antioxidant effects of vitamin E. Individuals with the *Hp2-2* phenotype and T2D are at increased risk to cardiovascular disease.

122. Mangialasche, F., Solomon, A., Kåreholt, I., et al., 2013. Exp. Gerontol. 48, 148–1435.

123. ALS ("Lou Gherig's Disease") is characterized by profound muscular weakness because of the selective death of upper and lower motor neurons; the disease is ultimately fatal, mostly because of respiratory failure.

TABLE 8.17 High Vitamin E Intakes Associated With Reduced Coronary Heart Disease Risks in Two Cohorts of Americans

	Quintile Group for Vitamin E Intake					*p* Value for Trend
Parameter	1	2	3	4	5	
Women[a] – Total Vitamin E (Diet + Supplements)						
Median intake (IU/day)	2.8	4.2	5.9	17	208	
Relative risk[c]	1.0	1.00	1.15	0.74	0.66	<0.001
Men[b] – Total Vitamin E (Diet + Supplements)						
Median intake (IU/day)	6.4	8.5	11.2	25.2	419	
Relative risk[c]	1.0	0.88	0.77	0.74	0.59	0.001
All – Vitamin E From the Diet						
Range of intakes (IU/day)	1.6–6.9	7.0–9.8	8.2–9.3	9.4–11.0	11.1	
Relative risk[c]	1.0	1.10	1.17	0.97	0.79	0.11
All – Vitamin E From Supplements						
Range of intakes (IU/day)[b]	0	<25	25–99	100–249	≥250	
Relative risk[c]	1.0	0.85	0.78	0.54	0.70	0.22

[a]*A total of 39,910 health professionals (139,883 per-years follow-up); Rimm, E.B., Stampfer, M.J., Ascherio, A., et al., 1993. N. Engl. J. Med. 328, 1450–1456.*
[b]*A total of 87,245 nurses (679,485 per-years follow-up); Stampfer, M., Hennekens, C.H., Manson, J.E., et al., 1993. N. Engl. J. Med. 328, 1444–1450.*
[c]*Ratio of events in each quintile to those in the lowest quintile; adjusted for age and smoking.*

TABLE 8.18 Reduced Low-Density Lipoprotein Susceptibility to Lipid Peroxidation by Oral Vitamin E in Humans

Treatment	Time of Sampling (weeks)	LDL α-Tocopherol (μmol/g) Protein	LDL Oxidation[a]	
			Lag Phase (h)	Rate (μmol/g) Protein (h)
Placebo	0	14.3 ± 5.0	2.1 ± 0.9	396 ± 116
	8	15.8 ± 6.1	2.0 ± 0.8	423 ± 93
Vitamin E[b]	0	13.8 ± 4.1	1.9 ± 0.6	373 ± 96
	8	32.6 ± 11.5[c]	2.9 ± 0.8[b]	367 ± 105

[a]*Lipid peroxide formation.*
[b]*Total of 1200 IU/day as* RRR-α-*tocopherol.*
[c]*Significantly different* ($p < .05$) *from corresponding placebo value.*
From Fuller, C.J., Chandalia, M., Garg, A., et al., 1996. Am. J. Clin. Nutr. 63, 753–759.

involve mitochondrial stress. However, a multicenter trial conducted on patients with Alzheimer disease in the United States found a daily supplement of 2000 IU α-tocopherol to be effective in retarding the functional decline of mild and moderate Alzheimer disease.[124]

124. Dysken, M.W., Sano, M., Asthana, S., et al., 2014. JAMA 311, 33–44.

Inflammatory Diseases

Despite the clear anti-inflammatory effects that have been demonstrated for these vitamers, clinical trials have generally not found α-tocopherol supplements effective in reducing risks to inflammatory diseases (e.g., RA, asthma, and hepatitis) or diseases in which chronic inflammation

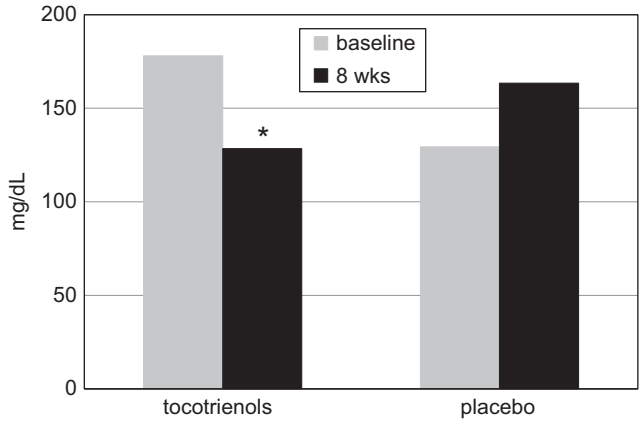


FIGURE 8.19 Reduction of serum triglycerides by mixed tocotrienols in hypercholesterolemic subjects. No effects on serum cholesterol of lipoproteins were noted. *From Zaiden, N., Yap, W.N., Ong, S., et al., 2010. J. Atherocler. Thromb. 17, 1019–1032.*

contributes (e.g., cardiovascular disease, cancer neurodegenerative diseases). The apparently different anti-inflammatory activities of e vitamers have made some to suggest that mixed tocopherols may have potential value in this regard. Studies with animal models of RA have found vitamin E supplementation to reduce joint swelling and γ-tocotrienol to reduce the pathogenesis of collagen-induced arthritis.[125] Randomized controlled trials have shown high-level supplementation with the vitamin (100–600 IU/day) to relieve pain and be anti-inflammatory for patients with RA, but not in reducing the risk of the disease.[126]

Air Pollution and Smoking. Vitamin E can be expected to protect the lungs against the continuous exposure to relatively high concentrations of O_2 and environmental oxidants and irritants. The respiratory epithelium contains vitamin E and relatively high concentrations of vitamin C, urate, GSH, extracellular superoxide dismutase, catalase, and GPX. Individuals living in smog-filled urban areas can be exposed to relatively high levels of ozone (O_3)[127] and NO_2, strong oxidants that provoke inflammatory responses of the airway, i.e., activating neutrophils and evoking macrophage respiratory bursts the overproduction of ROS, which causes peroxidative damage. Because vitamin E deprivation increases the susceptibility of experimental animals to the pathological effects of O_3 and NO_2,[128] it has been suggested that supplements of the vitamin may protect humans against chronic exposure to smog.

Supranutritional doses of vitamin E (up to 560 mg of α-tocopherol per day) have been found to reduce the peroxidation potential of erythrocyte lipids from smokers (Table 8.20). Smoking increases the oxidative burden on the lungs and other tissues because of the sustained exposure to free radicals from tars and gases.[129] This is characterized by increased levels of peroxidation products in the circulation (e.g., MDA) and breath (e.g., ethane, pentane), with decreased levels of ascorbic acid in plasma and leukocytes and of vitamin E in plasma and erythrocytes. High amounts of RNS in cigarette smoke cause nitration of vitamin E; 5-nitro-γ-tocopherol occurs at higher levels in smokers than nonsmokers (Table 8.15). This may be the basis for the enhanced turnover of tocopherols and reduced production of CECH by smokers.

Cataracts.[130] Vitamin E has been shown in animal models to protect against cataracts induced by galactose or aminotriazol and to reduce the photoperoxidation of lens lipids. These effects are thought to involve direct action of the vitamin as an antioxidant, including the maintenance of lens glutathione in the reduced state. Much of cataract lens opacification involves oxidations resulting in loss of sulfhydryls, formation of disulfide and nondisulfide covalent linkages, and oxidation of tryptophan residues. More than a dozen cohort studies have found plasma α-tocopherol level and/or vitamin E intakes to be inversely related to cataract risk.[131] These suggest that 5–10 years of high–vitamin E intake may be required for protection against nuclear cataracts. However, the only randomized controlled trial to have been conducted to date did not find α-tocopherol supplementation (500 IU/day) to reduce incidence of progression of cataract.[132]

Skin. Vitamin E is found in the skin mostly in the lower levels of the stratum corneum, where it is released by sebum. In animal models, the tocopherol content of dermal tissues decreases with UV irradiation, presumably a result of oxidative stress. Topical treatment with vitamin E may increase the hydration of the stratum corneum and confers protection against UV-induced skin damage, as measured by reduced erythemal responses and delayed

125. Radhakrishnan, A., Tudawe, D., Chakravarthi, S., et al., 2014. Exp. Therap. Med. 7, 1408–1414.
126. Karlson, E.W., Shadick, N.A., Cook, N.R., et al., 2008. Arthr. Rheum. 59, 1589–1596.
127. Ambient O_3 level on the top of Mt. Everest or in Los Angeles can be 70–80 ppb, whereas in Grand Forks, ND, the level is <50 ppb.
128. O_3 is produced in photochemical smog from nitrogen dioxide (NO_2) from internal combustion engines, O_2, and gasoline vapors. Both O_3 and NO_2 can generate unstable free radicals that damage lungs through oxidative attack on polyunsaturated membrane phospholipids.
129. Cigarette smoke contains a number of compounds that produce free radicals; it also increases the number of free radical–producing inflammatory cells in the lungs.
130. Cataracts result from the accumulation in the lens of damaged proteins that aggregate and precipitate, resulting in opacification of the lens. Cataract is a significant public health problem in the United States, where a million cataract extractions are performed annually at a cost of some $5 billion. The prevalence of cataracts among Americans increases from about 5% at age 65 years to about 40% at age 75 years. These rates are considerably (up to fivefold) greater in less developed countries.
131. Vishwanathan, R., Johnson, E.J., 2012. In: Erdman, J.W., Macdonald, I.A., Zeisel, S.H., (Eds.) Present Knowledge in Nutrition, tenth ed. Wiley-Blackwell, Ames, IA, p. 946–948.
132. McNeil, J.J., Robman, L., Tikellis, G., et al., 2004. Ophthalmology 111, 75–84.

TABLE 8.19 Relationship of Haptoglobin Genotype and Cardioprotection by Vitamin E

Treatment	Hp Genotype	n Events	Hazard Ratio (95% CI)		*p* Value
None	Hp2-1	1248	25	1.0	
	Hp1-1	285	6	1.0 (0.4–2.5)	0.92
Placebo	Hp2-2	708	33	2.3 (1.4–3.9)	0.001
Vitamin E	Hp2-2	726	16	1.1 (0.6–2.00)	0.81

From Milman, U., Blum, S., Shapira, C., et al., 2008. Arterioscler. Thromb. Vasc. Biol. 28, 341–348.

TABLE 8.20 Comparison of Effect of Vitamin E on Erythrocyte Lipid Peroxidation in Smokers and Nonsmokers

	Weeks of Vitamin E Administration[a]	Vitamin E in Erythrocytes (μmol/g Hb)	In vitro Lipid Peroxidation (nmol MDA[b]/g Hb)
Nonsmokers	0	20.0 ± 4.5	141 ± 54
	20	36.1 ± 8.2	86 ± 51
Smokers	0	18.0 ± 4.2	291 ± 102
	20	32.8 ± 8.2	108 ± 53

[a]*A total of 70 IU/day.*
[b]*Malonyldialdehyde.*
From Brown, K.M., Morrice, P.E., Duthie, C.G., 1977. Am. J. Clin. Nutr. 65, 496–502.

onset of tumorigenesis. One study found that regular topical application of vitamin E reduced wrinkle amplitude and skin roughness in about half of cases. For these reasons, α-tocopherol and α-tocopheryl acetate are widely used in skin creams and cosmetics.

Type 2 Diabetes (T2D). Vitamin E appears to play protective roles against T2D. α-Tocopherol appears to be less well absorbed and to turnover slower in subjects with metabolic syndrome[133] compared to controls.[134] Studies have found patients with diabetes to have low plasma tocopherols, higher urinary levels of tocopherol metabolites, and greater erythrocyte lipid peroxidation than healthy controls (Table 8.16). A randomized trial reported protection by vitamin E against the development of T2D among subjects with impaired glucose tolerance,[135] and high-level vitamin E supplements (e.g., 900 mg α-tocopherol per day) have been found to improve insulin responsiveness in both normal patients and patients with T2D . Oxidative stress is known to alter cellular serine/threonine kinase activities, reducing insulin signaling by suppressing phosphorylation of insulin receptor substrate-1. Supranutritional levels of α- and γ-tocopherols have been shown to upregulate an endogenous ligand involved in activating PPARγ and upregulate adiponectin, an adipokine that increases insulin sensitivity.[136] Vitamin E has been considered as a factor in protecting against diabetic complications (e.g., retinopathy, cardiac dysfunction) the etiologies of which are thought to involve oxidative stress. Clinical trials have found α-tocopherol supplementation to improve parameters of redox balance and endothelial function in nonobese (BMI < 30) diabetics (Table 8.21).[137]

Tocotrienols have been found effective in normalizing serum glucose and glycated hemoglobin (HbA_{1C}) in a diabetic rat model, and in reducing serum lipids, cholesterol, and LDL-cholesterol in patients with T2D with hyperlipidemia.[138] γ-Tocotrienol has been found to reduce insulin resistance associated with obesity in the rat; these effects appear to be because of reductions in adipose output of proinflammatory cytokines.[139]

133. Metabolic syndrome is associated with risks of developing T2D and cardiovascular disease. It is diagnosed on the basis of an individual having three or more of the following conditions: abdominal obesity, elevated blood pressure, high fasting serum glucose, high serum triglycerides, and low HDL levels.
134. Mah, E., Sapper, T.N., Chitchumroonchokchai, C., et al., 2015. Am. J. Clin. Nutr. 102, 1070–1080.
135. Mayer–Davis, E.J., Costacou, T., King, I., et al., 2002. Diabetes Care 25, 2172–2177.
136. Landrier, J.F., Gouranton, E., El Yazidi, C., et al., 2009. Endocrinol. 150, 5318–5328.
137. Montero, D., Walther, G., Stehouwwer, C.D.A., et al., 2014. Obes. Rev. 15, 107–116.
138. Baliarsingh, S., Bg, Z.H., Ahmad, J., 2005, Atherosclerosis 182, 367–374.
139. Zhao, L., Kang, I., Fang, X. et al., 2015. Int. J. Obesity 39, 438–446.

TABLE 8.21 Diabetes and Tocopherol Status

	Diabetics	Controls
Quilliot, D., Walters, E., Bonte, J.P., et al., 2005. Am. J. Clin. Nutr. 81, 1117–1125.		
Plasma α-tocopherol (μg/mL)	12.9 ± 2.9[a]	17.4 ± 3.7
Plasma LDL (mg/g)	5.5 ± 3.8[a]	6.4 ± 1.3
Sharma, G., Muller, D.P., O'Riordan, S.M., et al., 2013. Free Radic. Biol. Med. 55, 54–62.		
Urine α-CEHC glucuronide (nmol/mmol creatinine)	126 ± 16[a]	73 ± 19
Urine α-CEHC sulfate (nmol/mmol creatinine)	138 ± 33[a]	57 ± 12
Urine α-tocopheronolactore glucuronide (nmol/mmol creatinine)	1098 ± 279[a]	76 ± 13

[a]*Significantly different from control,* $p < .05$.

TABLE 8.22 Antioxidant Protection by Vitamin E in High-Altitude Climbers

	Change in Pentane Exhalation[a] (%)		
Treatment Group	Median	Lower Quartile	Upper Quartile
Placebo	104	26	122
Vitamin E, 400 IU/day	−3	−7	3

[a]*After 4 weeks at high altitude.*
From Simon-Schnass, I., Pabst, H., 1988. J. Vit. Nutr. Res. 58, 49–56.

Preeclampsia. That vitamin E plays a key role in pregnancy is suggested by the fact that circulating α-tocopherol levels correlate positively with fetal growth rate, particularly during the last trimester when O_2 utilization is increased. Increases in lipid peroxides of placental origin in the maternal circulation correlate with the severity of preeclampsia.[140] A systematic review of nine intervention trials found no evidence for vitamin E supplementation during pregnancy affecting risk of preeclampsia.[141]

Altitude. Vitamin E protects against the prooxidative effects of O_3. One study found that a daily supplement of 400 IU vitamin E prevented decreases in anaerobic thresholds of high-altitude mountain climbers, suggesting that exercise at altitude increases the need for vitamin E (Table 8.22).

Ischemia–Reperfusion Injury. Vitamin E and other free-radical scavengers have been found to affect the functions of mitochondria and sarcoplasmic reticula in animal models of myocardial injury induced by ischemia–reperfusion. The injury, occurring in tissues reperfused after a period of ischemia, appears to be because of the oxidative stress of reoxygenation, involving production of ROS. The phenomenon has been demonstrated for several tissues (heart, brain, skin, intestine, and pancreas) and has relevance for the preservation of organs for transplantation. ROS are thought to contribute to milder forms of tissue injury at the time of reperfusion (e.g., myocardial stunning, reperfusion arrhythmias). However, the extent to which free radicals are responsible for the acute tissue damage seen under those circumstances is not clear. Intervention trials with antioxidants have yielded conflicting results, but it is possible that preoperative treatment with vitamin E may be useful in reducing at least some of this type of injury. A randomized clinical trial demonstrated that vitamin E supplements (given by intramuscular injection) can be very effective in protecting premature infants against intraventricular hemorrhage,[142] which involves natural ischemia–reperfusion injury.

Antitumorigenesis

It would seem reasonable to expect that vitamin E may have a role in cancer prevention in as much as at least for some types of cancer are thought to involve ROS. Chemical carcinogenesis typically involves the electrophilic attack of free radicals with DNA. The generation of ROS, mutagenic to mammalian cells in vitro, correlates with the initiation, promotion, and progression of tumors in experimental animal models. MDA has also been found to increase tumor production in animals. Studies with two-stage, UV-, or chemically induced mammary, colon, oral, or skin tumor models have generally shown α-tocopherol to inhibit tumor promotion. These include studies showing topically applied vitamin E to reduce UV-induced skin cancers by as much as 58%.

Evidence indicates that vitamin E, particularly the tocotrienols, can selectively stimulate apoptosis in neoplastic cells.[143] Tocotrienols have been shown to be capable of targeting multiple cell signaling pathways. They

140. That is, pregnancy-induced hypertension associated with proteinuria. Preeclampsia is thought to involve endothelial dysfunction of maternal blood vessels induced by factors released from the placenta. It can develop in the last trimester of pregnancy and has the highest rates of morbidity and mortality of all complications of pregnancy.

141. Conde-Agudelo, A., Romero, R., Kusanovic, J.P., et al., 2011, Am. J. Obstet. Gynecol. 204 (503) e1–e12.

142. Hemorrhage in and around the lateral ventricles of the brain occurs in about 40% of infants born before 33 weeks of gestation.

143. McIntyre, B.S., Briski, K.P., Tirmenstein, M.S., et al., 2000. Lipids 35, 171–180.

have been shown to cause receptor-induced caspase-8 and -3 activation (a typical response to oxidative stress), leading to apoptosis in some cancer cells, and caspase-9 activation by mitochondrial stress in others.[144] They have been found to inhibit cytokine-stimulated activation of NF-κB by inducing its inhibitor, the anti-inflammatory protein A20;[145] to inhibit TGF-β, P38, and β-catenin/Tcf signaling; to downregulate Bcl2, cyclin D, and the Raf/Erk pathway; to inhibit HMGR; to induce DNA fragmentation, to inhibit angiogenesis; and to promote cell cycle arrest.[146] A polymorphism of the scavenger receptor class B member 1 associated with increased circulating levels of vitamin E was also found to be associated with reduced prostate cancer risk.[147] The α-TAP and apparent transcription factor TAP appears to have a role in regulating tumorigenesis. Liganded TAP is thought to serve as a tumor suppressor.[148] Its mRNA is inversely associated with tumor stage in breast cancer. Overexpression of TAPs has been found to suppress growth of prostate cancer cells,[149] apparently by sensitizing them to antiproliferative effects of α-tocopherol and allowing them to accumulate the vitamin.[150]

The results of most studies to date are inconsistent with respect to relationships of vitamin E and cancer incidence. Inverse epidemiological associations have been made between the consumption of vitamin E–rich foods and cancer risks, and longitudinal studies have found circulating tocopherol levels to be only slightly lower (~3%) in patients with cancer than in healthy controls. A pooled analysis of 15 prospective studies found serum/plasma α-tocopherol concentration to be positively associated with prostate cancer risk.[151] A difference in this magnitude was observed between patients with cancer and controls in a large trial in Finland,[152] which also found individuals with relatively low serum α-tocopherol levels to have a 1.5-fold greater risk of cancer than those with higher serum vitamin E levels.

Only a few clinical trials have evaluated the antitumorigenic potential of supplemental vitamin E. One study found the combination of vitamin E, selenium and β-carotene to reduce lung cancer risk by an apparent 45%.[153] Some small trials have suggested that vitamin E may reduce the risk of breast cancer among women with mammary dysplasia; however, those results have not been confirmed in larger trials of benign breast disease. A study with more than 29,000 male smokers found vitamin E treatment (50 mg α-tocopherol per day) to be associated with a 34% reduction in prostate cancer incidence;[154] however, large subsequent trials have not found supplemental vitamin E to affect prostate cancer incidence.[155]

Other Conditions. Studies with animal models have demonstrated that vitamin E can protect against radiation-induced chromosome damage, because of direct ionization of DNA and other cellular targets and indirect effects of ROS that are produced. This effect may be the basis of epidemiological associations between consumption of antioxidant-rich foods and low cancer risk. Vitamin E has frequently proven effective as a therapeutic measure in hemolytic anemia of prematurity, intermittent claudication,[156] and chronic hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency. In veterinary practice, vitamin E (most frequently administered with selenium) has been reported efficacious for treating "tying up"[157] in horses and postpartum placental retention in dairy cows. Formerly, vitamin E was thought to protect against retinopathy of prematurity;[158] however, a controlled clinical trial failed to confirm that. Low vitamin E status has been associated with osteoporosis and fracture risk,[159] supporting the hypothesis that oxidative stress may impair osteoblast function. A systematic review of the relevant scientific literature concluded that tocotrienols may have value in preventing/treating osteoporosis. Of the 11 studies meeting the inclusionary criteria, three

144. *See* review: Sylvester, P.W., 2007. Vit. Horm. 76, 329–356.
145. Wang, Y., Park, N.Y., Jang, Y., et al., 2015. J. Immunol. 195, 126–133.
146. Kannapan, R., Gupta, S.C., Kim, J.H., et al., 2012. Genes Nutr. 7, 43–52; Ling, M.T., Luk, S.U., Al-Ejeh, F., et al., 2012. Carcinogen. 33, 233–249; Xu, W., Du, M., Zhao, Y., et al., 2012. J. Nutr. Biochem. 23, 800–807; Ahsan, H., Ahad, A., Iqbal, J., et al., 2014. Nutr. Metab. 11, 52–74; Shibata, A., Nakagawa, K., Tsuduki, T., 2015. J. Nut. Biochem. 26, 832–840.
147. Major, J.M., Yu, K., Weinstein, S.J., et al., 2014. J. Nutr. 144, 729–733.
148. Wang, X., Ring, B.Z., Seitz, R.S., 2015. BMC Clin. Pathol. 15, 21–31.
149. Ni, J., Wen, X., Yao, J., et al., 2005. Cancer Res. 65, 9807–9816.
150. Morley, S., Thjakur, V., Denielpour, D., et al., 2010. J. Biol. Chem. 285, 35,578–35,589.
151. Key, T.J., Appleby, P.N., Travis, R.C., et al., 2015. Am. J. Clin. Nutr. 145, 1142–1157.
152. That is, the Finnish Mobile Clinic Health Survey, which involved more than 36,000 subjects; Kneckt, P., et al., 1991. J. Clin. Nutr. 53, 283S–289S.
153. Blot, W.J., Li, J.Y., Taylor, P.R., et al., 1993. J. Natl. Cancer Inst. 85, 1483–1492.
154. Albanes, D., Heinonen, O.P., Taylor, P.R., et al., 1996. J. Natl. Cancer Inst. 88, 1560–1570.
155. The HOPE-TOO Trial (Lonn, E., Bosch, J., Yusuf, S., et al., 2005. JAMA 293, 1338–1347); The Physicians' Health Study II (Gaziano, J.M., Glynn, R.J., Christen, W.G., et al., 2009. JAMA 301, 52–62); SELECT (Lippman, S.M., Klein, E.A., Goodman, P.J., et al., 2009. JAMA 301, 39–51); Wang, L., Sesso, H.D., Glynn, R.J., et al., 2014. Am. J. Clin. Nutr. 100, 915–923.
156. Nocturnal leg cramps.
157. That is, rhabdomyolysis involves the breakdown of striated muscle after exercise, characterized by soreness in the gluteal muscles and painful/stiff gait. It most frequently results from having had limited exercise or having been put in a stressful environment.
158. This disorder was formerly called **retrolental fibroplasia**. Its pathogenesis involves exposure to a hyperoxic environment during neonatal oxygen therapy. It can affect as many as 11% of infants with birth weights below 1500 g, resulting in blindness of about one-quarter of them.
159. Michaëlson, K., Wolk, A., Byberg, L., et al., 2014. Am. J. Clin. Nutr. 99, 107–114.

epidemiological studies and eight animal studies reported positive effects of tocotrienols.[160]

Healthy Aging. Studies in animal models have shown that vitamin E status reduces the accumulation of "age pigments", i.e., lipid-soluble, brown-yellow, autofluorescent pigments[161] collectively called lipofuscin, in several tissues (retinal pigment epithelium, heart muscle, brain, skin). That these changes are causally related to aging is suggested by interspecies observations that mammalian life span potentials tend to correlate inversely with metabolic rate and directly with tissue concentrations of tocopherols and other antioxidants (carotenoids, urate, ascorbic acid, and superoxide dismutase activity).[162] It has been proposed that cumulative damage by ROS and associated increases in redox tone lead to gradual decreases in repair capacity likely because of changes in gene expression, diminished immune function, enhanced inflammation, and enhanced programmed cell death.[163] Studies in rats have shown that the age-related decline in the major glucose transporter in neurons (Glut3) is exacerbated by deprivation of vitamin E.

TABLE 8.23 Upper Tolerable Limits (UL) for Vitamin E From Supplements

Age Group	UL (mg/day)
Infants	–[a]
Children, 1–3 years	200
4–8	300
9–13	600
14–18	800
Adults, 19+	1000
Pregnancy, ≤18	800
>18	1000
Lactation, ≤18	800
>18	1000

[a]*No UL was set for infants; however, an UL was recommended for premature infants (birth weight ≤1.5 kg) at 21 mg/day.*
From Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, p. 186–283.

11. VITAMIN E TOXICITY

Vitamin E has been viewed as one of the *least toxic* of the vitamins. Both animals and humans appear to be able to tolerate high levels.[164] For animals, intakes at least two orders of magnitude greater than nutritional requirements, e.g., to 1000–2000 IU/kg, are without untoward effects. For humans, daily doses as high as 400 IU have been be considered harmless, and large oral doses as great as 3200 IU have not been found to have consistent ill effects. These views were challenged by a meta-analysis (of 19 trials) suggesting that vitamin E supplements (≥400 IU/day) may increase all-cause mortality. A more recent meta-analysis, which included a larger number (57) of published trial results, concluded that supplemental vitamin E does *not* affect all-cause mortality at doses up to 5500 IU/day.[165]

It is known that at very high doses of vitamin E can antagonize the functions of other fat-soluble vitamins. Hypervitaminotic E animals have been found to show impaired bone mineralization, reduced hepatic storage of vitamin A, and coagulopathies. In each case, these signs could be corrected with increased dietary supplements of the appropriate vitamin (i.e., vitamins D, A, and K, respectively) and the antagonism seemed to be based at the level of absorption. Isolated reports of negative effects in human subjects consuming up to 1000 IU of vitamin E per day included headache, fatigue, nausea, double vision, muscular weakness, mild creatinuria, and gastrointestinal distress.

Potentially deleterious metabolic effects of high-level vitamin E status include inhibitions of retinyl ester hydrolase and vitamin K–dependent carboxylations. The former effect has been demonstrated in animals, where it results in impaired ability to mobilize vitamin A from hepatic stores. Supranutritional vitamin E treatment of rats has been shown to increase bleeding tendency; however, there is little evidence for comparable effects in humans. Patients given high doses of α-tocopherol (1200 IU/day) have shown increased blood clotting times because of hypoprothrombinemia, but that effect may be of concern only for patients on anticoagulant therapy. Although the metabolic basis of the effect is not clear, it likely involves inhibition of vitamins E and K competing for the CYP4F2-dependent ω-hydroxylation of their structurally similar phytyl side chains, inhibiting the conversion of vitamin K_1 to MK-4 and, thus, the vitamin K–dependent carboxylase (*see* Chapter 9).

Any concerns about the safety of high intakes of vitamin E must be about the use of nutritional supplements, as high intakes are virtually impossible to achieve from foods. Accordingly, the IOM has recommended safe upper limits on vitamin E intakes from supplements (Table 8.23)

160. Muhammad, N., Borhanuddin, B., Shuid, A.N., et al., 2012. Evid. Based Complem. Altern. Med. ID 250584, 14 pp.
161. Lipofuscins are thought to be condensation products of proteins and lipid. There is some evidence that the pigments isolated from the retinal pigment epithelium contain, at least in part, derivatives of vitamin A (e.g., *N*-retinylidene-*N*-retinylethanolamine).
162. Caloric restriction can increase longevity in animals, perhaps by reducing the metabolic rate, hence, endogenous production of ROS.
163. That is, **apoptosis**.
164. Hathcock, J.N., Azzi, A., Blumberg, J., et al., 2005. Am. J. Nutr. 81, 736–745; Cook-Mills, J.M., May, C.A., 2010. Endocr. Metab. Immune Disord. Drug Targets 10, 348–366.
165. Abner, E.L. Schmitt, F.A., Mendiondo, M.S., et al., 2011. Curr. Aging Sci. 4, 158–170.

12. CASE STUDIES

Instructions

Review the following case reports, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

Case 1

At birth, a male infant with acidosis[166] and **hemolytic anemia**[167] was diagnosed as having glutathione (GSH) synthetase[168] deficiency associated with 5-keto-prolinuria[169]; he was treated symptomatically. During his second year, he experienced six episodes of bacterial otitis media.[170] His white cell counts fell to 3000–4000 cells/μL during two of these infections, with notable losses of PMNs[171]. Between infections, the child had normal white and differential cell counts, and PMNs were obtained for study.

Functional studies of PMNs showed the following results:

Parameter	Finding
GSH synthetase activity	10% of normal
Phagocytosis of *Staphylococcus aureus*	Less than normal
Iodination of phagocytized zymosan particles	Much less than normal
H_2O_2 production during phagocytosis	Well above normal

The child was then treated daily with 30 IU of all-*rac*-α-tocopheryl acetate per kilogram body weight (about 400 IU/day). His plasma vitamin E concentration rose from 0.34 mg/dL (normal for infants) to 1.03 mg/dL. After 3 months of treatment, the same studies of his PMNs were performed. Although there were no changes in the activity of GSH peroxidase[172] or the concentration of GSH (which remained near 25% of normal during this study) in his plasma, the production of H_2O_2 by his PMNs had declined to normal levels, the iodination of proteins during phagocytosis had increased, and the bactericidal activity toward *Staphylococcus aureus* had increased to the control level. Before his vitamin E therapy, electron microscopy of his neutrophils had revealed defective cytoskeletal structure, with more than the usual number of *microtubules*[173] seen at rest and a disappearance of microtubules seen during phagocytosis. This ultrastructural defect was corrected after vitamin E treatment.

Case 2

A 23-year-old woman with a 10-year history of neurologic disease was admitted complaining of severe ataxia,[174] titubation of the head,[175] and loss of proprioceptive sense in her extremities.[176] Her history revealed that she had experienced difficulty in walking and was unsteady at age 10 years; there was no family history of ataxia, malabsorption, or neurologic disease. At 18 years of age, she had been hospitalized for her neurologic complaints; at that time, she had been below the fifth percentile for both height and weight. Her examination had revealed normal higher intellectual function, speech, and cranial nerve function; but her limbs had been found to be hypotonic[177] with preservation of strength and moderately severe ataxia. Her deep tendon reflexes were absent, plantar responses were abnormal, vibrational sense was absent below the wrists and iliac crests, and joint position sense was defective at the fingers and toes. Laboratory findings at that time had been negative, i.e., she showed no indications of hepatic or renal dysfunction. No etiologic diagnosis was made. Two years later, when she was 20 years old, the patient was reevaluated. By that time, her gait had deteriorated and her proprioceptive loss had become more severe.

Over the next 3 years, her symptoms worsened and, by age 23 years, she had trouble walking unassisted. Still, she showed no sensory, visual, bladder, respiratory, or cardiac signs and ate a normal and nutritious diet. Her only gastrointestinal complaint was of constipation, with bowel movements only once per week. Nerve conduction tests revealed that the action potentials of both her sensory and motor nerves, recorded from the median and ulnar nerves, were normal. Electromyography of the biceps, vastas medialis, and tibialis anterior muscles was normal. However, her cervical and cortical somatosensory-evoked responses to median nerve stimulation were abnormal; there was no peripheral delay and the nature of the response was abnormal. Furthermore, no consistent cortical responses could be recorded after stimulation of the tibial nerve at the ankle. These findings were interpreted as indicating

166. The condition of reduced alkali reserve.
167. Reduced number of erythrocytes per unit blood volume, resulting from their destruction.
168. This is the rate-limiting enzyme in the pathway of the biosynthesis of glutathione (GSH), a tripeptide of glycine, cysteine, and glutamic acid, and the most abundant cellular thiol compound. Oxidized glutathione (GSSG) is a dimer joined by a disulfide bridge between the cysteinyl residues.
169. This is the condition of abnormally high urinary concentrations of 5-ketoproline, the intermediate in the pathway of GSH biosynthesis (the γ-glutamyl cycle).
170. Inflammation of the middle ear.
171. The PMN is a type of white blood cell important in disease resistance, which functions by phagocytizing bacteria and other foreign particles.
172. An enzyme that catalyzes the reduction of hydroperoxides (including H_2O_2) with the concomitant oxidation of glutathione (2 mol of GSH converted to 1 mol of GSSG).
173. A subcellular organelle.
174. Loss of muscular coordination.
175. Unsteadiness.
176. Senses of position, etc., originating from the arms and legs.
177. Having abnormally low tension.

spinocerebellar disease characterized by delayed sensory conduction in the posterior columns.

Routine screening tests failed to detect α-tocopherol in her plasma, although she showed elevated circulating levels of cholesterol (448 mg/dL vs. normal: 150–240 mg/dL) and triglycerides (184 mg/dL vs. normal: 50–150 mg/dL). Her plasma concentrations of 25-hydroxyvitamin D_3 [25-(OH)-D_3], retinol, and vitamin K–dependent clotting factors were in the normal range. Tests of lipid malabsorption showed no abnormality. Her glucose tolerance and pancreatic function (assessed after injections of cholecystokinin and secretin) were also normal.

The patient was given 2 g of α-tocopheryl acetate with an ordinary meal; her plasma α-tocopherol level, which had been nondetectable before the dose, was in the subnormal range 2 h later and she showed a relatively flat absorption curve.[178] She was given the same large dose of the vitamin daily for 2 weeks, at which time her plasma α-tocopherol concentration was 24 μg/mL. When her daily dose was reduced to 800 mg of α-tocopheryl acetate per day for 10 weeks, her plasma level was 1.2 mg/dL, i.e., in the normal range. During this time, she showed marked clinical improvement.

Case Questions

1. What inborn metabolic error(s) was(were) apparent in the first patient?
2. What sign/symptom indicated a vitamin E–related disorder in each case?
3. Why are PMNs useful for studying protection from oxidative stress, as in the first case?
4. What inborn metabolic error might you suspect that led to vitamin E deficiency in the second patient?

13. STUDY QUESTIONS AND EXERCISES

1. Construct a concept map illustrating the nutritional interrelationships of vitamin E and other nutrients.
2. Construct a decision tree for the diagnosis of vitamin E deficiency in a human or animal.
3. What features of the chemical structure of vitamin E relate to its nutritional activity?
4. How might vitamin E utilization be affected by a diet high in polyunsaturated fat? Of a fat-free diet? Of a selenium-deficient diet?
5. Detail the role of vitamin E in maintaining oxidant (peroxide?) tone.
6. What kinds of prooxidants might you expect people or animals to encounter daily?
7. How can nutritional deficiencies of vitamin E and selenium be distinguished?

178. That is, the plot of plasma α-tocopherol concentration versus time.

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Chapter 9

Vitamin K

Chapter Outline

Anchoring Concepts

1. *Vitamin K* is the generic descriptor for 2-methyl-1,4-naphthoquinone and all its derivatives exhibiting qualitatively the antihemorrhagic activity of phylloquinone.
2. The K vitamers are side chain homologs; each is hydrophobic and, thus, insoluble in such aqueous environments as plasma, interstitial fluids, and cytoplasm.
3. The 1,4-naphthoquinone ring system of vitamin K renders it susceptible to metabolic reduction.
4. Deficiencies of vitamin K have a narrow clinical spectrum: hemorrhagic disorders.

…Then Almquist showed
A substance, phthiocol, from dread T.B.,
Would cure the chicks…
And so the microbes of tuberculosis,
That killed the poet Keats by hemorrhage,
Has yielded forth the clue to save the lives
Of infants bleeding shortly after birth.

T.H. Jukes[1]

1. Thomas H. Jukes (1906–1999) was a British born nutritional biochemist noted for his early work in elucidating the B-vitamins and later pioneering work in the field of molecular evolution. After holding a research position at the Lederle Laboratories of American Cyanamid Co., he spent a long and productive career on the faculty of the University of California at Berkeley where he was a respected and outspoken crusader for sound science in the field of nutrition. He wrote these lines on the occasion of Henrik Dam being awarded the Nobel Prize for elucidating vitamin K; obviously, Jukes, thought that his colleague H.J. Almquist should have shared the prize.

LEARNING OBJECTIVES

1. To understand the nature of the various sources of vitamin K
2. To understand the means of absorption and transport of the K vitamers
3. To understand the metabolic functions of vitamin K
4. To understand the physiological implications of impaired vitamin K status and/or function

VOCABULARY

Atherocalcin
γ-Carboxyglutamate
Chloro-K
Clotting time
Coagulopathy
Collagen
Coprophagy
Coumarins
Dicumarol
Dihydrovitamin K
Dysprothrombinemia
Enterotype
Extrinsic clotting system
Factor II
Factor VII
Factor IX
Factor X

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00009-5

Fibrin
Fibrinogen
Gas6
Gla
Hemorrhage
Hemorrhagic disease of the newborn
Heparin
Hydroxyvitamin K
Hypoprothrombinemia
Intrinsic clotting system
Matrix Gla protein (MGP)
Menadione
Menadione sodium bisulfite complex
Menadione pyridinol bisulfite (MPB)
Menaquinones (MKs)
2-Methyl-1,4-naphthoquinone
Microbiome
Naphthoquinone
Osteocalcin
Periostin
Phylloquinone
PIVKA (protein induced by vitamin K absence)
Protein C
Protein M
Protein S
Protein Z
Serine protease
Stuart factor
Sulfaquinoxaline
Thrombin (factor IIa)
Thromboplastin
Vitamin K deficiency bleeding (VKDB)
Vitamin K–dependent γ-glutamyl carboxylase (VKγGC)
Vitamin K epoxide
Vitamin K epoxide reductase (VKER)
Vitamin K hydroquinone
Vitamin K oxide
Vitamin K quinone
Vitamin K quinone reductase (VKQR)
Warfarin
Zymogen

1. THE SIGNIFICANCE OF VITAMIN K

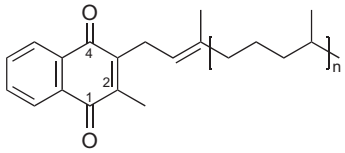
Vitamin K is synthesized by plants and bacteria, which use it for electron transport and energy production. Animals, however, cannot synthesize the vitamin; still, they require it for blood clotting, bone formation, and other essential functions. These needs are critical to good health; yet enteric microbial synthesis of the menaquinones (MK), including that occurring in the hindgut of humans and other animals (many of whom have **coprophagous**[2] eating habits), renders frank deficiencies of this vitamin rare. Asymptomatic low vitamin K status has been observed in as many as a third of Americans, and premature infants, born with low reserves of the vitamin, face risk of hemorrhagic disease. Vitamin K deficiency can also occur in poultry and other monogastric animals when they are raised on wire or slatted floors and treated with antibiotics that reduce their hindgut microbial synthesis of the vitamin.

The function of vitamin K in blood clotting was the first to be recognized and is widely exploited to reduce risks of thrombosis in cardiac and surgical patients. **Coumarin**-based drugs (e.g., **warfarin**, **dicumarol**)[3] and other inhibitors of the vitamin K oxidation/carboxylation/reduction cycle are valuable for this purpose. In addition, vitamin K has clear roles in the metabolism of both calcified and noncalcified tissues. Emerging evidence indicates that vitamin K functions with other vitamins in the regulation of intracellular Ca^{2+} metabolism, in signal transduction, and in cell proliferation, functions that have profound effects on health status.

2. PROPERTIES OF VITAMIN K

The term vitamin K describes **2-methyl-1,4-naphthoquinone** and its derivatives exhibiting the antihemorrhagic activity of phylloquinone. Naturally occurring forms of the vitamin consist of a side chain–substituted **naphthoquinone nucleus** (at C-3) and are characterized by the type and number of unsaturated isoprene units (not carbon atoms) that form the side chain. For these groups of vitamers, a numeric system is used to indicate side chain length – e.g., the abbreviations K-*n* for the phylloquinones and MK-*n* for the MKs, to indicate the number of isoprenoid units comprising the side chains (Table 9.1). There are three groups of K vitamers:

- **Phylloquinones** (**K-*n***) – forms with phytyl and further alkylated side chains consisting of several saturated isoprenoid units. Phylloquinones have only one double bond in their side chains, i.e., on the proximal isoprene unit. These vitamers are synthesized by green plants as a normal component of chloroplasts.



2. The term **coprophagy** describes the ingestion of excrement. This behavior is common in many species and exposes them to nutrients such as vitamin K produced by the microbial flora of their lower guts. Coprophagy can be easily prevented in some species (e.g., chicks) by housing with raised wire floors; it is very difficult to prevent in others (e.g., rats) without the use of such devices as tail cups.

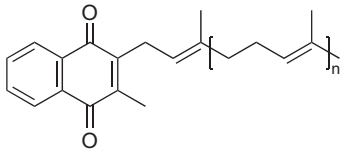
3. These 4-hydroxycoumarins have anticoagulant activities by inhibiting VKER.

TABLE 9.1 Systems of Vitamin K Nomenclature

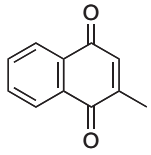
Chemical Name	Preferred System IUPAC[a]	Other Systems	
		IUNS[b]	Traditional
2-Methyl-3-phytyl-1,4-naphthoquinone (*n*)	Phylloquinone-*n* (K-*n*)	Phytylmenaquinone-*n* (PMQ-*n*)	$K_1(n)$
2-Methyl-3-multiprenyl-1,4-naphthoquinone (*n*)	Menaquinone-*n* (MK-*n*)	Prenylmenaquinone-*n* (MQ-*n*)	$K_2(n)$
2-Methyl-1,4-naphthoquinone	Menadione	MK	K_3

[a]*International Union of Pure and Applied Chemists.*
[b]*International Union of Nutritional Sciences.*

- Menaquinones (**MK-*n***) – forms with side chains consisting of variable numbers of isoprenoid units, each containing a double bond. MKs are synthesized only by bacteria (including those of the intestinal microbiota) and some spore-forming *Actinomyces* spp. The predominant vitamers are MK-*6* to MK-*10*; MKs with as many as 13 side chain isoprene units have been identified.



- **Menadione** – does not occur naturally, but is a common synthetic form, 2-methyl-1,4-naphthoquinone. It forms a water-soluble sodium bisulfite addition product, **menadione sodium bisulfite**, the practical utility of which is limited by its instability in complex matrices such as feeds. However, in the presence of excess sodium bisulfite, it crystallizes as a complex with an additional mole of sodium bisulfite (i.e., **menadione sodium bisulfite complex**), which has greater stability and, therefore, is used as a supplement to poultry and swine feeds. A third water-soluble compound is **menadione pyridinol bisulfite** (**MPB**), a salt formed by the addition of dimethylpyridinol.



Vitamin K Chemistry

Phylloquinone (K_1) is a yellow oil at room temperature, but the other K vitamers are yellow crystals. The K and MK vitamers and most forms of menadione are insoluble in water, slightly soluble in ethanol, and readily soluble in ether, chloroform, fats, and oils. The K vitamers are sensitive to light and alkali, but are relatively stable to heat and oxidizing environments. Their oxidation proceeds to produce the 2,3-epoxide form. Their reduction (e.g., with sodium hydrogen sulfite) produces the corresponding naphthohydroquinones, which can be reoxidized with mild oxidizing agents. The K vitamers show the characteristic UV spectra of the naphthoquinones, i.e., their oxidized forms having four strong absorption bands in the 240- to 270-nm range. The reduced (hydroquinone) forms show losses of the band near 270 nm and increases of the band around 245 nm. Extinction decreases with increasing side chain length.

Vitamin K Biopotency

The biopotencies of the K vitamers (Table 9.2) depend on both the nature and length of their isoprenoid side chains. In general, the MK-*n* tend to have greater biopotencies than the corresponding phylloquinone (MK-*n*) analogs, and members of each series with four or five isoprenoid side chains are the most biopotent. The reported biopotencies of the menadiones tend to be variable; this may be partly because of varying stabilities of the preparations tested, as well as to whether the vitamin K antagonist **sulfaquinoxaline** was used in the assay diet.

3. SOURCES OF VITAMIN K

Biosynthesis

Synthesis of MK by the gut **microbiome** contributes to vitamin K nutrition in most humans and other animals. Many bacteria in the large intestine can synthesize long-chain MKs.[4] These include the obligate anaerobes of the

4. MK confer essential roles in procaryotes: as electron carriers in respiratory electron transport chains; as antioxidants protecting cellular membranes from peroxidative degradation; and as facilitators of transmembrane movement of molecules.

TABLE 9.2 Relative Biopotencies of Vitamin K–Active Compounds

Compound	Biopotency[a] (%)
Phylloquinones (K-*n*)	
K-*1*[b]	5
K-*2*	10
K-*3*	30
K-*4*	100
K-*5*	80
K-*6*	50
Menaquinones (MK-*n*)	
MK-*2*[b]	15
MK-*3*	40
MK-*4*	100
MK-*5*	120
MK-*6*	100
MK-*7*	70
Forms of Menadione	
Menadione	40–150
Menadione sodium bisulfite complex	50–150
Menadione dimethylpyrimidinol bisulfite	100–160

[a]*Relative biopotency based on chick prothrombin/clotting time bioassays using K-4 as the standard.*
[b]*Indicates the number of side chain isoprenoid units (each containing five carbons).*

groups *Bacteroides*,[5] *Eubacterium*, *Propionibacterium*, and *Arachnia* and the facultative anaerobe *Escherichia coli*, which produces mostly MK-*10* and MK-*11* (Table 9.3). The contributions of the gut microbiome to the vitamin K nutriture of the host likely vary according to **enterotype**, i.e., the dominant taxonomic groups comprising that microbiome.[6] Studies of human fecal microbiota found that MK composition was determined mainly by the species composition of *Prevotella* spp. and *Bacteroides* spp.[7] Studies with the rat have shown that enteric production of long-chain MKs can be suppressed by high intakes of phylloquinone or MK-*4*.[8] A quantitative study of colonoscopy patients found total gut MK content to average 1.8 mg (range 0.3–5.1 mg, predominantly MK-*9* and MK-*10*),[9] an amount exceeding nutritional requirements by an order of magnitude. That MKs are absorbed from the large intestine is suggested by the fact that the liver normally contains significant amounts of those vitamers, that germ-free animals have greater dietary requirements for the vitamin than do animals with normal gut microbiomes (Table 9.4), and that prevention of coprophagy is necessary to produce vitamin K deficiency in animals. Humans seldom show signs of vitamin K deficiency; hypoprothrombinemia is rare *except* among patients given antibiotics. Species with short gastrointestinal tracts and very short intestinal transit times (e.g., about 8 h in the chick), less than the generation times of many bacteria, do not have well-colonized guts. Being thus unable to harbor vitamin K–producing bacteria, they depend on their diet as the source of vitamin K.

Dietary Sources

Data for the specific vitamin K contents of foods have been limited by available analytical methods, which have only recently been improved with the development of tandem liquid chromatography–mass spectrometry. Still, it is clear that many foods contribute to meeting vitamin K needs (Table 9.5). Phylloquinones comprise most of the vitamin K in diets, the richest sources being green leafy vegetables (e.g., spinach, kale, broccoli, Brussels sprouts), vegetable oils, and margarine.[10] MKs can comprise as much as a quarter of the vitamin K in many diets; those vitamers largely come from bacterially fermented foods (e.g., cheese, sauerkraut, natto[11]) (Table 9.6), which contain long-chain MKs (particularly MK-*8* and MK-*9*),[12] and poultry and pork products.[13] It is difficult to formulate a normal diet that does not provide some 100 µg of the vitamin per day.

5. The most quantitatively important groups are *Bacteroides* and *Bifidobacteria*, which collectively comprise more than half of the intestinal microfloral mass. *Bifidobacteria* do not produce MKs.
6. Three major human enterotypes have been described based on the species composition of the microbiome (Arumugam, M., Raes, J., Pelletier, E., et al., 2011. Nature 473, 174–180).
7. Karl, J.P., Fu, X., Wang, X., et al., 2015. Am. J. Clin. Nutr. 102, 84–93.
8. Koivu-Tikkanen, T.J., Schurgers, L.J., Thijssen, H.H.W., et al., 2000. Br. J. Nutr. 83, 185–190.
9. Conly, J.M., Stein, K., 1992. Am. J. Gastroenterol. 87, 311–316.
10. Hydrogenation of vegetable oils converts produces 2′,3′-dihydrophylloquinone from phylloquinone, which is less bioactive than the parent, being less well absorbed and not appearing to be metabolized to MK-*4*.
11. A traditional Japanese food made with soy beans fermented with *Bacillus subtilis* var. natto.
12. MK contents can vary because of differences in the MK-biosynthetic capacities of the strains of bacteria used. Various strains of lactic acid bacteria (*Lactococcus lactis* sp., *Leuconostoc lactis*) were found to vary by more than threefold in their capacity to produce long-chain MKs (Morishita, T., Tamura, N., Makino, T., et al., 1999. J. Dairy Sci. 82, 1897–1903).
13. These meats can contain MK-*4* to the extent that they are fed formula feeds in which menadione is used as a feed supplement.

TABLE 9.3 MKs Produced by Dominant Species of Enteric Bacteria

Organism	MK-*6*	MK-*7*	MK-*8*	MK-*9*	MK-*10*	MK-*11*	MK-*12*
Bacteroides fragilis		+	+	+	++	++	++
Bacteroides vulgatus		+	+	+	++	++	++
Eubacterium lentum	++						
Enterobacter sp.			++				
Enterococcus sp.	+	+	+	++			
Lactococcus lactis		+	++	++			
Leuconostoc lactis		+	++	++			
Veillonella sp.	+	++					

From Mathers, J.C., Fernandez, F., Hill, J.E., et al., 1990. Br. J. Nutr. 63, 639–652.

TABLE 9.4 Impaired Clotting in Germ-Free Rats

Treatment	Prothrombin Time (s)	Hepatic MK-*4* (ng/g)
Germ-Free		
Vitamin K deficient	∞	8.3 ± 2.3
+ MK-*4*	5.8 ± 1.4[a]	66.5 ± 25.9
+ Menadione	11.1 ± 2.5[a]	12.4 ± 2.0
Conventional		
Vitamin K deficient	12.7 ± 1.6	103.5 ± 44.9
+ MK-*4*	12.5 ± 2.0	207.6 ± 91.3
+ Mendione	12.6 ± 0.9	216.2 ± 86.5

[a]$p < .05$.
From Komai, M., Shirakawa, H., Kimura, S., 1987. Int. J. Vit. Nutr. Res. 58, 55–59.

Breast Milk

Breast milk is typically low in vitamin K, providing insufficient amounts of the vitamin to meet the vitamin K needs of infants (Table 9.7).[14] The extent to which such low levels may reflect low maternal intakes of vitamin K is not clear. Vitamin K levels in breast milk can be increased by vitamin K supplementation of the mother; a clinical study found a daily supplement of 5 mg phylloquinone to increase breast

14. In addition, vitamin K appears to be poorly transferred across the placenta. Accordingly, the American Academy of Pediatrics recommended in the early 1960s the administration of vitamin K intramuscularly (1 mg) or orally (1 mg weekly for 12 weeks.) at the time of birth to prevent hemorrhagic disease. This practice is now required by law in the United States and Canada. All commercial infant formulas are supplemented with vitamin K at levels in the range of 50–125 ng/mL.

TABLE 9.5 Vitamin K Contents of Foods

Food	Vitamin K (μg/100 g)
Vegetables	
Asparagus	51
Beans snap	48
Beets	0.2
Broccoli	141
Cabbage	109
Carrots	14
Cauliflower	14
Corn	0.4
Cucumbers	16
Kale	817
Tomatoes	2
Lettuce	103
Peas	30
Potatoes	0.3
Spinach	494
Sweet potatoes	2
Oils	
Canola	71
Corn	2
Olive	60
Peanut	0.7
Soybean	184

Continued

TABLE 9.5 Vitamin K Contents of Foods—cont'd

Food	Vitamin K (μg/100 g)
Fruits	
Apples	2[a]
Bananas	0.05
Cranberries	5
Oranges	0
Peaches	3
Strawberries	2
Meats	
Beef	1–3
Pork	0.1–0.2
Beef liver	3
Dairy Products and Eggs	
Milk, cow's	0.1–0.6
Eggs	0.3
Egg yolk	0.7
Grains	
Rice	0–0.2
Wheat bran	2
Wheat flour	2

[a]*~90% in the skin (peel).*
From USDA National Nutrient Database for Standard Reference, Release 28. http://www.nal.usda.gov/fnic/foodcomp/search/.

milk phylloquinone concentrations from 27 ± 12 ng/mL at 2 weeks of lactation to 59 ± 25 ng/mL at 26 weeks.[15]

Bioavailability

The bioavailability of vitamin K in most foods has not been determined. Studies have shown that >17% of the phylloquinone in boiled spinach or kale is absorbed by humans.[16] This may relate to its association with the thylakoid membrane in chloroplasts, as the free vitamer is well absorbed (~80%). This suggests that vitamin K may be poorly bioavailable from the foods thought to be the most important sources of the vitamin in most diets, green leafy vegetables. The relative biopotencies of K vitamers appear to depend on the nature of the side chain. Studies have found that phylloquinone concentrations in plasma peak at more than twice the levels of comparable oral doses of MK-*4* and MK-*9*, indicating better short-term bioavailability.[17] Studies using restoration of normal clotting in the vitamin K–deficient chick have shown that when administered orally, phylloquinone, MK-*3*, MK-*4*, and MK-*5* had greater activities than those with longer side chains. This effect reflected the relatively poor absorption of the long-chain vitamers. In contrast, long-chain MKs (especially MK-*9*) had the greatest activities

15. Greer, F.R., Marshall, S.P., Foley, A.L., et al., 1997. Pediatrics 99, 88–92.
16. Gijsbers, B.L.M.G., et al., 1996. Br. J. Nutr. 76, 223; Novotny, J.A., Kurilich, A.C., Britz, S.J., et al., 2010. Br. J. Nutr. 104, 858–862.
17. Schurgers, L.J., Vermeer, C., 2002. Biochim. Biophys. Acta 1570, 27–32.

TABLE 9.6 MKs Produced by Bacteria Used in Food Production

Organism	Food Application	MK-*5*	MK-*6*	MK-*7*	MK-*8*	MK-*9*	MK-*10*
Arthrobacter nicotianae	Cheese			+	++	+	
Bacillus subtilis var. *natto*	Natto			++	+		
Brevibacterium linens	Cheese				+		
Hafnia alvei	Cheese						
Lactococcus lactis ssp. *lactis*	Cheeses, sour cream, buttermilk	+		+	+	++	
Lactococcus lactis ssp. *cremoris*	Cheeses, sour cream, buttermilk			+	+	++	
Leuconostoc lactis	Cheese			+	+	++	+
Proprionobacterium shermanii	Cheese					+	
Staphylococcus xylosus	Sausage				+		
Staphylococcus equorum	Meat		+	++	+		

From Morishita, T., Tamura, N., Makino, T., et al., 1999. J. Dairy Sci. 82, 1897–1903; Walther, B., Karl, J.P., Booth, S.L., et al., 2013. Adv. Nutr. 4, 463–473.

TABLE 9.7 Vitamin K Contents of Human Milk

	Vitamin K (nM)
Colostrum (30–81 h)	7.52 ± 5.90[a]
Mature Milk	
1 month	6.98 ± 6.36
3 months	5.14 ± 4.52
6 months	5.76 ± 4.48

[a]*Mean ± SD.*
From Canfield, L.M., Hopkinson, J.M., Lima, A.F., et al., 1991. Am. J. Clin. Nutr. 53, 730–735.

TABLE 9.8 Vitamin K Transport in Humans

Fraction	Phylloquinones % Serum Total
Triglyceride-rich fraction	51.4 ± 17.0[a]
LDLs	25.2 ± 7.6
HDLs	23.3 ± 10.9

[a]*Mean ± SD.*
From Kohlmeier, M., Solomon, A., Saaupe, J., et al., 1996. J. Nutr. 126, 1192S–1196S.

when administered intracardially to the vitamin K–deficient rat. Of the synthetic forms, some studies have found MPB to be somewhat more effective in chick diets; however, each is generally regarded as of comparable biopotency to MK-*4*.

4. ABSORPTION OF VITAMIN K

Micellar Solubilization

The K vitamers are absorbed from the intestine into the lymphatic (in mammals) or portal (in birds, fishes, and reptiles) circulation by processes that first require that these hydrophobic substances be dispersed in the aqueous lumen of the gut via the formation of mixed micelles, in which they are dissolved. Vitamin K absorption, therefore, depends on normal pancreatic and biliary function, and the presence of some dietary fat. Therefore, conditions resulting in impaired luminal micelle formation (e.g., dietary mineral oil, pancreatic exocrine dysfunction, bile stasis) impair the enteric absorption of the vitamin. The mixtures of phylloquinones and MKs that diets typically contain appear to be absorbed with a wide range of efficiencies, e.g., 5–70%. K vitamers are absorbed across the brush border via noncarrier-mediated passive diffusion, the rates of which are affected by the micellar contents of lipids and bile salts. This occurs in both the distal part of the small intestine and the colon. Thus, noncoprophagous animals, including humans, appear to benefit from the bacterial synthesis of vitamin K in their lower guts by being able to absorb the vitamin at that location. The magnitude of this benefit remains the subject of debate.

5. TRANSPORT OF VITAMIN K

Lipoprotein Carriers

No specific carriers have been identified for any of the K vitamers. Instead, upon absorption into the enterocyte, vitamin K associates with nascent chylomicra, which transport the vitamin in the lymph ultimately to the liver. Vitamin K is rapidly taken up by the liver via an apolipoprotein E (apoE) receptor, which interacts with the apoE on the chylomicron surface. Phylloquinones and MK-*4* have relatively short half-lives in the plasma (c. 17 h); whereas, the longer chain MKs circulate in the plasma for much longer periods (up to 48 h) Ultimately, these vitamers are transferred to triglyceride-rich lipoproteins and, as they lose triglycerides, to low-density lipoproteins (LDLs) and high-density lipoproteins (HDLs) (Table 9.8). Studies have shown these transfers to occur at different rates for different K vitamers. In humans, radiolabeled MK-*4* and MK-*9* were both transferred from the triglyceride-rich fraction to HDLs, but MK-*9* first went to LDLs, which increased its residence time in the plasma.

Cellular Uptake

Uptake of vitamin K is thought to occur in the way that other lipids are taken up into hepatocytes, osteocytes, and other cells. This process involves vitamin K–bearing chylomicron remnants being bound to cell surface receptors of the LDL receptor family by a process referred to as secretion capture. This involves apoE,[18] which is acquired by chylomicron remnants from triglyceride-rich lipoproteins and HDLs, thus allowing it to act as a ligand to facilitate binding of that particle to high-affinity cell surface receptors. This binding leads to cellular internalization, which is facilitated by heparin sulfate proteoglycans. The rapid appearance of radiolabeled metabolites in the urine after an oral dose of labeled phylloquinone shows this to be a rapid process.

18. It has been suggested that apoE genotype may affect the cellular uptake of vitamin K. Experimental tests of that hypothesis have not yielded consistent results; some studies have found carriers of the *APOE4* allele to have the greatest (Yan, I., Shou, B., Nigidikar, S., et al., 2005. Br. J. Nutr. 94, 956–961) or lowest (Saupe, J., Shearer, M.J., Kohlmeier, M., 1993. Am. J. Clin. Nutr. 58, 204–208) circulating levels of phylloquinone.

TABLE 9.9 K Vitamers in Livers of Several Species

Vitamer	Human	Cow	Horse	Dog	Pig
Phylloquinone	+++	+	+++		+
MK-*4*					+
MK-*5*	+				
MK-*6*	+			+	
MK-*7*	+++			+	
MK-*8*	+			+++	+++
MK-*9*	+			+++	+++
MK-*10*	++	+++		+++	+++
MK-*11*	++	+++		+	
MK-*12*		+++		+	
MK-*13*				+	

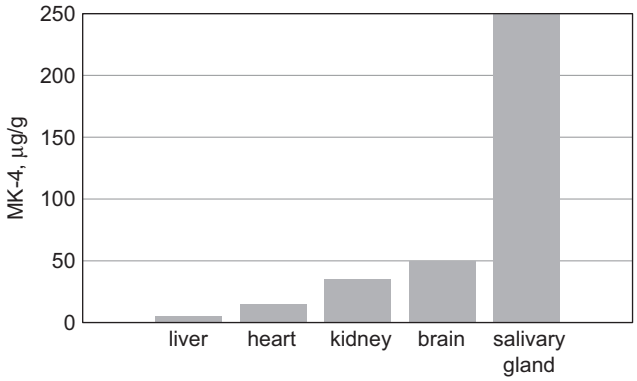


FIGURE 9.1 Detection of MK-*4* in tissues of gnotobiotic, vitamin K–deficient rats treated intraperitoneally with phylloquinone evidences conversion to MK-*4* in vivo. *After Davidson, R.T., Foley, A.L., Engelke, J.A., et al., 1998. J. Nutr. 128, 220–223.*

Tissue Distribution

The liver, the site of synthesis of the vitamin K–dependent coagulation proteins, is the primary storage organ for vitamin K. It rapidly takes up both phylloquinones and MKs, but not menadione, which is instead distributed in other tissues along with other vitamers (Table 9.9). Some 90% of the vitamin K in liver is comprised by MK-*10* and MK-*11*, with only a small portion (10%) as phylloquinones. Extrahepatic tissues of most animals ingesting plant materials contain phylloquinones and MKs with *6–13* isoprenoid units in their side chains. The vitamin is concentrated in several tissues such as the heart, pancreas, and adipose tissue.[19] In brain and kidney, MK-*4* levels typically exceed those of phylloquinone. Vitamin K is localized in cellular membranes (endoplasmic reticulum and mitochondria) where it is depleted only slowly under conditions of vitamin K deprivation. Transplacental movement of vitamin K is poor; it is frequently not detectable in the cord blood from mothers with normal plasma vitamin K levels. Accordingly, newborns are susceptible to hemorrhage.[20]

Animals fed phylloquinone show MK-*4* widely distributed in their tissues. Because MK-*4* is not a bacterial product, its presence in tissues evidences its production from phylloquinone, i.e., interconversion of the phytyl side chain to a geranylgeranyl side chain. Tissues can also contain longer–side chain MKs, even when the sole dietary form is MK-*4*. This raises the possibility of much of the vitamin in tissues being of enteric bacterial origin.

6. METABOLISM OF VITAMIN K

Side Chain Modification

Dealkylation–Alkylation. That tissues contain MK-*4* when the dietary source of vitamin K is phylloquinone evidences the dealkylation of the phylloquinone side chain. Studies have shown this to occur by first removing the side chain to produce menadione, which is re-alkylated to produce MK-*4*. The conversion appears greatest when phylloquinone is taken orally,[21] suggesting that it may involve the gut microbiome. However, this metabolism does not depend on the microbiome, as evidenced by the finding of MK-*4* in tissues of gnotobiotic animals given phylloquinone intraperitoneally (Fig. 9.1). The conversion has been demonstrated in several tissues of the rat in which MK-*4* levels exceed those of phylloquinone (e.g., brain, pancreas, salivary gland); it appears to be catalyzed by a homolog of a bacterial UbiA-prenyltransferase (UBIAD1), as small interfering RNA against the *UBIAD1* gene inhibited the conversion of phylloquinone to MK-*4* in human cells.[22] UBAID1 is also thought to catalyze the conversion of long-chain MKs to MK-*4*.

Menadione (either from dietary supplements or from microbial degradation of phylloquinone) can be alkylated by a process that uses geranyl pyrophosphate, farnesyl pyrophosphate, or geranylgeranyl pyrophosphate as the

19. Adults undergoing bariatric surgery were found to have phylloquinone levels of 148 ± 72 nmol/kg and 175 ± 112 nmol/kg in subcutaneous and visceral adipose tissue, respectively (Shea, M.K., Booth, S.L., Gundberg, C.M. et al., 2010. J. Nutr. 140, 1029).

20. Further, because human milk contains less vitamin K than cow's milk, infants who receive only their mother's milk are more susceptible to hemorrhage than are those who drink cow's milk.

21. Thijssen, H.H., Verrot, L.M., Schurgers, L.J., et al., 2006. Br. J. Nutr. 95, 260–266.

22. Nakagawa, K., Hirota, Y., Sawadaa, N., et al., 2010. Nature 468, 117–121.

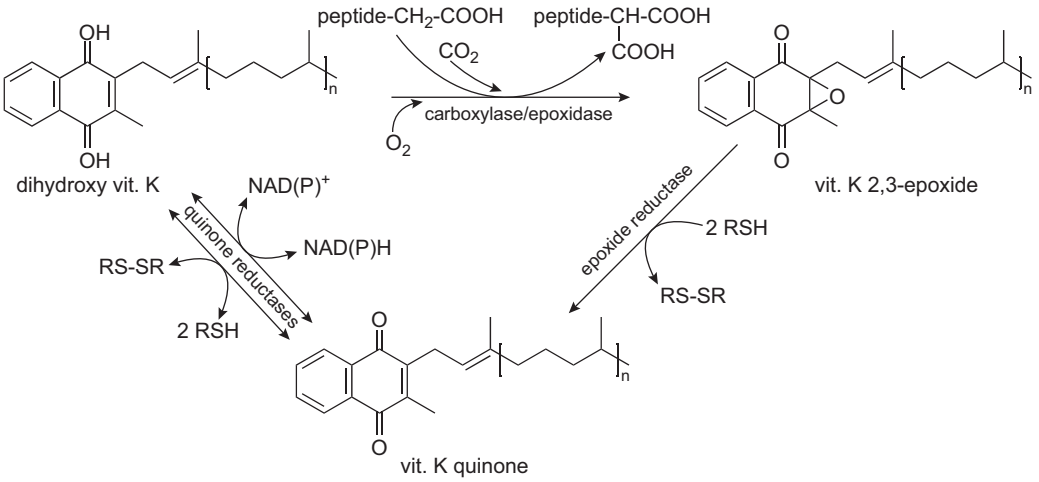


FIGURE 9.2 The vitamin K cycle.

alkyl donor and that can be inhibited by O_2 or warfarin. The main product of the alkylation of menadione is MK-*4*.

Redox Cycling

Vitamin K undergoes cyclic oxidation and reduction coupled to the carboxylation of peptidyl glutamyl residues to produce various functional γ-carboxylated proteins (Fig. 9.2). This redox cycling occurs in three steps:

1. **Vitamin K γ-glutamyl carboxylase (VKγGC)** catalyzes the oxidation of **dihydroxyvitamin K** (including phylloquinone, MK-*4*, and longer–side chain MKs) to the respective K 2,3-epoxide.[23] The enzyme is a 94-kDa protein located in the endoplasmic reticulum and Golgi apparatus. Vitamin K-2,3-epoxide comprises about 10% of the total vitamin K in the normal liver and can be the predominant form in the livers of rats treated with warfarin or other coumarin anticoagulants. Studies of the human, bovine, and rat VKγGC show high (88–94%) sequence homology, each with three γ-carboxyglutyl residues (Gla) per mole of enzyme.[24] Polymorphisms have been related to interindividual variation in sensitivity to warfarin anticoagulation and differences in the γ-carboxylation of osteocalcin.[25]
2. **Vitamin K epoxide reductase (VKER)** catalyzes the reduction of vitamin K-2,3-epoxides to their respective quinones. This is the rate-limiting step in the vitamin K cycle. VKER is an 18-kDa, dithiol-dependent, microsomal enzyme inhibited by coumarins. Genetic variation in the VKER subunit 1 has been shown to account for the variability observed in patient responses to warfarin therapy, to affect circulating levels of phylloquinones and undercarboxylated osteocalcin (ucOC),[26] and to be associated with cardiovascular risk.[27] Subjects with the VKER CG/GG (rs8050894) genotype show increased risk to progressive coronary artery calcification and poorer survival to those effects.[28] The chick has been noted for having relatively low hepatic VKER activity compared to the rat (Table 9.10); that this condition reduces its ability to recycle the vitamin is consistent with its greater hepatic levels of vitamin K-2,3-epoxide, and its greater dietary requirement for vitamin K than the rat.

TABLE 9.10 Species Differences in Vitamin K Metabolism

Enzyme	Substrate	V_{max} (μmoles/min/mg)	
		Chick	Rat
VKγGC	Phylloquinone	14 ± 2	26 ± 1
	MK-*4*	41 ± 3	40 ± 2
VKER	Phylloquinone	26 ± 2	280 ± 2
	MK-*4*	55 ± 7	430 ± 10

From Will, B.H., Usui, Y., Suttie, J.W., 1992. J. Nutr. 122, 2354–2360.

23. Also **vitamin K epoxide** or **vitamin K oxide**.
24. Berkner, K.L., Pudota, B.N., 1998. Proc. Natl. Acad. Sci. USA 95, 466–471.
25. Kinoshita, H., Nakagawa, K., Narusawa, K., 2007. Bone 40, 451–456.
26. Individuals with the minor allele of rs8050894 (G) had significantly higher plasma phylloquinone levels than those with the C allele; GG homozygotes have slightly lower levels of ucOC than other genotypes (Crosier, M.D., Peter, I., Booth, S.L., et al., 2009. J. Nutr. Sci. Vitaminol. 55, 112–119).
27. Individuals with the rs8050894TT genotype had 60% fewer atherothrombotic events than those with other genotypes (Suh, J.W., Baek, S.H., Park, J.S., et al., 2009. Am. Heart Assoc. J. 157, 908–912).
28. Holden, R.M., Booth, S.L., Tuttle, A., et al., 2014. Arterioscler. Thromb. Vasc. Biol. 34, 1591–1596.

3. **Reduction of vitamin K quinone** – This final reductive step leading to the production of the active hydroquinone, dihydroxyvitamin K, can be catalyzed in two ways:
   a. by **vitamin K quinone reductase (VKQR)**, a dithiol-dependent microsomal enzyme inhibited by the coumarin-type anticoagulants
   b. by **DT diaphorase**[29], a microsomal flavoprotein that uses NAD(P)H as a source of reducing equivalents to catalyze the 2-electron reduction of vitamin K quinone and other quinones. The enzyme also catalyzes the reduction of phylloquinones and MKs, but has highest affinity for menadione. In doing so, it protects against menadione cytotoxicity by competing with cytochrome P_{450}–catalyzed single-electron reductions that produce the semiquinone, which can redox cycle and produce ROS. It is relatively insensitive to coumarins, such that reduction of vitamin K quinone persists in anticoagulant-treated individuals.

Catabolism

Catabolism of K vitamers involves metabolism of the isophytyl side; there is no evidence of catabolism of the naphthoquinone ring. The total body pool of phylloquinone, c. 100 mg in an adult, turns over in about 1.5 days. It is thought to be catabolized by side chain removal by the same pathways used to degrade tocopherols: ω-hydroxylation followed by progressive chain shortening by way of β-oxidation. The initial ω-hydroxylation has been found to be catalyzed by a cytochrome *P*-450 isoform, CYP4F2, polymorphisms of which have been found to affect the efficacy of warfarin therapy.[30] The most abundant phylloquinone metabolite is its 2,3-epoxide, formed by the vitamin K–dependent γ-carboxylation of proteins. This metabolite and other phylloquinones and MKs undergo oxidative shortening of the side chain to 5- or 7-carbon carboxylic acids and a variety of other, more extensively degraded metabolites. A fifth of phylloquinone is ultimately excreted in the urine; however, the primary route of excretion of these metabolites is the feces, which contain mostly 7-C and 5-C aglycones and glucuronic acid conjugates excreted via the bile. Warfarin treatment greatly increases the excretion of phylloquinone metabolites in the urine while decreasing the amounts of metabolites in the feces. Little is known about MK metabolism, but it is likely that it also undergoes side chain degradation in the same manner as phylloquinone. MK catabolism, particularly that of the long-chain MKs, appears to be much slower than that of menadione, which is rapidly metabolized and excreted primarily in the urine (e.g., 70% of a physiological dose within 24 h) as the phosphate, sulfate, or glucuronide of menadiol and also in the bile as the glucuronide conjugate. A ring-altered metabolite has been identified: 3-hydroxy-2,3-dihydrophylloquinone, also called **hydroxyvitamin K** (Fig. 9.3).

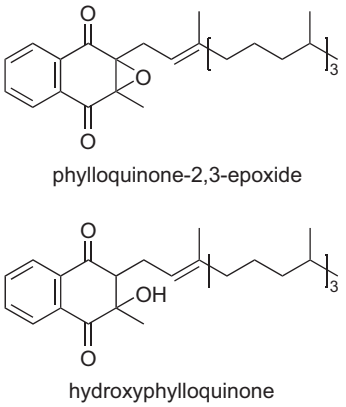


FIGURE 9.3 Ring-altered vitamin K metabolites.

Vitamin K Antagonists

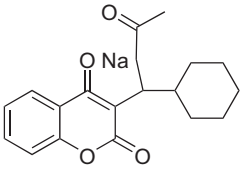
The coumarin anticoagulants were developed after 3,3′-methylbis-(4-hydroxycoumarin) was identified as the active principle in spoiled sweet clover responsible for the hemorrhages and prolonged clotting times of animals consuming that feedstuff. Compounds in this family block the thiol-dependent, redox recycling of the vitamin by inhibiting VKQR. This reduces carboxylation of the Gla protein precursors, including those involved in clotting.[31] Several substituted 4-hydroxycoumarins have been widely used in anticoagulant therapy in clinical medicine, and rodenticides (effective by causing fatal hemorrhaging). The most widely used has been warfarin (3-[a-acetonylbenzyl]-4-hydroxycoumarin)[32], an analog of the naturally occurring hemorrhagic factor dicumarol, and its sodium salt[33].

29. **NAD(P)H:(vitamin K quinone) oxidoreductase; also called** menadione reductase, phylloquinone reductase, and quinone reductase.

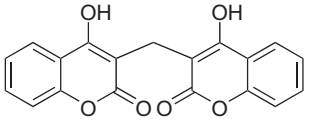
30. Carriers of the V433M allele (rs2108622) require higher warfarin doses. This appears to be related to their reduced hepatic capacity to oxidize phylloquinone, leaving them with relatively high hepatic concentrations of the vitamin (McDonald, M.G., Reider, M.J., Nakano, M., et al., 2009. Mol. Pharmacol. 75, 1337–1346).

31. This effect is different from that of another anticoagulant, heparin, a polysaccharide that complexes with thrombin in the plasma to enhance its inactivation.

32. This analog of the naturally occurring vitamin K antagonist, dicumarol, warfarin (4-hydroxy-3-[3-oxo-1-phenylbutyl]-2*H*-1-benzopyran-2-one) was synthesized by Link's group at the University of Wisconsin and named for the Wisconsin Alumni Research Foundation.



Warfarin



dicumarol

33. Others include ethyl biscoumacetate (3,3′-carboxymethylenebis-[4-hyroxycoumarin] ethyl ester and phenprocoumon (3-[1-phenylproyl]-4-hydroxycoumarin).

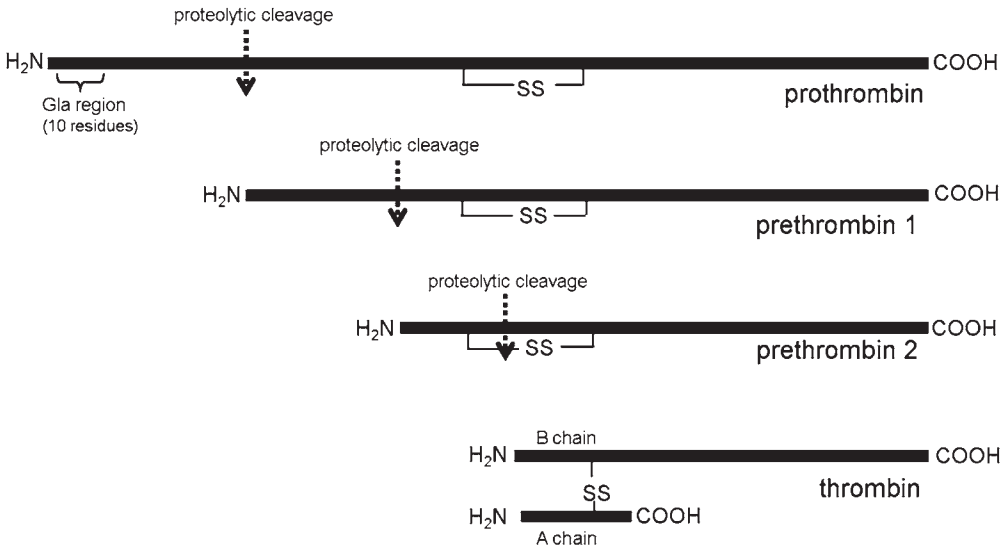


FIGURE 9.4 Thrombin is formed by the successive proteolytic removal of sequences from prothrombin.

Warfarin therapy has been important in preventing strokes; the drug is prescribed for a million patients each year in the United States alone. However, its effectiveness can vary among individuals by as much as 20-fold, and it is estimated that 12% of treated patients continue to experience major bleeding episodes.[34] Warfarin resistance is thought to involve polymorphisms in VKQR and the cytochrome *P*-450 isoform (CYP2C9) that metabolizes the drug. Warfarin-resistant rats have been found to respond to competitive inhibitors of one or more steps in the vitamin K redox cycle: 2-chloro-3-phytyl-1,4-naphthoquinone (chloro-K); 2,3,5,6-tetrachloro-pyridinol and 2-phylloquinone derivatives;[35] and other coumarins.[36]

7. METABOLIC FUNCTIONS OF VITAMIN K

Vitamin K–Dependent γ-Carboxylations

Vitamin K is the cofactor of a specific microsomal carboxylase that uses the energy of oxygenation of vitamin K hydroquinone to drive the γ-carboxylation of peptide-bound glutamic acid residues (Fig. 9.4). This **vitamin K–dependent γ-glutamyl carboxylase (VKγGC)** is a unique, integral membrane protein with no apparent homology with other proteins. It is found in the endoplasmic reticulum predominantly not only in the liver but also in several other organs.[37] In their reduced forms, K vitamers provide reducing equivalents for the reaction, undergoing oxidation to the 2,3-epoxide form. This is coupled to the cleavage of a C–H bond and formation of a carbanion at the γ-position of a peptide-bound glutamyl residue and followed by carboxylation. The VKγGC requires reduced forms of the vitamin (the hydroquinones), CO_2 as the carboxyl precursor, and molecular oxygen (O_2).[38] It is frequently referred to as vitamin K carboxylase/epoxidase to indicate the coupling of the γ-carboxylation step with the conversion of vitamin K to the 2,3-epoxide. Normally, this coupling is tight; full carboxylation of candidate proteins occurs through a progressive mechanism in which all their Glu sites are converted to Gla. However, under conditions of low CO_2 levels or in the absence of peptidyl-Glu, the epoxidation of the vitamin proceeds without concomitant carboxylation, yielding undercarboxylated proteins.

The vitamin K–dependent γ-carboxylation of specific glutamyl residues on the **zymogen**[39] precursors of each blood clotting factor occurs posttranslationally at the N-terminus of the nascent polypeptide. In the case of prothrombin, all 10 glutamyl residues in positions 7–33 (but none of the remaining 33 glutamyl residues) are γ-carboxylated. Carboxylation confers Ca^{2+}-binding capacity, facilitating the formation of Ca^{2+} bridges between the clotting factors

34. Anticoagulant efficacy, typically measured by prothrombin time, i.e., the time for a blood sample to clot after the addition of thromboplastin. As the latter reagents can vary in their sensitivity to clotting factors, such results are normalized to an international average.

35. For example, desmethylphylloquinone, 2-ethylphylloquinone, 2-fluromethylphylloquinone, 2-hydroxymethylphylloquinone, 2-methoxymethylphylloquinone, and 2-trifluromethylphylloquinone.

36. For example, difenacoum (3-[3-*p*-diphenyl-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxycoumarin); bromodifenacoum (3-[3-{4′-bormodiphenyl-4-yl}-1,2,3,4-tetrahydronaphth-1-yl]-4-hydroxycoumarin).

37. For example, lung, spleen, kidney, testes, bone, placenta, blood vessel wall, and skin.

38. The in vitro activity of the carboxylase is stimulated almost fourfold by pyridoxal phosphate when the substrate is a pentapeptide. It is doubtful whether that cofactor is important in vivo, as no stimulation was observed in the carboxylation of endogenous microsomal proteins.

39. The term "**zymogen**" was coined in 1875 by the German physiologist Rudolf Heidenhain to mean "producing ferment." It is used to indicate an inactive form of an enzyme, which must be converted biochemically to show catalytic activity, i.e., a proenzyme.

and phospholipids on membrane surfaces of blood platelets and endothelial and vascular cells, and between Gla residues (i.e., glutamyl residues that have been carboxylated) to form internal Gla–Gla linkages. Polymorphisms of the VKγGC have been associated with variations in circulating levels of ucOC.[22]

Vitamin K–Dependent Gla Proteins

Vitamin K functions in the posttranslational modification of at least 20 proteins via γ-carboxylation of 10–12 specific glutamate residues to produce **Gla** residues (Table 9.11).[40,41] The Gla proteins function by binding negatively charged phospholipids via Ca^{2+} held by their Gla residues. Each mammalian Gla protein contains a short, carboxylase recognition sequence (cleaved after carboxylation) that binds covalently to glutamate-containing propeptides to enhance catalysis. The VKγGC binds these propeptides with different affinities: compared to the most tightly bound factor X, most other propeptides are bound 2–10 times less tightly, whereas protein C and prothrombin are bound 100 times less tightly.[42] This suggests that competition for the VKγGC underlies variations in the amounts of Gla proteins produced, as has been observed in warfarin-treated cells. In the absence of vitamin K, these proteins can be secreted into the circulation in non- and undercarboxylated forms. These biologically inactive forms continue to be referred to by the name given when they were first recognized, **proteins induced by vitamin K deficiency**.

TABLE 9.11 Vitamin K–Dependent Gla Proteins

Clotting regulatory proteins	Prothrombin (factor II)
	Factor VII
	Factor IX
	Factor X
	Protein C
	Protein M
	Protein S
	Protein Z
Bone proteins	Osteocalcin
	Matrix Gla protein (MGP, periostin)
	Protein S
	Periostin
Transmembrane proteins	Proline-rich Gla protein 1 (PRGP1)
	Proline-rich Gla protein 2 (PRGP2)
	Transmembrane Gla protein 3
	Transmembrane Gla protein 4
Other Gla proteins	Gas6
	Gla-rich protein
	Transthyretin
	Atherocalcin
	PRGPs
	Renal Gla protein

Physiological Functions of the Gla Proteins

Blood clotting. Blood clotting is produced by a complex system of proteins that function to prevent hemorrhage and lead to thrombus formation. The system confers coagulation at the site of injury and curtails the process upon the formation of a clot. The process is initiated by injury to tissues through the release of collagen fibers and a cell surface protein (tissue factor), which interact with vitamin K–dependent Gla proteins in the plasma. These signals are amplified via the clotting pathway, ultimately, to form the clot.

The eight vitamin K–dependent plasma proteins comprising this system (Table 9.12) have homologous amino acid sequences in their first 40 positions, each containing 9–13 Gla residues in their amino-terminal domain.[43] All require Ca^{2+} for activity. Most circulate as a zymogen, i.e., an inactive precursor of the respective functional form, which is a serine protease. Each participates in a cascade of proteolytic activation of a series of factors ultimately leading to the conversion of a soluble protein, fibrinogen, to insoluble fibrin, which cross-links with platelets to form the clot (Figs. 9.4 and 9.5). The activation of proteases in this cascade involves the Ca^{2+}-mediated association of the active protein, its substrate, and another protein factor with a phospholipid surface.

40. This rare amino acid was discovered in studies of the molecular basis of abnormal clotting in vitamin K deficiency. Although it had been known that vitamin K deficiency and 4-hydroxycoumarin anticoagulant treatment each caused hypoprothrombinemia, studies in the early 1970s revealed the presence in each condition of a protein that was antigenically similar to prothrombin but did not bind Ca^{2+} and, therefore, was not functional. Studies of the prothrombin Ca^{2+}-binding sites revealed them to have Gla residues, which were replaced by glutamate residues in the abnormal prothrombin. Subsequently, Gla residues were found in each of the other vitamin K–dependent clotting factors and in several other Ca^{2+}-binding proteins in other tissues.

41. Gla-rich proteins have also been found in the venomous cone snail, some poisonous snakes, and some urochordates. These Gla-proteins serve as paralyzing neurotoxins used to subdue prey.

42. Huber, P., Schmitz, T., Griffen, J., et al., 1990. J. Biol. Chem. 265, 12,467–12,473.

43. Undercarboxylation of the clotting factors is rare, except in cases of patients on anticoagulant therapy.

TABLE 9.12 Characteristics of the Vitamin K–Dependent Plasma Proteins

Parameter	II[a]	VII[b]	IX[c]	X[d]	C	M	S	Z
Concentration (μg/mL)	100	1	3	20	10	<1	1	<1
Molecular mass (kDa)	72	46	55	55	57	50	69	55
Carbohydrate (%)	8	13	26	13	8	+	+	+
Number of chains	1	1	1	2	2	1	1	1
Number of Gla residues	10	10	12	12	11	+	10	13

[a]*Prothrombin.*
[b]*Proconvertin*
[c]*Plasma thromboplastin component, also: Christmas factor, antihemophilic factor B, platelet cofactor II, antiprothrombin II.*
[d]*Stuart factor.*

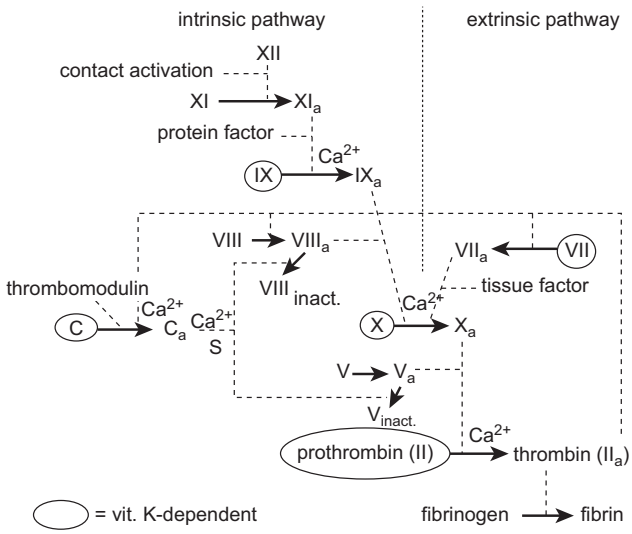


FIGURE 9.5 Roles of vitamin K–dependent proteins (circled factors) in blood clotting.

The key step in this system the activation of **factor X** (also, Stuart factor) by the proteolytic removal of a short polypeptide from the zymogen. This step is effected by two mechanisms:

- **intrinsic clotting system – factor IX** (also, Christmas factor or plasma thromboplastin component) is activated by **plasma thromboplastin** upon contact with a foreign surface.
- **extrinsic clotting system – factor VII**[44] is activated by **tissue thromboplastin** released as the result of tissue injury.

Once activated, factor X[45], after binding Ca^{2+} and phospholipid, can catalyze the activation of other coagulation factors:

- **prothrombin** (factor II) to its active form, **thrombin** (**factor IIa**), which catalyzes the proteolytic change in fibrinogen that renders it insoluble (as fibrin) for clot formation.
- **factor V** to its active form, factor Va; and
- **factor VIII** to its active form factor VIIIa.

Control of clotting is accomplished by the downregulation of thrombin production via thrombin binding to thrombomodulin, which complex activates protein C,[46] which, in turn, inactivates factors Va and VIIIa.

Two components of this system (Proteins S and Z) are not serine proteases. Protein S is found in the plasma both in free form and as a bimolecular complex with a regulatory component (C4b-binding protein) of the complement system. Individuals with inherited protein S deficiency have been reported to have recurrent thromboses. Protein Z is a cofactor for inhibition of activated factor X. Protein Z deficiency has been associated with a bleeding tendency in patients with factor V Leiden mutation.[47]

Bone Health. The presence of vitamin K–dependent Gla proteins in bone suggests a role of the vitamin in bone health. Several studies have shown individuals with

44. Also called **proconvertin**, factor VII is also activated by a high-fat meal and has been associated with increased risk to ischemic heart disease in some studies (e.g., Junker, R., Heinrich, J., Schulte, H., et al., 1997. Arterioscler. Thromb. Vasc. Biol. 1, 1539–1544).

45. Polymorphisms of factor X have been identified; however, these do not appear to affect circulating factor X levels.

46. Protein C also has anti-inflammatory and antiapoptotic activities involving activation of a protease-activated receptor (PAR1) and inhibition of the NF-κB pathway. Individuals with inherited protein C deficiency have elevated risk of thrombosis.

47. This condition involves a point mutation in the gene encoding coagulation factor V; it causes expression of a form of the factor resistant to activated protein C and results in a hypercoagulable state. The mutation occurs in 4–6% of the US population and is associated with increased risk to venous thromboembolism.

low circulating vitamin K levels or low vitamin K intakes to be at elevated risk to osteoporosis or fracture.[48] The Nurses' Health Study, a 10-year prospective study of more than 72,000 women, found the age-adjusted risk of hip fracture to be 30% less in women with vitamin K intakes >109 µg/day compared to those consuming greater amounts.[49] A similar relationship was observed in the Framingham Heart Study: subjects in the highest quartile of vitamin K intake (254 µg/day) had a significant reductions in hip fracture risk compared to those in the lowest quartile (56 µg/day).[50] Low vitamin K status has also been associated with articular cartilage and meniscus damage in men and women.[51] At least a dozen randomized clinical trials have been conducted to determine the efficacy of vitamin K supplementation in reducing bone mineral loss and/or fracture risk. Those have found MK-*4* to reduce fracture risk[52] and improve bone mineral density (Table 9.13).[53]

Such effects appear to be mediated by three vitamin K–dependent Gla proteins in bone:

- **Osteocalcin**[54] is the best characterized vitamin K–dependent protein of calcified tissues, although its function remains unclear. It shows no homology with the vitamin K–dependent plasma proteins, but has been strongly conserved between various species, consisting of a 5.7-kDa protein with three Gla residues in a 49–50 amino acid segment. It binds Ca^{2+} weakly and hydroxyapatite strongly; this serves to maintain its secondary structure while allowing it to bind mineralized bone matrix. It is the second most abundant protein[55] in the bone matrix, comprising ~2% of total bone protein and 10–20% of noncollagen protein. Osteocalcin is expressed relatively late in development, i.e., with the onset of bone mineralization. Its carboxylation is inhibited by warfarin treatment and is stimulated by MK supplementation and (in vitro) 1,25-$(OH)_2$-vitamin D_3. An estimated 20% of osteocalcin is not bound to bone and is free to enter the plasma.
- In most species, osteocalcin is fully carboxylated at each of its Gla sites. This is not the case in humans; analysis of human bone revealed each site to be 67%, 88%, and 93% carboxylated.[56] Similarly, much of the osteocalcin in human plasma is undercarboxylated – estimates approaching 50% have been made. Osteocalcin undercarboxylation may reflect low vitamin K intake; however, high intakes of vitamin K (10-fold the levels thought to be nutritionally required) are needed to support full carboxylation. Plasma levels of ucOC are subject to genetic variability associated with polymorphisms in both the vitamin K–dependent carboxylase and epoxide reductase. Greatest circulating ucOC levels are found in young children and in patients with Paget disease[57] and other disorders involving increased bone resorption/mineralization (Table 9.14). The degree of osteocalcin undercarboxylation has been found to be positively correlated with muscle strength and measures of bone health in older women, suggesting that carboxylated osteocalcin plays a role in muscular skeletal health.[58]
- It has been suggested that osteocalcin may function in the regulation of calcification, perhaps by acting as an attractant for osteoclast progenitor cells. Support for this hypothesis includes that association of plasma ucOC level with increased risk of hip fracture (Table 9.15) and

TABLE 9.13 Efficacy of Vitamin K Supplementation in Reducing Fracture Risk and Bone Mineral Loss in Older Adults

Study	Treatment	n	Fractures (%)	Bone Mineral Density Change (%)
Shiraki et al.[a]	MK-4[d]	86	14.3	−0.5
	Control	94	30.3	−3.3
Iwamoto et al.[b]	MK-4[d]	22	8.7	−0.1
	Control	20	25.0	−1.7
Ishida et al.[c]	MK-4[d]	63	14.3	−1.9
	Control	60	28.3	−3.3

[a]*Shiraki, M., Shiraki, Y., Aoki, C., et al., 2000. J. Bone Min. Res. 15, 515–521.*
[b]*Iwamoto, J. Takeda, T., Ichimura, S., 2001. J. Orthop. Sci. 6, 487–492.*
[c]*Ishida, Y., Kawai, S., 2004. Am. J. Med. 117, 549–555.*
[d]*45 mg/day.*

48. *See* review: Weber, P., 2001. Nutr. 17, 880–887.
49. Feskanich, D., Weber, P., Willett, W.C., et al., 1999. Am. J. Clin. Nutr. 69, 74–79.
50. Booth, S.L., Tuckcer, K.L., Chen, H., et al., 2000. Am. J. Clin. Nutr. 71, 1201–1208.
51. She, M.K., Kritchevsky, S.B., Hsu, F.C., et al., 2014. Osteoarth. Cartilage 23, 370–378.
52. A meta-analysis found seven studies to show an average risk reduction of 60% (Cockayne, S., Adamson, J., Lanham-New, S., et al., 2006. Arch. Intern. Med. 166, 1256–1261).
53. Iwamoto, J., Takeda, T., Sato, Y., 2006. Nutr. Rev. 64, 509–517.
54. Sometimes, "bone GLA protein."
55. Collagen is the most abundant protein in bone.
56. Dowd, T.L., Rosen, J.F., Li, L., et al., 2003. Biochemistry 42, 7769–7779.
57. Paget disease, also called osteitis, affects 3% of adults over 40 years of age. It involves dysfunctional bone remodeling, with bone continually breaking down and rebuilding at rates faster than normal. This results in bone being replaced with soft, porous, highly vascularized bone that can be weak and easily bent, leading to shortening of the affected part of the body, or with excess bone that can be painful and easily fractured. The disease most commonly affects the spine, pelvis, skull, femur, and tibiae.
58. Levinger, I., Scott, D., Nicholson, G.C., et al., 2014. Bone 64, 8–12.

TABLE 9.14 Plasma Osteocalcin Concentrations in Humans

	Osteocalcin (ng/mL)
Children	10–40[a]
Adult men and women <60 years	4–8
Women 60–69 years	7
Women 80–89 years	8
Patients with Paget disease	39
Patients with secondary hyperparathyroidism	47
Patients with osteopenia	9

[a]*Higher levels at 10–15 years.*
From Power, M.J., Fottrell, P.F., 1991. Crit. Rev. Clin. Lab. Sci. 28, 287–335.

TABLE 9.15 Total and Undercarboxylated Osteocalcin in Fracture and Nonfracture Patients[a]

Parameter	Nonfracture	Fracture
n	153	30
Age (years)	82.5 ± 5.9[b]	85.8 ± 6.5[b,c]
Body weight (kg)	56.6 ± 11.5	49.4 ± 10.7
Plasma Osteocalcin (ng/mL)		
Total	6.18 ± 3.34	7.90 ± 4.34[c]
Undercarboxylated	0.89 ± 0.89 (14)[b]	1.47 ± 1.65[c] (19)[b]
Carboxylated	5.29 ± 2.69 (86)[b]	6.43 ± 2.94[c] (81)[b]
25-OH-D_3 (ng/mL)	17.4 ± 14.1	15.9 ± 10.8
Parathyroid hormone (pg/mL)	47.0 ± 23.8	60.0 ± 40.9
Alkaline phosphatase (IU/L)	78 ± 37	92 ± 40[a]

[a]*Szulc, P., Chapuy, M.C., Meunier, P.J., et al., 1996. Bone 18, 487–488.*
[b]*Mean ± SD.*
[c]$p < .05$.

from studies in vitro that showed osteocalcin to inhibit the deposition of hydroxyapatite crystals reminiscent of the smaller crystals observed in bones of osteocalcin-deficient mice.[59] Studies have found phylloquinone supplementation to reduce plasma ucOC level, but effects on bone mineral density have been inconsistent. Relatively high intakes of vitamin K (up to 5 mg/day) appear to be required to support full carboxylation. Although osteocalcin undercarboxylation does not impair bone mineralization in animal models, osteocalcin deficiency (because of warfarin treatment or genetic ablation) increases bone mineralization in the rat. Therefore, it has been suggested that osteocalcin may function as a negative regulator of bone formation.[60]

- **Matrix Gla Protein (MGP)**[61] is a small (9.6 kDa), insoluble polypeptide structurally related to osteocalcin, with Ca-binding activity conferred by five Gla residues among its 79–amino acid sequence. It is expressed in many soft tissues, including vascular and smooth muscle cells, but accumulates only in calcified tissues. It has clear affinities for demineralized bone matrix and nonmineralized cartilage, where it is thought to function as an inhibitor in the regulation of calcification. MGP is posttranslationally modified by VKγCG to have five Gla residues and by a casein kinase which phosphorylates three of its serine residues; these groups are thought to participate in Ca-binding. Normally, MGP is thought to be fully carboxylated; however, it has been found incompletely carboxylated (and, thus, inactive) in patients undergoing hemodialysis, presumably because of their loss of vitamin K. Genetic ablation of MGP in the mouse led to early arterial calcification and fatal hemorrhages.[62] The mechanistic basis of this effect remains unclear.
- **Protein S** is synthesized by osteoblasts. It contains a thrombin-sensitive region, an epidermal growth factor–like domain, and a steroid hormone–binding domain. It has been shown to bind tyrosine kinase receptors.[63] A role in bone function was suggested by the finding of severe osteopenia, low bone mineral density, and vertebral compression fractures in two pediatric cases with very low protein S levels.
- **Periostin** is produced and secreted by bone-derived mesenchymal stromal cells and is abundant in mineralized bone nodules.[64] It is believed to function in the formation of the extracellular bone matrix.

Cardiovascular Health. Supplementation with phylloquinone has been found to improve the functional characteristics of the carotid artery,[65] reduce risk of coronary artery calcification,[66] and reduce risk of cardiovascular

59. Boskey, A.L., Gadaleta, S., Gundberg, C., et al., 1998. Bone 23, 187–196.

60. Price, P.A., 1988. Ann. Rev. Nutr. 8, 565–583; Ducy, P., Desbois, C., Boyce, B., et al., 1996. Nature 382, 448–452.
61. Sometimes referred to as **periostin**.
62. Luo, G., Ducy, P., McKee, M.D., et al., 1997. Nature 386, 78–81.
63. Nakamura, Y.S., Hakeda, Y., Takakura, N., et al., 1998. Stem Cells 16, 229–238.
64. Coutu, D.L., Wu, J.H., Monetter, A., et al., 2008. J. Biol. Chem. 283, 17,991–18,001.
65. Braam, L.A., Knapen, M.H., Geusens, P., et al., 2003. Calcif. Tissue Int. 73, 21–26.
66. Shea, M.K., Gundberg, C.M., Meigs, J.B., et al., 2009. Am. J. Clin. Nutr. 90, 1230–1235.

death.[67] Such effects may be mitigated by vitamin K–dependent Gla proteins that impair vascular intimal calcification:

- **MGP** is also expressed in vascular smooth muscle and appears to play a dominant role in maintaining the rate of arterial calcification as low as possible. Its impaired carboxylation by warfarin treatment has been shown to lead to arterial calcification in animal models. Its genetic deletion in the mouse led to the fragmentation and calcification of vascular smooth muscle, the loss of contractility, and death. Mutations in MGP are associated with calcification of cartilage in Keutel syndrome.[68] It is thought that both the Gla and phosphoserine groups of MGP participate in Ca-binding at internal nucleation sites within collagen and elastin fibrils and extracellular matrix components.
- **Atherocalcin** was discovered in calcified atherosclerotic tissue. It has been suggested that atherocalcin may inhibit VKγGC, which is found in the walls of arteries but not veins, and may be involved in the development of atherosclerosis.
- **Osteocalcin**, normally expressed only in bone, is upregulated in arterial calcification.
- **Gas6**[69] is a 75-kDa protein with 11–16 Gla residues. Similar to protein S, it contains an epidermal growth factor–like domain. Gas6 functions as a ligand for the reception tyrosine kinases Ax1 and Sky/Rse and protects cells from apoptosis by activating Ark phosphorylation and inducing MAP kinase[70] activity.
- **Protein S** has a 44% sequence homology with Gas6; but it also has thrombin-sensitive motifs. It has also been suggested as protecting arterial intimal cells from apoptosis.

Other Gla proteins have been identified, although their functions remain unknown:

- **Gas6** is widely distributed in nervous tissue where is thought to protect cells from apoptosis. It has also been shown to have neurotrophic activity toward hippocampal neurons and to promote growth and survival of several types of neural cells. That brain microsomes lack γ-carboxylase activity means that the posttranslational glutamation of Gas6 must occur in other tissues.
- **A Gla-rich protein** has been identified in cartilage.[71] It has more Gla residues (15–16) than other known Gla proteins. Its function remains unknown.
- **Proline-rich Gla proteins** are small (17–23 kDa), single-pass, transmembrane proteins expressed in a variety of extrahepatic tissues.
- **Transthyretin** binds retinol and thyroxine.
- **Renal Gla protein** is thought to have a role in the renal transport/excretion of Ca^{2+}.
- **Thrombin**, factor VII, and **factor Xa** have been shown to interact with tissue factor to activate protease-activated receptors (PARs) to promote platelet aggregation, activate protein C, and promote anti-inflammatory and antiapoptotic responses.
- **Other Gla proteins** have been reported in sperm, urine, hepatic mitochondria, and snake venom.

Prospective functions. Vitamin K may have a role in neurologic function. Warfarin has been shown to reduce brain sphingolipid contents and MK-*4*, which comprises virtually all (98%) the vitamin K in neural tissue, and is strongly correlated with the contents of sphingolipids, sulfatides, sphingomyelin, and gangliosides in neural tissues.[72] That vitamin K can have anti-inflammatory effect is suggested by the observations of phylloquinone reducing the inflammatory response to lipopolysaccharide and of MK-*4* reducing the in vitro production of prostaglandins and IL-6.

8. BIOMARKERS OF VITAMIN K STATUS

Useful biomarkers of vitamin K status include:

- **Plasma phylloquinone** concentration reflects recent dietary intake of that vitamer (Fig. 9.6). Levels are correlated with those of triglycerides and α-tocopherol and have been found to be related to polymorphisms in genes involved in lipoprotein and phylloquinone metabolism.[73] In healthy humans, circulating phylloquinone concentrations are in the range of 0.1–0.7 ng/mL.
- **Prothrombin time** is informative only to detect advanced, subclinical vitamin K deficiency. A 50% loss of plasma prothrombin level is required to affect prothrombin time.
- **Plasma ucOC**, because it is synthesized only by osteoblasts, has been used as a marker of bone formation. High circulating levels predict low bone mineral density and fracture risk and are frequently elevated among postmenopausal women.[74] Percentage undercarboxylation, but not total osteocalcin, can also indicate vitamin K status, as that parameter responds to increasing intakes of the vitamin over the nutritional range (Fig. 9.7).

67. Juanola-Falgarona, M.J., Salas-Salvadó, J., Martínez-González, M.A., et al., 2014. J. Nutr. 144, 743–750.
68. A rare autosomal recessive disorder characterized by cartilage calcification, peripheral pulmonary stenosis, nasal bridge depression, hearing loss, mild mental retardation, and shortened distal phalanges.
69. Named for its gene, Growth Arrest Specific gene 6.
70. Mitogen-activated protein kinase.
71. Viegas, C.S.B., Simes, D.C., Laize, M.K., et al., 2008. J. Biol. Chem. 283, 36,655–36,664.
72. Carrié, I., Portoukalian, J., Vicaretti, R., et al., 2004. J. Nutr. 134, 167–172.
73. Dashti, H.S., Shen, M.K., Smith, C.E., et al., 2014. Am. J. Clin. Nutr. 100, 1462–1469.
74. Affected individuals respond to vitamin K supplements with increased bone formation and decreased bone resorption.

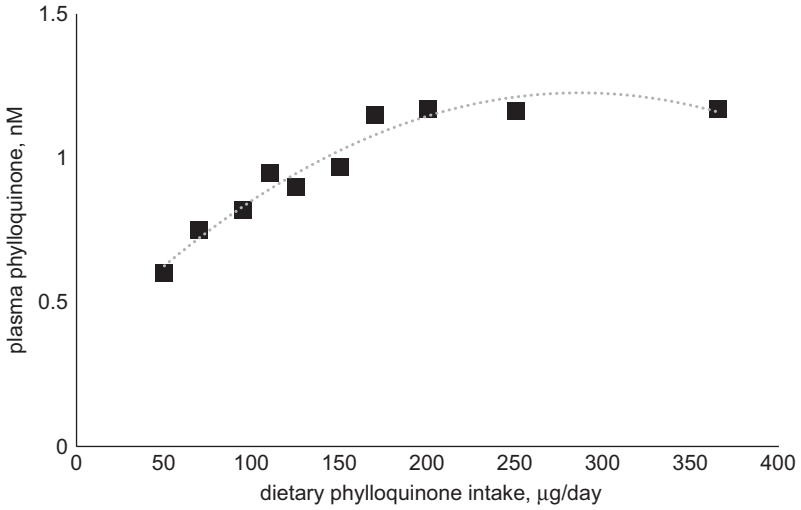


FIGURE 9.6 Relationship of plasma phylloquinone concentration to estimated dietary phylloquinone intake in healthy adults. *After McKeown, N.M., Jacques, P.F., Gundberg, C.M., et al., 2002. J. Nutr. 132, 1329–1334.*

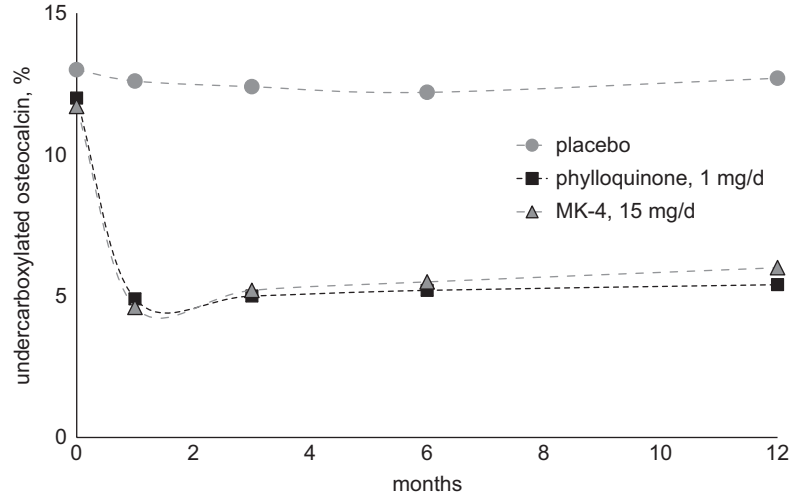


FIGURE 9.7 Effect of phylloquinone supplementation on undercarboxylation of plasma osteocalcin in humans. *After Binkley, N., Harke, J., Krueger, D., et al., 2008. J. Bone Miner. Res. 24, 983–991.*

9. VITAMIN K DEFICIENCY

Vitamin E deficiency can have primary (privational) and secondary (nonprivational) causes.

- **Primary causes** involve inadequate vitamin K supply
  - **Dietary patterns** that fail to provide vitamin K in adequate amounts, e.g., low amounts of green leafy vegetables, cheese, and other fermented foods.
- **Secondary causes** relate to impaired absorption, metabolism, or metabolic function of the vitamin
  - **Very low fat diets** do not support the development of intestinal luminal micelles upon which enteric vitamin K absorption is dependent.
  - **Lipid malabsorption** because of loss of pancreatic exocrine function (e.g., pancreatitis, pancreatic tumor, nutritional pancreatic atrophy in severe Se deficiency, *Ascarid* infection), lumenal deficiencies of bile (e.g., biliary stasis because of mycotoxicosis, biliary atresia), defects in lipoprotein metabolism (e.g., abetalipoproteinemia).
  - **Anticoagulant therapy** including treatment with warfarin, other 4-hydroxycoumarin anticoagulants, or large doses of salicylates, which inhibit the redox cycling of the vitamin.
  - **Insufficient intestinal microbiome** providing little or no vitamin K occurs in neonates and can be produced as a result of antibiotic therapy (with sulfonamides and broad-spectrum antibiotic drugs).
  - **Insufficient placental transport** of vitamin K giving neonates limited vitamin K reserves.

TABLE 9.16 General Signs of Vitamin K Deficiency

Organ System	Sign
General	Decreased growth
Dermatologic	Hemorrhage
Muscular	Hemorrhage
Gastrointestinal	Hemorrhage
Vascular	
Erythrocytes	Anemia
Platelets	Prolonged clotting time

Groups at Risk to Vitamin E Deficiency

Neonates.

Individuals with:

- very low fat intakes
- low intakes of plant oils and nuts
- lipid malabsorption syndromes
- dyslipidemias

Signs of Vitamin K Deficiency

The predominant clinical sign of vitamin K deficiency is **coagulopathy**, presenting as widespread subcutaneous and cerebral hemorrhage (Table 9.16), which can lead to a fatal anemia. The blood shows prolonged clotting time and **hypoprothrombinemia**. Because a 50% loss of plasma prothrombin level is required to affect prothrombin time, prolongation of the latter is a useful biomarker for advanced subclinical vitamin D deficiency.

Vitamin K deficiency can be prevented by consuming the recommended intakes of vitamin K established by expert bodies (Table 9.17).

Signs of Vitamin K Deficiency in Humans

Vitamin K deficiency presents as **hypoprothrombinemia** and prolonged **clotting time**. Coagulopathies now associated with vitamin K deficiency are not new; they were documented in the 19th century and are likely to have been historic problems. Low vitamin K status has also been associated with elevated risks to osteoporosis and fracture, damage to articular cartilage, and osteoarthritis.[75] These risks in adults are typically greater for older individuals, including those with chronic kidney disease.

75. Misra, D., Booth, S.L., Tolstykh, I., et al., 2013. Am. J. Med. 126, 243–248.

TABLE 9.17 Recommended Vitamin K Intakes

US		FAO/WHO	
Age, Sex	AI[a] (µg/day)	Age, Sex	RNI[b] (µg/day)
0–6 months	2	0–6 months	5
7–11 months	2.5	7–11 months	10
1–3 years	30	1–3 years	15
4–8 years	55	4–6	20
9–13 years	60	7–9 years	25
14–18 years	75	10–18 years, Female	35–55
>18 years, female	90	Male	35–65
Male	120	>18 years, female	55
Pregnancy, ≤18 years	75	Male	65
>18 years	90	Pregnancy	55
Lactation, ≤18 years	75	Lactation	55
>18 years	90		

[a]*Adequate Intakes values are given, as Recommended Dietary Allowances (RDAs) have not been established; Food and Nutrition Board, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. National Academy Press, Washington, DC, 773 pp.*

[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*

Newborns are at increased risk of vitamin K deficiency. The frequency of vitamin K–responsive hemorrhagic disease in 1-month-old infants is 1/4000 overall, but 1/1700 among breast-fed infants. Several factors contribute to their risk:

- **limited vitamin K reserves** because of poor placental transport of the vitamin. Their serum levels are typically about half those of their mothers.
- **no enteric microbial synthesis** of the vitamin, as their **intestines are sterile** for the first few days of life.
- **limited hepatic synthesis of the clotting factors** (Fig. 9.8), e.g., their **p**lasma prothrombin levels are typically a quarter those of their mothers.
- Breast milk is typically an inadequate source of vitamin K.

Some exclusively breast-fed infants not given vitamin K prophylaxis will develop **vitamin K deficiency bleeding (VKDB)**, also called **hemorrhagic disease of the**

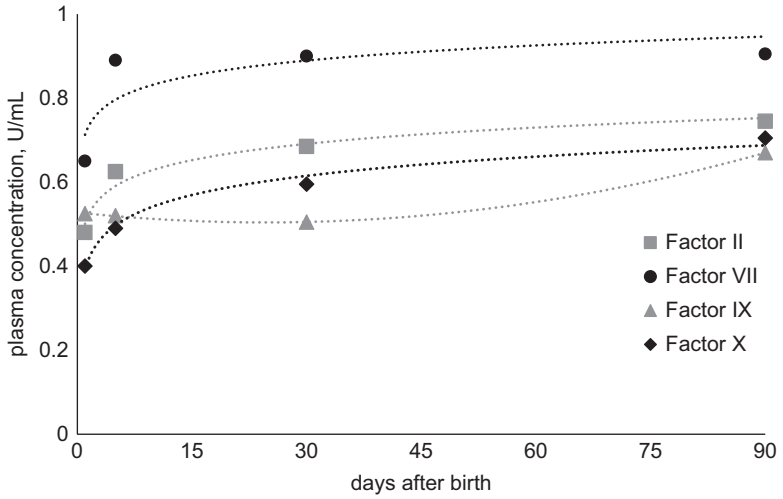


FIGURE 9.8 Developmental expression of vitamin K–dependent coagulation factors in infants. *After Zipursky, A., 1999. Br. J. Haematol. 104, 430–437.*

newborn.[76] This can present in different ways, depending on infant age:[77]

- newborns (first 24 h): cephalohematoma;[78] intracranial, intrathoracic, or intra-abdominal bleeding.
- newborns (first week): generalized ecchymoses;[79] bleeding from the gastrointestinal tract, umbilical cord stump, or circumcision site.
- infants (1–12 weeks): intracranial, skin, or gastrointestinal bleeding.

Infants with disorders involving lipid malabsorption (cystic fibrosis, biliary atresia, α_1-antitrypsin deficiency) can show potentially fatal signs within several weeks: intracranial hemorrhage with liver disease, bilirubinemia, and central nervous system damage. Hemorrhagic disease has also been reported for newborns of mothers on anticonvulsant therapy. It has become a common practice in many countries to treat all infants at birth with phylloquinone administered intramuscularly (0.5–1 mg) or orally (1 mg at birth followed by 50 μg/day for three months). This practice has greatly reduced the incidence of hemorrhagic disease of the newborn. Infants fed formula diets are at lower risk, as the amounts of vitamin K in infant formulas typically exceed those in human milk by as much as 50-fold.

Several congenital disorders of vitamin K–dependent proteins have been identified. Individuals with the VKER CG/GG genotype face increased risk to progressive coronary artery calcification and poorer survivals. Those with mutations in VKQR and VKγGC face **dysprothrombinemia** because of combined deficiencies of the coagulation factors. Genetic variants have also been identified for factor VII, and a congenital deficiency of protein C has been described. These conditions present as a range of spontaneous bleeding symptoms. In some cases, high-level vitamin K supplementation may provide effective management.

Signs of Vitamin K Deficiency in Animals

Monogastric species show hypothrombinemia when deprived of vitamin K. The clinical signs include prolonged clotting times, hemorrhages. Poultry are more likely than other species to show signs of vitamin K. This may be in part because of their hindgut microbial synthesis of MKs (because of their short gut and short transit time) and their susceptibility to intestinal coccidiosis for which sulfaquinoxaline is used. It is also likely because of their relatively low VKQR activities, which results in their inefficient recycling of the vitamin and gives them relatively high needs for dietary vitamin K. The use of sulfa drugs and antibiotics has also been associated clinical signs in young pigs, which are therefore typically given supplemental vitamin K.

Ruminants appear to obtain all their vitamin K needs from their rumen microbiota, which synthesize large amounts of the vitamin. Hypoprothrombinemia and spontaneous bleeding caused by vitamin K deficiency are seen only when they have been exposed to an antagonist such as dicumarol from molded clover in the condition called "spoiled sweet clover disease."

76. Without vitamin K prophylaxis, the risk of hemorrhage for healthy, nontraumatized infants in the first two weeks of life has been estimated to be 1–2/1000, and for older infants a third of that level.
77. VKDB differs from hemophilia by its earlier presentation (within a couple of days after birth) and absence of family history.
78. That is, subperiosteal bleeding.
79. Sheet hemorrhages of the skin, **ecchymoses**, differ from the smaller **petechiae** only in size.

10. VITAMIN K HEALTH AND DISEASE

Antibiotic Therapy

Hypoprothrombinemia has been associated with the use of antibiotics. The prevalence of hypoprothrombinemia increased in the 1980s with the introduction of the β-lactam antibiotics.[80] Although these drugs are administered intravenously, it is possible that they may affect enteric bacterial metabolism via biliary release. They do not alter fecal MKs in all patients, but they increase circulating vitamin K-2,3-epoxide levels in patients treated with vitamin K. The cephalosporin-type antibiotics have been found to inhibit the VKγGC to produce coumarin-like depressions of vitamin K–dependent clotting factors. Unlike the coumarins, the β-lactam antibiotics are weak anticoagulants; their effects are observed only in patients of low vitamin K status.

Anticoagulation Control

Low vitamin K status appears to contribute to unstable anticoagulation control in the use of warfarin in the management of thrombotic disorders. This affects as many as half of the patients and can be reversed by reducing warfarin dose and treating with phylloquinone.[81]

Anticarcinogenesis

That vitamin K status can play an anticarcinogenic role was suggested some six decades ago when MK-*4* treatment was found to increase the survival of patients with inoperable bronchial carcinoma. Since then it has been observed that patients with hepatocellular carcinoma typically have abnormally high circulating levels of under-γ-carboxylated prothrombin. Recently, a large prospective study found that cancer mortality was significantly less in individuals with the highest intakes of phylloquinone, although no effect were observed for MKs;[82] whereas, an earlier study found inverse association of cancer incidence and mortality for intake of MKs, but not phylloquinone.[83] In an 8-year randomized clinical trial, MK (45 mg/day) reduced the risk of hepatocellular carcinoma in 43 women with viral cirrhosis of the liver by 87% compared to controls.[84] Studies with animal models have shown all K vitamers capable of inhibiting tumor cell growth through several mechanisms:

- **Oxidative stress in malignant cells** is thought to be increased by menadione redox cycling.
- **Modulation of transcription factors** by phylloquinone and MKs. In cell culture, these vitamers have been shown to induce proto-oncogenes, increasing the levels of c-myc, c-jun, and c-fos; delaying the cell cycle; and enhancing apoptosis. Menadione can also induce protein tyrosine kinase activation and directly inhibit extracellular signal-regulated kinase protein tyrosine phosphatases. These effects are associated with reduced proliferation.
- **Cell cycle arrest** has been shown to be caused by menadione, which can inhibit cyclin-dependent kinases by binding to sulfhydryls at their active sites. This effect is associated with inhibition of malignant cell proliferation at the G1/S and S/G2 phases of the cell cycle. MKs have been found to affect cyclin function and also manifest as cell cycle inhibition.

Obesity Diabetes

Obesity has been associated with low vitamin K status. Adipose tissue stores vitamin K at relatively high levels.[85] Parameters of glucose metabolism have been inversely associated with serum concentrations of total, but not undercarboxylated, osteocalcin.[86] Phylloquinone levels have been found to vary inversely with percentage body fat in women (Fig. 9.9); higher levels were associated with greater insulin sensitivity and glycemic control as indicated by measures from oral glucose tolerance tests in men and women.[87] A 3-year intervention study found that phylloquinone supplementation reduced insulin resistance in men.[88] However, no significant benefits were found in women in that or another trial.[89]

11. VITAMIN K TOXICITY

No upper tolerable limits have been established for vitamin K. Phylloquinone exhibits no adverse effects when administered to animals in massive doses by any route, although it has been associated with increased risk of chronic kidney disease in humans.[90] The MKs are also thought to have negligible toxicity.

Menadione, however, can be toxic. At high doses - at least three orders of magnitude greater than those levels required for normal physiological function - it can produce

80. This group includes penicillin derivatives (penams), cephalosporins (cephams), monobactams, and carbapenems.
81. Baker, P., Gleghorn, A., Tripp, T., 2006. Br. J. Haematol. 133, 331–336.
82. Juanola-Falgarona, M.J., Salas-Salvadò, J., Martínez-González, M.A., et al., 2014. J. Nutr. 144, 743–750.
83. The European Prospective Investigation into Cancer and Nutrition (Nimptsch, K., Rohrmann, S., Linseisen, J. 2008. Am. J. Clin. Nutr. 87, 985–992).
84. Habu, D., Shiomi, S., Tamori, A., et al., 2004. JAMA 292, 358–361.
85. Adults undergoing bariatric surgery were found to have phylloquinone levels of 148 ± 72 nmol/kg and 175 ± 112 nmol/kg in subcutaneous and visceral adipose tissue, respectively (Shea, M.K., Booth, S.L., Gundberg, C.M., et al., 2010. J. Nutr. 140, 1029–1034).
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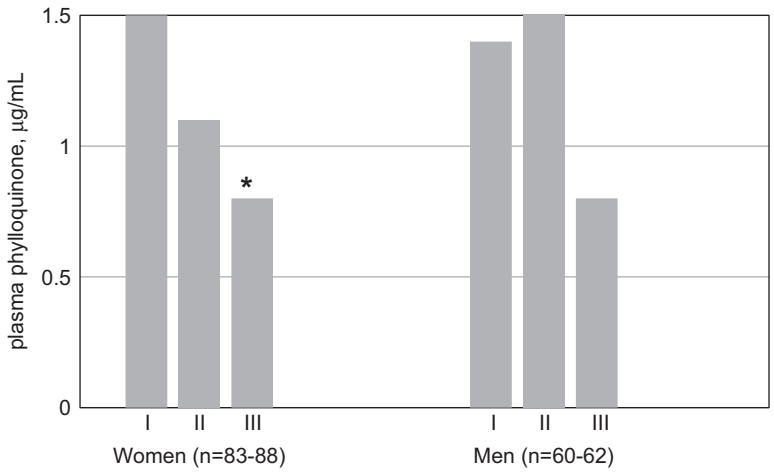

FIGURE 9.9 Relationship of vitamin K status and adiposity (by tertile of % body fat) in older adults (*$p < .05$). *After Shea, M.K., Booth, S.R., Gundberg, C.M., et al., 2020. J. Nutr. 140, 1029–1034.*

oxidative stress and manifests as hemolytic anemia, hyperbilirubinemia, and severe jaundice. This occurs as a result of its redox cycling to produce the superoxide radical anion. At high levels, it can also react with free sulfhydryl groups to deplete reduced glutathione (GSH) and reduce cellular antioxidant capacity.

A review of the US Food and Drug Administration database revealed 2236 adverse reactions reported for 1019 patients receiving intravenous vitamin K from 1968 to 1997.[91] Of those cases, 192 were anaphylactoid reactions and 24 were fatalities; those numbers were only 21 and 4, respectively, for patients given vitamin K doses <5 mg. Persistent, localized eczematous plaque has been reported at the site of injection for some patients given phylloquinone intramuscularly or subcutaneously[92].

12. CASE STUDIES

Instructions

Review the following case report, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

Case 1

A 60-year-old woman involved in an automobile accident sustained injuries to the head and compound fractures of both legs. She was admitted to the hospital, where she was treated for acute trauma. Her recovery was slow, and for the next 4 months she was drowsy and reluctant to eat. Her diet consisted mainly of orange and glucose drinks with a multivitamin supplement that contained no vitamin K. Her compound fractures became infected and she was treated with a combination of antibiotics (penicillin, gentamicin, tetracycline, and cotrimoxazole). She then developed intermittent diarrhea, which was treated with codeine phosphate. After a month, all antibiotics were stopped, and 46 days later (6 months after the injury), she experienced bleeding from her urethra. At that time other signs were also noted: bruising of the limbs, bleeding gums, and generalized *purpura*.[93] The clinical diagnosis was scurvy until it was learned that the patient was taking 25 mg of ascorbic acid per day via her daily vitamin supplement.

Laboratory Results

Parameter	Patient	Normal Range
Hb	9.0 g/dL	12–16 g/dL
Mean RBC volume	79 fL	80–100 fL
White cells	6.3×10^9/L	$5–10 \times 10^9$/L
Platelets	320×10^9/L	$150–300 \times 10^9$/L
Plasma iron	22 µg/dL	72–180 µg/dL
Total iron-binding capacity	123 µg/dL	246–375 µg/dL
Calcium	7.6 mg/dL	8.4–10.4 mg/dL
Inorganic phosphate	2.4 mg/dL	2.4–4.3 mg/dL
Folate	1.6 ng/mL	3–20 ng/mL
Vitamin B_{12}	110 ng/L	150–1000 ng/L
Prothrombin time	273 s	13 s (control)
Thrombin time	10 s	10 s (control)

When her abnormal prothrombin time was noted, specific coagulation assays were performed. These showed that the activity of each of the vitamin K–dependent factors (factors II, VII, IX, and X) was <1% of the normal level and that the activity of factor V was 76% of normal.

91. Fiore, L.D., Sccola, M.A., Cantillon, C.E., et al., 2001. J. Thromb. Thromboysis 11, 175–183.
92. Wilkins, K., DeKoven, J., Assaad, D., 2000. J. Cutan. Med. Surg. 4, 164–168.
93. Subcutaneous hemorrhages.

A xylose tolerance test (to measure small bowel absorption), performed with a single oral dose of 5 g of xylose, showed tolerance within normal limits. A stool culture showed normal fecal flora. The patient was then given phylloquinone (10 mg daily, administered intravenously, for 3 days) and showed a complete recovery of all coagulation factor activities to normal. She was given a high-protein/high-energy diet supplemented with $FeSO_4$ and, for a week, daily oral doses of 10 mg of phylloquinone. Her diarrhea subsided, her wounds healed, and she returned to normal health.

Case 2

A 55-year-old man with arteriosclerotic heart disease and type IV hyperlipoproteinemia was admitted to the hospital with a hemorrhagic syndrome. Six months earlier, he had suffered a myocardial infarction[94] complicated by pulmonary embolism[95] for which he was treated with heparin[96] followed by warfarin. Two months earlier, he had been admitted for a cardiac arrhythmia, at which time his physical examination was normal and chest radiograph showed no abnormalities, but his electrocardiogram showed first-degree atrioventricular block[97] with frequent premature ventricular contractions. At that time, he was taking 5 mg of warfarin per day.

Laboratory Findings 2 Months Before Third Admission[a]

Parameter	Patient	Normal
Prothrombin time	16.6 s	12.7 s
Plasma triglycerides	801 mg/dL	20–150 mg/dL
Serum cholesterol	324 mg/dL	150–250 mg/dL

[a]Blood count, blood urea nitrogen, blood bilirubin, and urinalysis were all normal.

He was treated with warfarin (5 mg/day), digoxin,[98] diphenylhydantoin,[99] furosemide,[100] potassium chloride,[101] and clofibrate.[102] Within a month, quinidine gluconate[103] was substituted for diphenylhydantoin because the patient showed persistent premature ventricular beats, but that drug was discontinued because of diarrhea and procainamide[34] was used instead. At that time, his prothrombin time was 31.5 s, and his warfarin dose was reduced first to half the original dose and then to one-quarter of that level.

At the time of the third admission, the patient appeared well nourished, but had ecchymoses on his arms, abdomen, and pubic area. He had been constipated with hematuria[104] for the preceding 2 days. His physical examination was unremarkable except for occasional premature beats, and his laboratory findings were similar to those observed on his previous admission, with the exception that his prothrombin time had increased to 36.6 s. In questioning the patient, it was learned that he had been taking orally as much as 1200 mg of all-*rac*-α-tocopheryl acetate each day for the preceding 2 months.

Both his warfarin and vitamin E treatments were discontinued, and 2 days later his prothrombin time had dropped to 24.9 s and his ecchymoses began to clear. The patient consented to participate in a clinical trial of vitamin E (800 mg of all-*rac*-α-tocopheryl acetate per day) in addition to the standard regimen of warfarin and clofibrate. The results were as follows:

Effect of Vitamin E on the Activities of the Patient's Coagulation Factors[a]

Activity	Initial Value	+Vit E, 6 weeks	−Vit E, 1 week	Normal Range
Factor II (prothrombin)	11	7	21	60–150
Factor VII[a]	27	16	23	50–150
Factor X[a]	15	10	—	50–150
Prothrombin time (sec)	20.7	29.2	22.3	11.0–12.5

[a]% mean of normal.

Case Questions

1. What signs indicated vitamin K–related problems in each case?
2. What factors probably contributed to the vitamin K deficiency of the patient in case one? Why was phylloquinone, rather than menadione, chosen for treatment of that patient?
3. What factors may have contributed to the coagulopathy of the patient in case two? What might be the basis of the effect of high levels of vitamin E seen in that case?

13. STUDY QUESTIONS AND EXERCISES

1. Construct a concept map to illustrate the ways in which vitamin K affects blood coagulation.
2. Construct a decision tree for the diagnosis of vitamin K deficiency in a human or animal.
3. What features of the chemical structure of vitamin K relate to its metabolic function?

94. Dysfunction because of necrotic changes resulting from an obstruction of a coronary artery.
95. Obstruction or occlusion of a blood vessel by a transported clot.
96. A highly sulfated mucopolysaccharide with specific anticoagulant properties.
97. Impairment of normal conduction between the atria and ventricles.
98. A cardiotonic.
99. A cardiac depressant (and anticonvulsant).
100. A diuretic.
101. That is, to correct for the loss of K^+ induced by the diuretic.
102. An antihyperlipoproteinemic.
103. A cardiac depressant (antiarrhythmic).
104. The presence of blood in the urine.

4. Discuss factors affecting the vitamin K requirement of humans, including infants.
5. What relevance to their vitamin K nutrition would you expect of the rearing of experimental animals in a germ-free environment or fed a fat-free diet?
6. How does the concept of a coenzyme relate to vitamin K?

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Chapter 10

Vitamin C

Chapter Outline

Anchoring Concepts

1. Vitamin C is the generic descriptor for all compounds exhibiting qualitatively the biological activity of ascorbic acid.
2. Vitamin C-active compounds are hydrophilic and have an oxidizable/reducible 2,3-enediol grouping.
3. Deficiencies of vitamin C are manifest as connective tissue lesions (e.g., capillary fragility, hemorrhage, muscular weakness).

I still had a gram or so of hexuronic acid. I gave it to [Svirbely] to test for vitaminic activity. I told him that I expected he would find it identical with vitamin C. I always had a strong hunch that this was so but never had tested it. I was not acquainted with animal tests in this field and the whole problem was, for me, too glamorous, and vitamins were, to my mind, theoretically uninteresting. "Vitamin" means that one has to eat it. What one has to eat is the first concern of the chef, not the scientist. Anyway, Svirbely tested hexuronic acid…after one month the result was evident: hexuronic acid was vitamin C.

Albert Szent-Györgyi[1]

LEARNING OBJECTIVES

1. To understand the nature of the various sources of vitamin C.
2. To understand the means of vitamin C synthesis by most species.
3. To understand the means of enteric absorption and transport of vitamin C.
4. To understand the functions of vitamin C in connective tissue metabolism, in drug and steroid metabolism, and in mineral utilization.
5. To understand the physiologic implications of low and high intakes of vitamin C.

VOCABULARY

Antioxidant
Ascorbate
Ascorbate–cytochrome b_5 reductase
Ascorbate phosphate
Ascorbate sulfate
Ascorbic acid
Ascorbyl free radical
Carnitine
Cholesterol 7α-hydroxylase
Collagen
Dehydroascorbic acid
Dehydroascorbic acid reductase
DNA oxidation
Dopamine β-monooxygenase
Ecchymoses
2,3-Enediol
Elastin
Erythorbic acid
Glucose transporters (GLUTs)
Glucuronic acid pathway
Guinea pig
L-gulonolactone oxidase

1. Albert Szent-Györgyi de Nagyrápolt (1893–1986) was a Hungarian physiologist credited with the discovery of vitamin C and elucidating the citric acid cycle in metabolism for which he received the 1937 Nobel Prize in physiology or medicine.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00010-1

Histamine
Homogentisate 1,2-dioxygenase
Hydroxylysine
4-Hydroxyphenylpyruvate
Hydroxyproline
Hypoascorbemia
Indian fruit bat
Insulin
Iron
Ischemia–reperfusion injury
Lipid peroxidation
Lordosis
Lysyl hydroxylase
Moeller–Barlow disease
Monodehydroascorbate
Monodehydroascorbate reductase
Nitric oxide
Oxalic acid
Oxaluria
Peptidylglycine α-amidating monooxygenase
Petechiae
Prolyl hydroxylases
Prooxidant
Protein oxidation
Rebound scurvy
Red-vented bulbul
L-saccharoascorbic acid
Semiascorbic acid
Scoliosis
Scurvy
Sodium-dependent vitamin C transporters (SVCTs)
Systemic conditioning
ε-N-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase
Tropoelastin
Tyrosine
Vitamin C

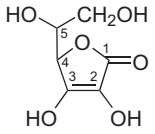
1. THE SIGNIFICANCE OF VITAMIN C

Vitamin C is a dietary essential for only a few species, which, by virtue of a single enzyme deficiency, cannot synthesize it. For most species, **ascorbic acid** is a normal metabolite of glucose, not an essential dietary constituent. Whether it is obtained from exogenous sources biosynthesized by the host, ascorbic acid is important for several physiological functions. Many, if not all, of these functions involve its redox characteristics, such that ascorbic acid, the major water-soluble antioxidant in plasma and tissues, functions with tocopherols, reduced glutathione, and other factors in the antioxidant protection of cells and is thought to support the redox recycling of α-tocopherol and promote the utilization of dietary nonheme iron. It also supports the maintenance of enzyme-bound metals in oxidation states appropriate for their enzymatic functions in the biosynthesis of collagen, carnitine, and nonepinephrine. Compromise of these functions underlies the pathophysiology of vitamin C deficiency. Hypovitaminosis C affects some 5–15% of people worldwide; estimated prevalence in some industrialized countries has been higher.[2] Other beneficial health effects of ascorbic acid have been reported: reductions in hypertension, atherogenesis, diabetic complications, colds and other infections, and carcinogenesis. Although some of these claims have become widely accepted, the empirical evidence remains incomplete for many.

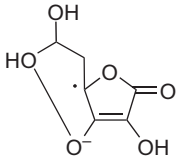
2. PROPERTIES OF VITAMIN C

The term **vitamin C** describes all compounds exhibiting the biological activity of **ascorbic acid** (2,3-didehydro-l-threo-hexano-1,4-lactone; also L-**ascorbic acid**).[3] The vitamin also occurs in the oxidized form, L**-dehydroascorbic acid** or **dehydroascorbic acid**. Biological activity depends on this 6-carbon lactone having a ***2,3*-enediol** structure.

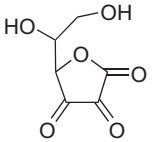
Chemical structure of vitamin C:



Ascorbic acid



Semidehydroascorbic acid



Dehydroascorbic acid

Vitamin C Chemistry

Ascorbic acid is a dibasic acid (pK_a values,[4] 4.1 and 11.8) because both enolic hydroxyl groups can dissociate. It

2. Hampl, J.S., Taylor, C.A., Johnston, C.S., 2004. Am. J. Pub. Health 94, 870–875; Mosdol, A., Erens, B., Brunener, E.J., 2008. J. Pub. Health 30, 456–460; Cahill, L., Corey, P.N., El-Sohemy, A., 2009. Am. J. Epidemiol. 170, 464–471.

3. Formerly, **hexuronic acid**.

4. The quantitative strength of an acid in solution is expressed in terms of its dissociation constant, K_a. The dissociation behavior of an acid is described: $HA + H_2O \leftrightarrow A^- + H_3O^+$, and $K_a = ([A^-]\,[H_3O^+])/([HA]\,[H_2O])$, or its log value, $pK_a = -\log 10\ K_a$.

forms salts, the most important of which are the sodium and calcium salts, the aqueous solutions of which are strongly acidic. A strong reducing agent, ascorbic acid is oxidized under mild conditions to dehydroascorbic acid via the radical intermediate **semidehydroascorbic acid** (also, **monodehydroascorbic acid**). The semiquinoid ascorbic acid radical is a strong acid (pK_a –0.45); after the loss of a proton, it becomes a radical anion that, owing to resonance stabilization, is relatively inert but disproportionates to ascorbic acid and dehydroascorbic acid. Thus, the three forms (ascorbic acid, semidehydroascorbic acid, and dehydroascorbic acid) compose a reversible redox system. Thus, it is an effective quencher of free radicals such as singlet oxygen ($^1O_2$). It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron (and other metals analogously), and the superoxide radical (O_2^-) to H_2O_2 and is oxidized to monodehydroascorbic acid in the process. Ascorbic acid complexes with disulfides (e.g., oxidized glutathione, cystine) but does not reduce those disulfide bonds. At physiological pH, ascorbic acid exists primarily as the ascorbate monoanion, while its reduced form, dehydroascorbic acid, is not ionized.

Dehydroascorbic acid is not ionized in environments of weakly acidic or neutral pH; therefore, it is relatively hydrophobic and is better able to penetrate membranes than is ascorbic acid. In aqueous solution, dehydroascorbic acid is unstable and is degraded by hydrolytic ring opening to yield 2,3-dioxo-L-gulonic acid. Dehydroascorbic acid reacts with several amino acids to form brown colored products, a reaction contributing to the spoilage of food.

TABLE 10.1 Relative Biopotencies of Vitamin C-Active Substances

Compound	Relative Biopotency (%)
Ascorbic acid	100
Ascorbyl-5,6-diacetate	100
Ascorbyl-6-palmitate	100
6-Deoxy-6-chloro-L-ascorbic acid	70–98
Dehydroascorbic acid	80
6-Deoxyascorbic acid	33
Ascorbic acid 2-sulfate	±[a]
Isoascorbic acid	5
L-glucoascorbic acid	3

[a]*This form is active in fishes, which have an intestinal sulfohydrase that liberates ascorbic acid; it is inactive in guinea pigs, rhesus monkeys, and humans, which lack that enzyme.*

Vitamin C Biopotency

Several synthetic analogues of ascorbic acid have been made. Some (e.g., 6-deoxy-L-ascorbic acid) have biological activity, whereas others (e.g., D-isoascorbic acid and L-glucoascorbic acid) have little or no activity. Several esters of ascorbic acid are converted to the vitamin in vivo and thus have good biological activity (e.g., ascorbyl-5,6-diacetate, ascorbyl-6-palmitate, 6-deoxy-6-chloro-L-ascorbic acid; see Table 10.1). Esters of the C-2 position show variable vitamin C activity among different species.

3. SOURCES OF VITAMIN C

Biosynthesis of Ascorbic Acid

Most higher animals (and probably all green plants) can synthesize vitamin C. They make it from glucose via the **glucuronic acid pathway** (Fig. 10.1, Table 10.2). The enzymes of this pathway are localized in the kidneys of amphibians, reptiles, egg-laying mammals, and the more primitive orders of birds; in both the kidneys and livers of many marsupials; but only in the livers of passerine birds and other mammals. The transfer of ascorbic acid synthesis from the kidney to the larger liver has been interpreted as an evolutionary

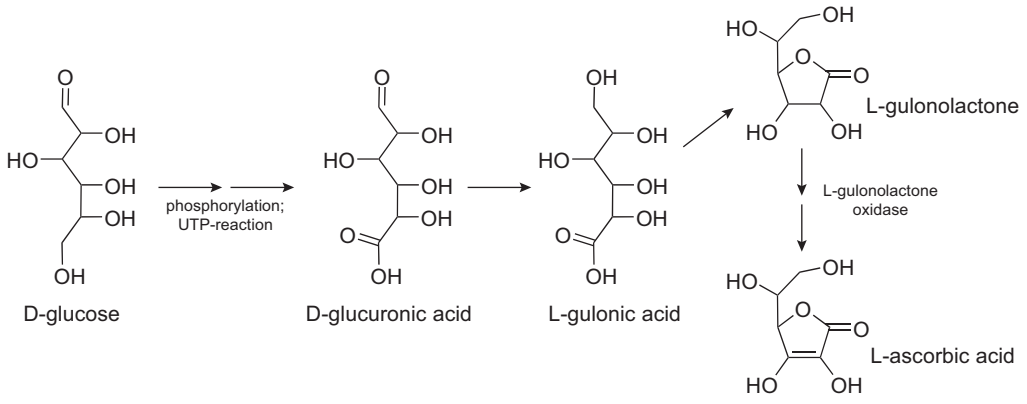


FIGURE 10.1 Biosynthesis of ascorbic acid.

TABLE 10.2 Estimated Rates of Ascorbic Acid Biosynthesis in Several Species

Species	Synthetic Rate, mg/kg BW	$T_{1/2}$,[a] days	Turnover, %/day
Mouse	125	1.4	50
Golden hamster	20	2.7	26
Rat	25	2.6	26
Rabbit	5	3.9	18
Guinea pig	0	3.8	18
Human	0	10–20	3

[a]*half-life in the body.*

adaptation that provided increased synthetic capacity to meet the increased needs associated with homeothermy. The biosynthesis of ascorbic acid is coupled to glycogenolysis. It can be stimulated by xenobiotic compounds including drugs (e.g., barbiturates, aminopyrine, antipyrine, chlorobutanol) and carcinogens (e.g., 3-methylcholanthrene, benzo-α-pyrene) due to induction of the glucuronic acid pathway, which is needed for xenobiotic detoxification by conjugation.[5] It can be inhibited by deficiencies of vitamins A or E, or biotin. Some species may not express this key enzyme early in development; the fetal rat, for example, is incapable of ascorbic acid biosynthesis until the 16th day of gestation. There is no evidence that ascorbic acid can be synthesized by the gut microbiome of any species.

Evolutionary loss of ascorbic acid biosynthetic capacity appears to have occurred in invertebrates, teleost fishes,[6] several species of birds (e.g., **red-vented bulbul**[7]), and some mammals (humans, other primates,[8] **guinea pigs**, most bats,[9] and a few mutant strains of rats[10]). These species do not express the last enzyme in the biosynthetic pathway, **L-gulonolactone oxidase**.[11] That microsomal flavoenzyme catalyzes the oxidation of L-gulonolactone[12] to L-2-ketogulonolactone, which, in turn, yields L-ascorbic acid by spontaneous isomerization. While all species studied have the gene, in some it is so highly mutated that it yields no gene product.[13] The loss of this single enzyme renders ascorbic acid, an otherwise normal metabolite, a vitamin. Therefore, **scurvy** can correctly be considered a congenital metabolic disease, **hypoascorbemia**.

Distribution in Foods

Vitamin C is widely distributed in both plants and animals, occurring mostly (80–90%) as **ascorbic acid** but also as **dehydroascorbic acid**. The proportions of both species tends to vary with food storage time, due to the time-dependent oxidation of ascorbic acid. Fruits, vegetables,[14] and organ meats (e.g., liver and kidney) are generally the best sources; only small amounts are found in muscle meats (Table 10.3). Plants synthesize L-ascorbic acid from carbohydrates; most seeds do not contain ascorbic acid but start to synthesize it on sprouting. Some plants accumulate high levels of the vitamin (e.g., fresh tea leaves, some berries, guava, rose hips). For practical reasons, citrus and other fruits are good daily sources of vitamin C, as they are generally eaten raw and are, therefore, not subjected to cooking procedures that can destroy vitamin C. Ascorbic acid is frequently added at low levels to processed foods to enhance shelf-life or preserve flavor. The analogue, **erythorbic acid**,[15] is also used as a food preservative. While it has no vitamin C activity, it can yield false positives in some analyses for plasma ascorbic acid.[16]

Stability in Foods

The vitamin C contents of most foods decrease dramatically during storage owing to the aggregate effects of several processes by which the vitamin can be destroyed (Table 10.4). Ascorbic acid is susceptible to oxidation to dehydroascorbic acid, which itself can be rapidly and irreversibly degraded at neutral pH by irreversible hydrolytic opening of the lactone ring to yield 2,3-diketogulonic acid. These reactions occur in the presence of O_2, even traces of metal ions, and

5. Because ascorbic acid synthesis and excretion are increased by exposure to xenobiotic inducers of hepatic, cytochrome *P*450-dependent, mixed-function oxidases (MFOs), it has been suggested that the urinary ascorbic acid concentration may be useful as a noninvasive screening parameter of MFO status.

6. Although most fish appear to be able to synthesize ascorbic acid, only carp (*Cyprinidae*) and Australian lungfish (*Neoceratodus forsteri*) appear to be able to do so at rates sufficient to meet their physiologic needs.

7. The bulbuls (*Pycnonotidae*) comprise 13 genera and 109 species distributed in Africa, Madagascar, and southern Asia. While the red-vented bulbul is often cited as being unable to biosynthesize ascorbic acid, it is not known how widely distributed in this family and the class *Aves* is the dietary need for vitamin C.

8. Gorilla, orangutan, gibbon, macaque, marmoset, owl monkey.

9. Including the **Indian fruit bat**, *Pteropus giganteus*, also known as the "flying fox."

10. A gulonolactone null mouse has been developed; and the osteogenic disorder Shionogi rat (ODS-*od*/*od*) derived from the Wistar strain has a dysfunctional form of that enzyme.

11. Whether loss of this enzyme may underlie inabilities of other species to synthesize ascorbic acid is still speculative.

12. Replacement (by injection) of this substrate prevents scurvy in guinea pigs.

13. This may be due to the presence of retrovirus-like sequences, identified in the human gene, that may have caused its activation. It has been suggested that mutations in this gene may have been driven by disadvantageous effects of H_2O_2 generated during the oxidation of gulono-1,4-lactone.

14. Historically, the potato was the best source of vitamin C in North America and Europe.

15. Also referred to as D-isoascorbic acid or D-araboascorbic acid.

16. This is not a problem for blood sampled after an overnight fast, as erythorbic acid is cleared from the blood within 12 h.

TABLE 10.3 Vitamin C Contents of Foods

Food	Vitamin C, mg/100 g
Fruits	
Apple	5
Banana	9
Cherry	7–10
Grapefruit	34
Guava	228
Lemon	53
Melons	8–37
Orange	59
Peach	7
Raspberry	26
Rose hips	426
Strawberry	59
Tangerine	27
Vegetables	
Asparagus	6
Broccoli	89
Cabbage	37
Carrot	3
Cauliflower	48
Celery	3
Collards	35
Corn	7
Kale	120
Leek	12
Potato	11
Onion	7
Pea	40
Parsley	133
Pepper	80–128
Cereals	(none)
Animal Products	
Beef	0
Milk cow	0–1
Milk, human	5

Adapted from Uncooked; USDA National Nutrient Database for Standard Reference, Release 28 http://www.ars.usda.gov/ba/bhnrc/ndl.

TABLE 10.4 Two-Day Storage Losses of Vitamin C

	% Lost	
Food	4°C	20°C
Beans	33	53
Cauliflower	8	26
Lettuce	36	42
Parsley	13	70
Peas	10	36
Spinach	32	80
Spinach (winter)	7	22

are enhanced by heat and conditions of neutral to alkaline pH. The vitamin is also reduced by exposure to oxidases in plant tissues. Therefore, substantial losses of vitamin C can occur during storage and are enhanced greatly during cooking. For example, stored potatoes lose 50% of their vitamin C within 5 mos. and 65% within 8 mos. of harvest. Apples and cabbage stored for winter can lose 50% and 40%, respectively, of their original vitamin C contents. Losses in cooking are usually greater with such methods as boiling, as the stability of ascorbic acid is much less in aqueous solution. For example, potatoes can lose 40% of their vitamin C content by boiling. Alternatively, quick heating methods can protect food vitamin C by inactivating oxidases, and acidic conditions stabilize dehydroascorbic acid.

Vitamin C Bioavailability

Vitamin C in most foods appears to have biological activities comparable to that of purified L-ascorbic acid at doses in the nutritional range (15–200 mg). At higher doses, bioavailability declines due to declining absorption efficiency. In humans, doses up to 200 mg are nearly completely absorbed, but doses of 1000 mg are utilized with only 50% efficiency. Because dehydroascorbic acid can be reduced metabolically to yield ascorbic acid (after enteric absorption and subsequent cellular uptake), both forms present in foods have vitamin activity. Several synthetic ascorbic acid derivatives also have vitamin C activity and offer advantages of superior chemical stability. Forms, such as ascorbate 2-sulfate, ascorbate 2-monophosphate, ascorbate 2-diphosphate, and ascorbate 2-triphosphate (mixtures of the latter three are referred to as **ascorbate polyphosphate**), are useful as vitamin C supplements for fish diets where the intrinsic instability of ascorbic acid in aqueous environments is a problem. The more highly biopotent of these vitamers appears to be effectively hydrolyzed in the digestive tract and tissues to yield ascorbic acid (Table 10.5).

TABLE 10.5 Vitamin C-Active Derivatives of Ascorbic Acid

Strongly Biopotent[a]	Weakly Biopotent[b]
Ascorbic acid 2-*O*-α-glucoside	L-ascorbyl palmitate
6-Bromo-6-deoxy-L-ascorbic acid	L-ascorbyl-2-sulfate
L-ascorbate 2-phosphate	L-ascorbate-*O*-methyl ether
L-ascorbate 2-triphosphate	

[a]*>50% antiscorbutic activity of ascorbic acid.*
[b]*<50% antiscorbutic activity of ascorbic acid.*

4. ABSORPTION OF VITAMIN C

Species that can synthesize ascorbic acid do not have active transport mechanisms for its enteric absorption. They absorb the vitamin across the mucosal brush border strictly by passive diffusion.

Species unable to synthesize ascorbic acid (e.g., humans, guinea pigs) absorb the vitamin by both passive and active means. Passive diffusion is important at high doses. At low doses, the most important means of absorbing the vitamin involves saturable, carrier-mediated active transport mechanisms. Thus, the efficiency of absorption of physiological doses (e.g., ≤180 mg/day for a human adult) of vitamin C is high, 80–90%, and declines markedly at vitamin C doses greater than about 1 g.[17] The reduced and oxidized forms of the vitamin are absorbed by different mechanisms of active transport, which occur throughout the small intestine:

- **Na^+-dependent vitamin C transporters (SVCTs)**[18] move ascorbate by an electrogenic process involving two Na^+ ions per ascorbic acid molecule. This family of surface glycoproteins comprises multiple isoforms; SVCT1 is the predominant form expressed the intestinal mucosa where it is localized on the brush border. It is inhibited by aspirin.[19] Its genetic deletion does not block ascorbic acid uptake,[20] suggesting other means of absorbing the vitamin are available. A genetic variant has been associated with susceptibility to Crohn's disease.[21]
- **Glucose transporter 1 (GLUT1)** facilitates vitamin C uptake by mucosal cells. The uptake of dehydroascorbic acid is 10- to 20-fold faster than that of ascorbic acid.[22] Upon entry into the cell, dehydroascorbic acid is quickly reduced to ascorbate.

Efflux of ascorbate across the basolateral side of the mucosal epithelial cell into the portal circulation can occur in several ways:

- by **SVCT2**-facilitated transport;
- by volume- or Ca^{+2}-sensitive **anion channels** that form pores in the plasma membrane;
- by **glutamate–ascorbate exchange**; and
- by **exocytosis** of ascorbate-containing vesicles and gap junction hemichannels.[23]

5. TRANSPORT OF VITAMIN C

Transport in Reduced Form

Vitamin C is transported in the plasma predominantly (80–90%) as ascorbate. Also present are small amounts of dehydroascorbic acid formed by the oxidation of ascorbate by diffusible oxidants of cellular origin (Fig. 10.2). Plasma ascorbate shows a sigmoid relationship with the level of vitamin C intake, saturation in humans is achieved at daily doses of 1000 mg or more (Fig. 10.3).[24] Levels in healthy humans are typically 30–70 μM and appear to be inversely related to adiposity, although that affect may simply reflect the fact that lower-energy diets tend to be richer in vitamin C-rich fruits and vegetables.[25]

Cellular Uptake

The uptake of vitamin C into cells occurs by the same mechanisms as those responsible for its enteric absorption. Uptake by simple diffusion is negligible due to the charge of ascorbate (which is ionized under physiological conditions) and the oil:water partitioning characteristics of dehydroascorbic acid, which excludes it from lipid membranes.

17. The efficiency of vitamin C absorption declines from about 75% of a 1-g dose, to about 40% of a 3-g dose, and about 24% of a 5-g dose; net absorption plateaus at 1–1.2 g at doses of at least 3 g.
18. These are members of the SLC23 human gene family.
19. For example, in humans, a 900-mg dose of aspirin blocks the expected rises in plasma, leukocyte, and urinary levels of ascorbic acid owing to a simultaneous dose of 500 mg of vitamin C.
20. Corpe, C.P., Tu, H., Eck, P., et al., 2010. J. Clin. Invest. 120, 1069–1083.
21. This variant (rs10063949-G) compromises the absorption and cellular uptake of ascorbate, which is thought to be needed to address the oxidative stress induced by excess ROS produced from aberrant immune responses in individuals genetically susceptible to inflammatory bowel disease (Shaghaghi, M.A., Bernstein, C.N., Leon, A.S., et al., 2014. Am. J. Clin. Nutr. 99, 378–383.).
22. Studies with cultured cells have shown that D-isoascorbic acid has only 20–30% of the activity of L-ascorbic acid in stimulating collagen production. The basis of this difference involves the much slower cellular uptake of the D-form, as, once inside the cell, both vitamers behaved almost identically.
23. See review: Corti, A., Casini, A.F., Pompella, A., 2010. Arch. Biochem. Biophys. 500, 107–115.
24. Levine, M., Conry-Cantilena, Wang, Y., et al., 1996. Proc. Natl. Acad. Sci. U.S.A. 93, 3704–3079) found that 200-mg/day doses produced only 80% saturation and that RDA-level doses supported plasma ascorbic acid concentrations on the lower third of the response curve.
25. Plasma ascorbic acid levels were inversely related to waist-to-hip ratio and to waist and hip circumferences but not to body mass index in a large European cohort (Canoy, D., Wareham, N., Welch, A., et al., 2005. Am. J. Clin. Nutr. 82, 1203–1209).

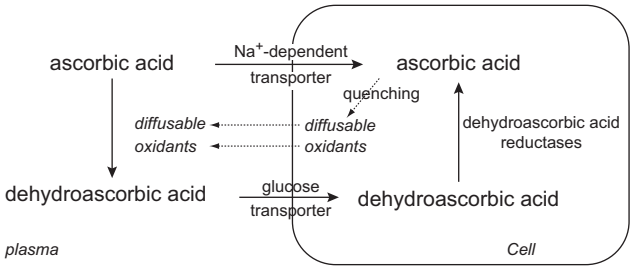


FIGURE 10.2 Redox cycling of ascorbic acid.

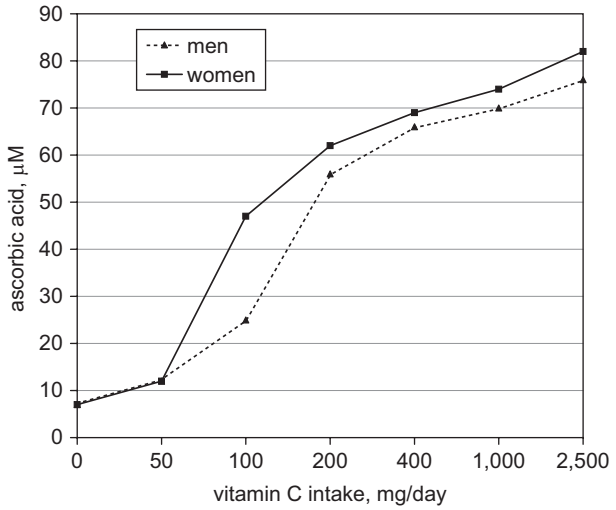


FIGURE 10.3 Relationship of plasma ascorbic acid (steady state) level and vitamin C intake. *From Levine, M., Wang, Y., Padayatty, S.J., et al., 2001. Proc. Natl. Acad. Sci. U.S.A. 98, 9842–9846.*

Nevertheless, cells accumulate ascorbic acid to levels 5- to 100-fold those of plasma; human cells become saturated at vitamin C intakes of about 100 mg/day. This is accomplished by facilitated uptake:

- **Ascorbate is taken up by SVCT1 and SVCT2**.[26] Each transporter is highly specific for ascorbate. SVCT1 is a high-affinity, high-capacity transporter expressed in epithelial tissues including the intestine, liver, and kidney; it is responsible for most ascorbate transport. SVCT2 is a high-affinity, low-capacity transporter expressed in brain, lung, heart, eye, placenta, neuroendocrine and exocrine tissues, and endothelial tissues. It appears to be responsible for the accumulation of ascorbate in tissues[27] and is upregulated under conditions of oxidative stress.[28] Genetic deletion of SVCT2 results in low-placental vitamin C transport and fetal death.[29] Both isoforms contain multiple potential N-glycosylation and protein kinase C (PKC) phosphorylation sites, suggesting regulation via glycosylation and/or PKC pathways. In the absence of ascorbate, the SVCTs can facilitate the unitransport of Na^+, allowing that ion to leak from cells. The SVCTs are noncompetitively inhibited by flavonoids and can be affected by cytokines and steroids. Their expression varies inversely with intracellular ascorbate levels.[30] SVCT1 appears to be principally involved in the maintenance of whole body vitamin C homeostasis by affecting enteric absorption and renal reabsorption;[31] while SVCT2 appears to be of principal importance in the protection of metabolically active tissues from oxidative stress.
- **Dehydroascorbic acid is taken up by GLUTs**. These transmembrane proteins are widely expressed (GLUT1 ubiquitously;[32] GLUT3 predominantly in brain and nerve cells; and GLUT4 predominantly in adipose, and cardiac and skeletal muscle).[33] They have similar affinities for ascorbate and glucose. Therefore, by competing for uptake by the transporter, hyperglycemia inhibits dehydroascorbic acid uptake. Accordingly, diabetics can have abnormally high plasma levels of dehydroascorbic acid.[34] Because the dehydroascorbic acid content of plasma is typically low, GLUT-facilitated uptake is thought to be a means of rapidly scavenging the oxidized form of the vitamin [e.g., as a result of reactive oxygen species (ROS) released by phagocytic cells] from the circulation so that it may be recycled to ascorbate intracellularly. This is best demonstrated by human erythrocytes, which have GLUT but no SVCTs.

Tissue Distribution

Nearly all tissues accumulate vitamin C, including some that lack ascorbic acid-dependent enzymes (Table 10.6).

26. These members of the solute carrier family 2 are designated SLC23A1 and SLC23A2, respectively.
27. Its genetic deletion rendered the mouse mortally depleted of ascorbate in every tissue (Sotirious, S., Gispert, S., Cheng, J. et al., 2002. Nature Med. 8, 514–517).
28. May, J.M., Li, L., Qu, Z., 2010. Mol. Cell. Biochem. 343, 217–222.
29. Harrison, F.E., Dawes, S.M., Meredith, M.E., et al., 2010. Free Rad. Biol. Med. 49, 821–829.
30. MacDonald, L., 2002. Br. J. Nutr. 87, 97–100.
31. Genetic deletion of SVCT1 resulted in massive losses of ascorbate from the plasma into the urine (Corpe, C.P., Tu, H., Eck, P., et al., 2010. J. Clin. Invest. 120, 1069–1083).
32. Congenital deficiency of GLUT1, a rare condition, is manifest in infancy as seizures and delayed development, presumably due to insufficient supply of glucose to the brain.
33. These are also members of the solute carrier family 2, designated SLC2A1, SLC2A3, and SCL2A4, respectively. GLUT2, previously considered only as a high-affinity transporter of glucosamine with low affinities for glucose and fructose, has been shown to transport dehydroascorbate with low affinity in hepatocytes (Mardones, L. Ormazabal, V., Romo, X., et al., 2011. Biochem. Biophys. Res. Commun. 410, 7–12).
34. Impaired cellular uptake of vitamin C, due to competition with glucose, may contribute to pathology in diabetes.

TABLE 10.6 Ascorbic Acid Concentrations of Human Tissues

Tissue	Ascorbic Acid, mg/100 g
Adrenals	30–40
Pituitary	40–50
Liver	10–16
Thymus	10–15
Lungs	7
Kidneys	5–15
Heart	5–15
Muscle	3–4
Brain	3–15
Pancreas	10–15
Lens	25–31
Plasma	0.4–1

Certain cell types (e.g., peripheral mononuclear leukocytes) can accumulate millimolar concentrations. Tissue levels are decreased by virtually all forms of stress, which also stimulate the biosynthesis of the vitamin in those animals able to do so.[35] The concentration of ascorbate in the adrenals is particularly high (72–168 mg/100 g in the cow); one-third of the vitamin is concentrated at the site of catecholamine formation, from which it is released with newly synthesized corticosteroids in response to stress.[36] The ascorbate concentration of brain tissue also tends to be high (5–28 mg/100 g), particularly in regions also rich in catecholamines.[37] Brain ascorbate levels are among the last to be affected by dietary deprivation of vitamin C in nonsynthesizing species. A relatively large amount of ascorbate is also found in the eye where it is thought to protect critical protein sulfhydryl groups from oxidation.[38] The ascorbate levels of white blood cells reach plateaus at vitamin C doses of 2 g/day, with lymphocytes, platelets, monocytes, and neutrophils showing decreasing plateau levels in that order[39]. Leukocyte ascorbate concentrations correlate with tissue levels of the vitamin.[40] There is no stable reserve of vitamin C; excesses are quickly excreted. At saturation, the total body pool of the human has been estimated to be 1.5–5 g,[41] the major fractions being found in the liver and muscles by virtue of their relatively large masses.

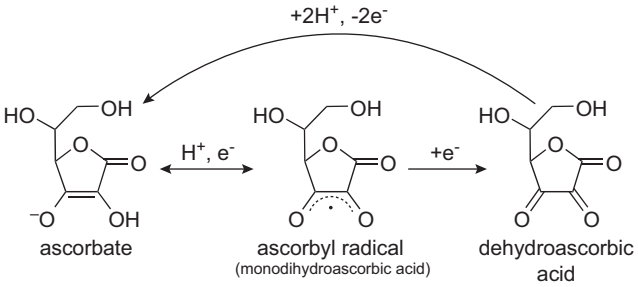


FIGURE 10.4 Oxidation–reduction reactions of vitamin C.

6. METABOLISM OF VITAMIN C

Oxidation

Ascorbate can be oxidized in vivo by two successive losses of single electrons. The first monovalent oxidation results in the formation of the **ascorbyl free radical**.[42] That partially reduced form can establish a reversible electrochemical couple with ascorbate, or it can be further oxidized to dehydroascorbic acid (Fig. 10.4).

The partially oxidized form, ascorbyl radical, can be reduced to dehydroascorbic acid by the cytosolic selenoenzyme thioredoxin reductase to from dehydroascorbic acid. It can also undergo nonenzymatic dismutation of 2 mol of ascorbyl radical to form equimolar amounts of dehydroascorbic acid and ascorbate. The completely oxidized form, dehydroascorbic acid, is unstable at physiological pH. If not reduced back to the ascorbate state, it undergoes irreversible ring opening 2,3-diketo-L-gulonic acid, which can undergo decarboxylation to CO_2 and five-carbon fragments (xylose, xylonic acid, lyxonic acid), or be oxidized to form oxalic acid and 4-C fragments (e.g., threonic acid). In addition, the formation of L-ascorbic acid 2-sulfate from ascorbic acid occurs in humans, fishes, and rats; and the oxidation of the ascorbate 6-carbon to form L-saccharoascorbic acid has been demonstrated in monkeys. Ascorbate may also undergo oxidation by reaction with tocopheroxyl or urate radicals (Fig. 10.5).

35. The ascorbic acid content of brown adipose tissue of rats can increase 60% during periods of cold stress.
36. That is, in response to the release of adrenocorticotropic hormone (ACTH).
37. Phenylalanine and tyrosine metabolites with hormonal functions, including epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine.
38. Lenses of cataract patients have lower lens ascorbic acid concentrations (e.g., 0–5.5 mg/100 g) than those of healthy patients (e.g., 30 mg/100 g).
39. Levine, M., Wang, Y., Padayatty, S.J., et al., 2001. Proc. Nat. Acad. Sci. U.S.A. 98, 9842–9846.
40. Leukocyte ascorbic acid concentrations are usually greater in women than men, and decrease with age and in some diseases.
41. The first signs of scurvy are not seen until this reserve is depleted to 300–400 mg.
42. The ascorbyl radical is also called monodehydroascorbic acid; it is relatively stable with a rate constant for its decay of about $10^5\ M^{-1}\ s^{-1}$.

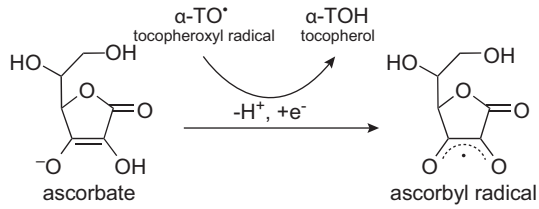


FIGURE 10.5 Coupling of ascorbate oxidation to reduction of α-tocopheroxyl radical.

Ascorbate Regeneration

Ascorbate can be regenerated from dehyroascorbic acid. Multiple reductase activities in mitochondria, endoplasmic reticulum, and erythrocyte plasma membranes promote a favorable ascorbate redox potential, indirectly preserving other antioxidants such as tocopherol. With the presence of an effective scavenging system specific for the oxidized form, a cycle is effectively established to maintain intracellular levels of the reduced vitamin (Fig. 10.2).

Although dehydroascorbic acid appears to have no metabolic function per se, its recycling to ascorbate renders it physiologically important in protecting cells from ROS. This appears to be the basis of ascorbate-stimulating osteoid formation by osteoblasts in response to ROS released by osteoclasts and of vitamin C protection of intestinal mucosa and mitochondria against ROS generated there. Impairments in this recycling can occur in uncontrolled diabetes due to excessive plasma glucose, which competes with dehydroascorbic acid for cellular uptake by GLUTs and leads to reduced intracellular ascorbate levels that weaken antioxidant defenses.

Excretion

Ascorbate is thought to pass unchanged through the glomeruli and to be actively reabsorbed in the tubules by SCVT1. Little, if any, ascorbic acid is excreted in the urine of humans consuming less than 100 mg/day and only one-fourth of the dose is excreted at twice that intake. At doses greater than about 500 mg/day (i.e., when blood ascorbic acid concentrations exceed 1.2–1.8 mg/dL), virtually all ascorbic acid above that level is excreted unchanged in the urine, thus producing no further increases in body ascorbate stores. The fractional excretion of a parenteral dose of ascorbic acid approaches 100% at doses >2 g.

The epithelial cells of the renal tubules reabsorb dehydroascorbic acid after it has been filtered from the plasma. Species vary in their routes of disposition of the vitamin. Guinea pigs and rats degrade it almost quantitatively to CO_2,[43] which is lost across the lungs. Humans, however, normally degrade only a very small amount via that route,[44] excreting mostly ascorbic acid, dehydroascorbic acid, and 2,3-diketogulonic acid, with relatively small amounts of oxalate and ascorbate 2-sulfate. Excretion of oxalate is relevant to risk of renal stone formation. It has been thought that healthy individuals convert no more than 1.5% of ingested ascorbic acid to oxalic acid within 24 h; however a careful study showed that subjects given ascorbic acid intravenously excreted <0.5% as oxalate.[45] It is estimated that, of the oxalate excreted daily by humans consuming nutritional amounts of vitamin C (e.g., 30–40 mg), 35–50% comes from ascorbic acid degradation, the balance coming from glycine and glyoxylate. Not all individuals show increased urinary oxalate excretion in response to ascorbic acid supplementation; only 40% of adults consuming very high doses (1000 mg/day) of vitamin C increased their urinary oxalate levels more than 16% (Table 10.7).

TABLE 10.7 Effect of High-level Ascorbic Acid Supplementation on Urinary Oxalate Excretion

Subject Group (n)	Treatment[a]	Oxalate, μmoles
Responders (19)	Control	513 ± 97
	Ascorbic acid, 1000 mg/day	707 ± 165[b]
Nonresponders (29)	Control	560 ± 110
	Ascorbic acid, 1000 mg/day	551 ± 129[b]

[a]*Each subject experienced alternating 6-day control and ascorbic acid treatments.*
[b]*Significantly different (p<.05) from control treatment within subject group.*
Adapted from Massey, L.K., Liebman, M., Kynast-Gales, S.A., 2005. J. Nutr. 135, 1673–1637.

Ascorbic acid is also excreted in the gastric juice. In healthy adults, that concentration is typically three times that of plasma, although it is low in patients with atrophic gastritis or *Heliobacter pylori* infection.[46]

7. METABOLIC FUNCTIONS OF VITAMIN C

The metabolic functions of vitamin C can be categorized as those of its properties as a biochemical antioxidants and those of its properties as an enzyme cosubstrate.

Antioxidant Functions

Ascorbic acid loses electrons easily and, because of its reversible monovalent oxidation to the ascorbyl radical,

43. The C-1 carbon of ascorbic acid is the main source of CO_2 derived from the vitamin, whereas C-1 and C-2 are the precursors of oxalic acid.

44. Degradation by this path is increased in some diseases and can then account for nearly half of ascorbic acid loss.

45. Robitaille, L., Mamer, O.A., Miller, Jr., W.H., et al., 2009. Metab. Clin. Exp. 58, 263–269.

46. Sobala, G.M., Schorah, C.J., Shires, S., et al., 1993. Gut 34, 1038–1041.

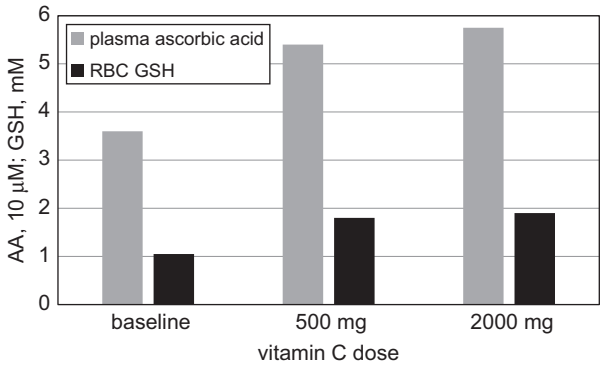


FIGURE 10.6 Enhancement of reduced glutathione (GSH) by vitamin C in men. *After Johnston, S.C., Meyer, C.G., Srilakshmi, J.C., 1993. Am. J. Clin. Nutr. 58, 103–105.*

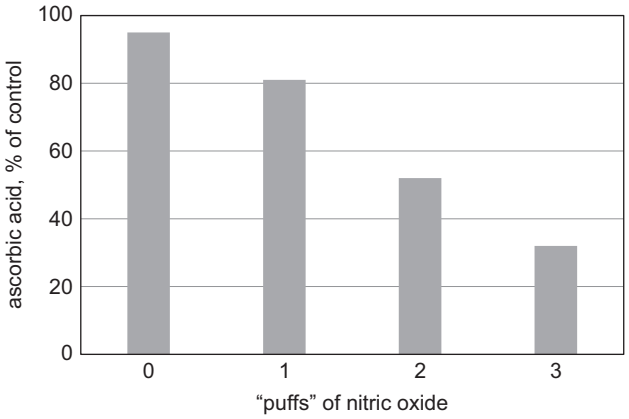


FIGURE 10.7 *In vitro* oxidation of ascorbic acid simulating the pro-oxidative effects of atmospheric nitric oxide. *After Eiserich, J.P., Cross, C.E., van der Vliet, A., 1997. In: Packer, L., Fuchs, J., (Eds.). Vitamin C in Health and Disease. Marcel Dekker, New York, pp. 399–412.*

it can serve as a biochemical redox system. The redox potential of the dehydroascorbic acid–ascorbate couple is in the range of 0.06–0.1 V. That of the ascorbyl radical–ascorbate couple is −0.17 V. These redox potentials mean that ascorbate can act as an **antioxidant** by reacting with free radicals and undergoing a single-electron oxidation to yield a relatively poorly reactive intermediate, the ascorbyl radical, which disproportionates to ascorbate and dehydroascorbic acid. In this way, ascorbate can reduce toxic ROS ($O_2^{\cdot -}$, $OH^{\cdot}$, $RO_2^{\cdot}$) and RNS ($NO_2^{\cdot}$). Those reactions are of fundamental importance in all aerobic cells, which must defend against the toxicity of the very element depended on as the terminal electron acceptor for energy production via the respiratory chain enzymes. One such reaction is important in extending the antioxidant protection to the hydrophobic regions of cells: ascorbate reduction of the semistable chromanoxyl radical, thus, regenerating the metabolically active form of the lipid antioxidant vitamin E.[47] Such quenching of oxidants protects glutathione in its reduced form (Fig. 10.6).

The antioxidant efficiency of ascorbate is significant at physiological concentrations of the vitamin (20–90 μM). Under those conditions, the predominant reaction is a radical chain-terminating one of ascorbate (AH^-) with a peroxyl radical to yield a hydroperoxide (ROOH) and ascorbyl radical (A^-), which proceeds to reduce a second peroxyl radical and yield the vitamin in its oxidized form, dehydroascorbic acid (A). At low concentrations of the vitamin, 2 moles of peroxyl radical are reduced for every mole of ascorbate consumed:

$$AH^- + RO^{\cdot} \rightarrow A^{\cdot -} + ROO^{\cdot} \left(+ H^+ \rightarrow ROOH\right)$$

$$A^{\cdot -} + R'OO^{\cdot} \rightarrow A + R'OO^- \left(+ H^+ \rightarrow R'OOH\right)$$

Dehydroascorbic acid is inherently unstable, with a half-life of only minutes in physiological conditions and undergoing ring opening to yield 3,4-diketogulonic acid. Therefore, exposure to free radicals can lead to the consumption of vitamin C (Fig. 10.7).[48] This type of direct effect appears to be moderated by the presence of other antioxidants (e.g., reduced glutathione) but is exacerbated by inflammatory oxidants such as $O_2\cdot$, H_2O_2, and hypochlorous acid (HOCl) produced by activated phagocytes. For this reason, smokers, who expose themselves to various highly reactive free radicals in tobacco smoke,[49,50] show a 40% greater turnover of ascorbate than do nonsmokers with similar vitamin C intakes.[51] Even nonsmokers exposed passively to tobacco smoke have been found to have lower circulating ascorbate levels than nonexposed persons.

At relatively high vitamin C concentrations, the slower radical chain-propagating reaction of ascorbyl radical and O_2 become significant. It yields dehydroascorbic acid and superoxide radical, which, in turn, can oxidize ascorbate to return ascorbyl radical:

$$A^{\cdot -} + O_2 \rightarrow A + O_2^{\cdot -}$$

$$O_2^{\cdot -} + AH^- \rightarrow HOO^- + A^{\cdot -}$$

It is thought that, at high-vitamin C concentrations, this two-reaction sequence can develop into a radical chain

47. Evidence for such an effect comes from demonstrations in vitro of the reduction by ascorbic acid of the tocopheroxyl radical to tocopherol, as well as from findings in animals that supplemental vitamin C can increase tissue tocopherol concentrations and spare dietary vitamin E.

48. Eiserich, J.P., Cross, C.E., van der Vliet, A., 1997. In: Packer, L., Fuchs, J., (Eds.), Vitamin C in Health and Disease. Marcel Dekker, New York. pp. 399–412.

49. For example, nitric oxide (NO·); nitrogen dioxide (·NO_2); and alkyl, alkoxyl, and peroxyl radicals.

50. Free radical-mediated processes are thought to be involved in the pathobiology of chronic and degenerative diseases associated with cigarette smoking, e.g., chronic bronchitis, emphysema, cancer, cardiovascular disease.

51. Smith, J.L., Hodges, R.E., 1987. Ann. N.Y. Acad. Sci. 498, 144–152.

autoxidation process that consumes ascorbate, thus, wasting the vitamin. Hence, in aerobic systems, the efficiency of radical quenching of ascorbate is inversely related to the concentration of the vitamin. At physiological concentrations, ascorbate serves as one of the strongest reductants and radical scavengers, reducing oxy, nitro, and ethyl radicals.

Cellular Antioxidant Functions

As the most effective aqueous antioxidant in plasma, interstitial fluids, and soluble phases of cells, ascorbate appears to be the first line of defense against ROS arising in those compartments. Those species include superoxide and hydrogen peroxide arising from activated polymorphonuclear leukocytes or other cells and from gas-phase cigarette smoke,[52] which can promote the oxidation of critical cellular components.

- **Lipid peroxidation**. The LDL (low-density lipoprotein)-protective action of vitamin E appears to be dependent on the presence of ascorbate, which, by reducing the tocopheroxyl radical, prevents the latter from acting prooxidatively, i.e., from abstracting hydrogen from a cholesteryl–polyunsaturated fatty esters to yield peroxyl radicals.
- **Protein oxidation**. At least in vitro, ROS species can oxidize proteins to produce carbonyl derivatives and other oxidative changes associated with loss of function. Whether ascorbate provides such protection in vivo has been suggested as the basis of effects reported for vitamin C in reducing risks to cataracts and other illnesses.
- **DNA oxidation**. Ascorbate contributes to the prevention of oxidative damage to DNA, which is elevated in cells at sites of chronic inflammation and in many preneoplastic lesions. In fact, the continuous attack of DNA by unquenched ROS is believed to contribute to cancer, as elevated steady-state levels of oxidized DNA bases are estimated to cause mutational events.[53] The levels of one base damage product, 8-hydroxy-2'-deoxyguanosine, have been found to be elevated in scorbutic individuals[54] and to be reduced by supplementation with vitamins C and E.[55]
- **NO oxidation**. Ascorbic acid protects **nitric oxide** (NO) from oxidation, supporting the favorable effects of the latter on vascular epithelial function, and lowering blood pressure. This may also involve ascorbate participating in the reductive recycling of tetrahydrobiopterin, an essential cofactor of endothelial nitric oxide synthase. Ascorbate also reacts with nitrites and nitrates formed from NO and commonly found in vegetables and cured foods. In this way, ascorbate prevents the formation of carcinogenic *N*-nitroso compounds.

Improving iron utilization. Ascorbic acid can reduce ferric iron (Fe^{3+}) to the ferrous form (Fe^{2+}) and form a stable chelate with the latter. This allows the vitamin to convert the dominant form of iron in the acidic environment of the stomach to a form that is soluble in the alkaline environment of the small intestine. These effects result in increased enteric absorption[56] of both nonheme and heme iron. In these ways, vitamin C increases the bioavailability of iron in foods. Studies with iron-deficient rats, which have upregulated enteric iron absorption, have shown vitamin C to promote the mucosal uptake of iron but not its mucosal transfer. This effect depends on the presence of both ascorbic acid and iron in the gut at the same time, e.g., the consumption of a vitamin C-containing food with the meal. Thus, the low bioavailability of nonheme iron and the iron-antagonistic effects of such food factors as polyphenols and phytates, or of calcium phosphate, can be overcome by the simultaneous consumption of vitamin C (Fig. 10.8).[57] Similarly, ascorbic acid administered parenterally has been found useful as an adjuvant therapy to erythropoietin in hemodialysis patients.

Ascorbic acid also promotes the utilization of heme iron, which appears to involve enhanced incorporation of iron into its intracellular storage form, ferritin.[58] This effect involves facilitation of ferritin synthesis; ascorbate enhances the iron-stimulated translation of ferritin mRNA by maintaining the iron-responsive element-binding protein[59] in its enzymatically active form. Studies with cultured cells have shown that ascorbic acid also enhances the stability of ferritin by blocking its degradation through reduced lysosomal autophagy of the protein. Thus, the decline in ferritin and accumulation of **hemosiderin**[60] in scorbutic animals is reversed by ascorbic acid treatment.[61]

52. Indeed, genetically scorbutic rats have been found to have elevated levels of LDL lipid peroxidation products (i.e., thiobarbituric-reactive substances, TBARS), which respond to vitamin C supplementation.
53. ~1 per 10^5 bases (Halliwell, B., 2000. Am. J. Clin. Nutr. 72, 1082–1087).
54. Rehman, A., Collis, C.S., Yang, M., et al., 1998. Biochim. Biophys. Res. Commun. 246, 293–298.
55. Moller, P., Viscovich, M., Lykkesfeldt, J., et al., 2004. Eur. J. Nutr. 43, 267–274.
56. This effect can be 200–600%.
57. Anemia, much of it due to iron deficiency, is an enormous global problem, affecting more than 40% of all women. Yet, iron is the fourth most abundant element in the earth's crust and few diets do not contain the element in nonheme form. The problem of iron-deficiency anemia is associated with inadequate iron bioavailability and, thus, vitamin C inadequacy may be a contributing factor.
58. A soluble, iron–protein complex found mainly in the liver, spleen, bone marrow, and reticuloendothelial cells. With 23% iron, it is the main storage form of iron in the body; when that capacity is exceeded, iron accumulates as the insoluble hemosiderin.
59. This is a dual-function protein that also has aconitase activity.
60. A dark yellow, insoluble, granular, iron–storage complex found mainly in the liver, spleen, and bone marrow.
61. The reverse relationship is apparently not significant, i.e., iron loading has been found to have no effect on ascorbic acid catabolism in guinea pigs.

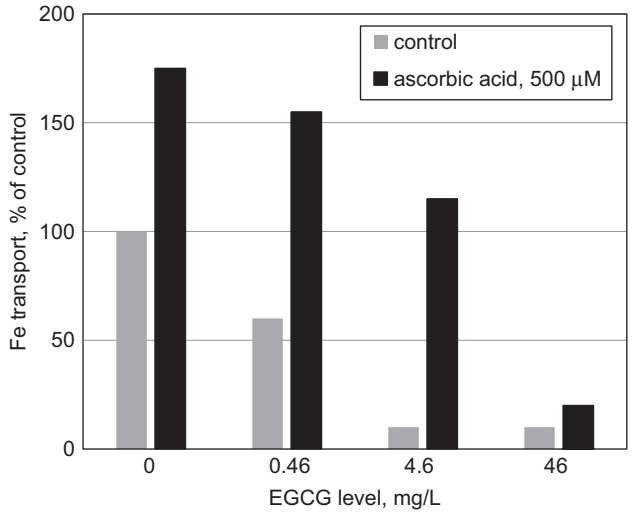


FIGURE 10.8 Effect of vitamin C in promoting iron transport and countering the inhibitory effect of epigallocatecin (EGCG) in the CaCo-2 cell model. *After Kim, E.Y., Ham, S.K., Bradke, D., et al., 2011. J. Nutr. 141, 828–834.*

Interactions with other mineral elements. Ascorbic acid can also interact with several essential trace elements. It can reduce the toxicities of high levels of selenium, copper, nickel, lead, vanadium, and cadmium—elements whose reduced forms are poorly absorbed or more rapidly excreted. In the case of copper, ascorbic acid enhances the postabsorptive utilization of copper for the synthesis of cuproproteins,[62] perhaps by increasing the balance of reduced *versus* oxidized forms of glutathione. Ascorbic acid can also enhance the utilization of low doses of selenium, and increase tissue levels of manganese.

Support of pulmonary function. Its redox properties give ascorbic acid an important role in the antioxidant protection of the lung,[63] which is consistently exposed to high concentrations of oxygen and inhaled toxic gases.[64] Lung parenchymal cells also generate ROS via cytochrome *P*450-dependent metabolism and inflammatory cell invasion. Accordingly, patients with asthma or acute respiratory distress syndrome typically show lower than normal concentrations of ascorbic acid in both plasma and leukocytes.

Support of neurologic function. The brain and spinal cord are among the richest tissues in ascorbic acid contents, with concentrations of 100–500 µM. An estimated 2% of the ascorbic acid in the brain turns over each hour. Plasma ascorbic acid concentrations have been positively associated with cognitive performance in older subjects and with memory in patients with dementia. These relationships may involve protection from inflammation, as such inflammatory mediators as cytokines and free radicals are important in the pathogenesis of neurodegenerative disease. Controlled intervention trials have not been conducted to evaluate the effects of vitamin C on cognitive function, but oral vitamin C was found to improve psychiatric rating in schizophrenics.

TABLE 10.8 Relationship of Vitamin C Intake and Cataract Risk

Quintile of Vitamin C Intake, mg/day	Odds Ratio[a]
≤102	1
>102–135	0.88 (0.56–1.40)[b]
>135–164	0.66 (0.41–1.07)
>164–212	0.60 (0.37–1.07)
>212	0.70 (0.44–1.13)

[a]*Ratio of cataracts incidence in each quintile group to that of the lowest (reference) quintile group; P value for trend, 0.04.*
[b]*95% Confidence interval.*
Adapted from Valero, M.P., Fletcher, A.E., De Stavola, B.L., et al., 2002. J. Nutr. 132, 1299–1306.

Prevention of cataracts. Cataracts, involving opacification of the ocular lens, are thought to result from the cumulative photooxidative effects of ultraviolet light from which the lens is protected by three antioxidants: ascorbic acid, tocopherol, and reduced glutathione. The lens typically contains relatively high concentrations of ascorbic acid (e.g., as much as 30-fold those of plasma), which are lower in aged and cataractous lens.[65] At least 10 cohort studies have found cataract risk to be inversely related to vitamin C intake (Table 10.8).[66] Some, but not all, case–control studies have found inverse associations of cataract risk and ascorbic acid intake and serum ascorbic acid level.[67] A large intervention trial found that high doses of vitamin C (increasing serum ascorbate levels >49 µM) reduced the incidence of cataract by 64%.[68] Scorbutic guinea pigs have been found to develop early cataracts; and ascorbate has been shown to protect against ultraviolet light-induced oxidation of lens proteins.

Diabetes prevention. Diabetic patients typically show lower serum concentrations of ascorbic acid than nondiabetic, healthy controls. Accordingly, reduced serum antioxidant activity has been implicated in the pathogenesis of the

62. Including two that use ascorbate as a cosubstrate (dopamine β-monooxygenase, peptidylglycine α-amidating monooxygenase).
63. See review by Brown, L.A.S., Jones, D.P., 1997. In: Packer, L., Fuchs, J., (Eds.), Vitamin C in Health and Disease. Marcel Dekker, New York, pp. 265–278.
64. For example, ozone, nitric oxide, nitrogen dioxide, cigarette smoke.
65. The ascorbic acid content of the oldest portion of the lens (the nucleus), where most senile cataracts originate, is typically only one-quarter the concentration in the lens cortex.
66. Vishwanathan, R., Johnson, E.J., 2012. In: Erdman, J.W., Macdonald, I.A., Zeisel, S.H., (Eds.), Present Knowledge in Nutrition, tenth ed. Wiley-Blackwell, Ames, IA, pp. 942–946.
67. Simon, J.A., Hudes, E.S., 1999. J. Clin. Epidemiol. 52, 1207–1211.
68. Agte, V., Tarwadi, K., 2010. Ophthalmic. Res. 44, 166–172.

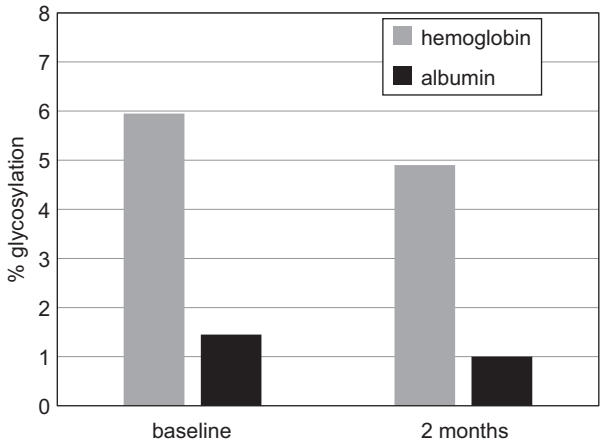


FIGURE 10.9 Protein glycosylation reduced by supplemental vitamin C (1 g/day). *After Davie, S.J., Gould, B.J., Yudkin, J.S., 1992. Diabetes 41, 167–173.*

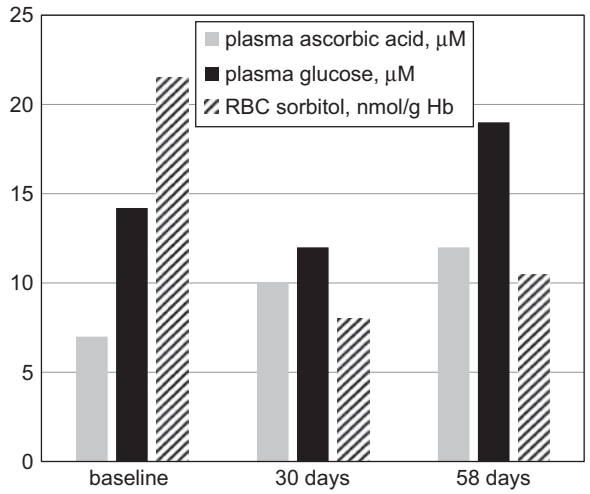


FIGURE 10.10 Responses of diabetics to supplemental vitamin C. *After Cunningham, J.J., Mearkle, P.L., Brown, R.G., 1994. J. Am. Coll. Nutr. 13, 344–350.*

disease. That vitamin C supplementation can reduce glycosylation of plasma proteins (Fig. 10.9) suggests a role of the vitamin in preventing diabetic complications. Intervention trials have shown that vitamin C supplementation can be effective in reducing erythrocyte sorbitol accumulation[69] (Fig. 10.10) and urinary albumin excretion[70] in noninsulin-dependent diabetics, although one found no effect on microvascular reactivity[71]. Treatment with vitamin C has also been shown to prevent arterial hemodynamic changes induced by hyperglycemia.

69. Because the sorbitol or its metabolites is thought to underlie the pathologic complications of diabetes, reduction of tissue sorbitol accumulation is a strategy for managing diabetes.

70. Gaede, P., Poulsen, H.E., Parving, H.H., et al., 2001. Diabetic Med. 18, 756–760.

71. Lu, Q., Bjorkhem, I., Wretlind, B., et al., 2005. Clin. Sci. 108, 507–513.

Prooxidant Potential

In the presence of oxidized metal ions (e.g., Fe^{3+}, Cu^{2+}), high concentrations of ascorbic acid can have prooxidant functions at least in vitro. It does so by donating an electron to reduce such ions to forms that, in turn, can react with O_2 to form oxy radicals (the metal ions being reoxidized in the process):

$$AH^- + Fe^{3+} \rightarrow A^{\cdot -} + Fe^{2+}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

In this way, ascorbate can react with copper or iron salts in vitro and lead to the formation of H_2O_2, $O_2^{\cdot -}$, and OH·, which can damage nucleic acids, proteins, and polyunsaturated fatty acids (PUFAs). Accordingly, iron–ascorbate mixtures are often used to stimulate lipid peroxidation in vitro, and such prooxidative reactions with transition metals are likely to be the basis of the cytotoxic and mutagenic effects of ascorbate observed in isolated cells in vitro. It has been suggested that high serum ascorbate may reduce Fe^{3+} in ferritin[72] to the catalytically active form Fe^{2+}.

The physiological relevance of these prooxidative reactions is unclear. Under physiological conditions, tissue concentrations of ascorbate acid greatly exceed those of dehydroascorbic acid, which greatly exceed those of ascorbyl radical. Thus, the redox potentials of the ascorbyl radical/ascorbate and dehydroascorbic acid/ascorbyl radical couples are sufficient to reduce most oxidizing compounds.

Enzyme Cosubstrate Functions

Ascorbate functions as a cosubstrate for at least 10 enzymes that function in electron transport reactions involved in the synthesis of collagen, norepinephrine,[73] peptide hormones, and carnitine; and the metabolism of tyrosine, xenobiotics, steroids, and fatty acids (Table 10.9). Two of these are monooxygenases, which incorporate a single atom of oxygen into a substrate and require at their active sites copper atoms that are reduced by ascorbate. The others are dioxygenases, which incorporate both atoms of molecular oxygen in different ways;[74] many of these also use α-ketoglutarate as a cosubstrate. In each, the electron acceptor, ascorbyl radical, is subsequently reduced by microsomal **monodehydroascorbate reductase** or **ascorbate–cytochrome b_5 reductase** to regenerate

72. This would require that ascorbate enter the pores of the ferritin protein shell to react with iron on the inner surface.

73. Also, noradrenaline.

74. In most cases, those oxygen atoms are incorporated into different acceptor substrates; in the single case of 4-hydroxyphenylpyruvate dioxygenase, the oxygens are incorporated in different locations in the same acceptor substrate (4-hydroxyphenylpyruvate).

TABLE 10.9 Enzymes That Require Ascorbic Acid as a Cosubstrate

Metabolic Role	Enzyme
Collagen synthesis	Prolyl 4-hydroxylase
	Prolyl 3-hydroxylase
	Lysine hydroxylase
Tyrosine metabolism	4-Hydroxyphenylpyruvate dioxygenase
Catecholamine synthesis	Dopamine β-monooxygenase
Peptide hormone synthesis	Peptidylglycine α-amidating monooxygenase
Carnitine synthesis	γ-Butyrobetaine 2-oxoglutarate 4-dioxygenase
	Trimethyllysine 2-oxoglutarate dioxygenase
Transcriptional responses to hypoxia	HIF-1α prolyl 4-hydroxylases
	HIF-1α asparaginyl hydroxylase
Drug and steroid metabolism	Cholesterol-7α-hydroxylase

ascorbic acid. In many of these functions, ascorbate is not required per se, i.e., it can be replaced by other reductants in vitro.[75] However, these enzymes show high affinities for ascorbate, suggesting that their activities are diminished only by chronic deprivation of the vitamin.

Connective tissue health. Vitamin C is required for wound healing. The vitamin is accumulated at wound sites where it is rapidly utilized.[76] This reflects the function of ascorbic acid in the synthesis of collagen proteins,[77] specifically, in the hydroxylation of specific prolyl and lysyl residues of the unfolded (nonhelical) procollagen chain catalyzed by **prolyl 4-hydroxylase**, **prolyl 3-hydroxylase**, and **lysyl hydroxylase**. These dioxygenases[78] require O_2, Fe^{2+}, and ascorbate, which are stoichiometrically linked to the oxidative decarboxylation of α-ketoglutarate. Each has a specific binding site for ascorbate near its iron center. The role of ascorbate in each reaction is to maintain iron in the reduced state (Fe^{2+}), which dissociates from a critical region (an SH group) of the active site to reactivate the enzyme after catalysis. The posttranslational hydroxylation of these procollagen amino acid residues is necessary for folding into the triple helical structure that can be secreted by fibroblasts. Hydroxyproline residues contribute to the stiffness of the collagen triple helix and hydroxylysine residues bind (via their hydroxyl groups) carbohydrates to form intramolecular cross-links that give structural integrity to the collagen mass. Vitamin C deprivation results in underhydroxylation of procollagen, which accumulates[79] and is degraded; this appears to be the basis of the poor wound healing characteristic of scurvy. Vitamin C-deficient subjects usually show reduced urinary excretion of hydroxyproline. The function of ascorbic acid in collagen synthesis makes the vitamin important in the synthesis of surfactant apoproteins, which have collagen-like domains that require ascorbic acid-dependent hydroxylation for proper folding and stability. Wound repair typically decreases with aging; this had been viewed as indicative of increasing needs for vitamin C by older individuals.

Studies have indicated modest effects of vitamin C deprivation on the hydroxylation of proline in the conversion of the soluble **tropoelastin** to the soluble **elastin**.[80] A component of complement, C1q, resembles collagen in that it contains **hydroxyproline** and **hydroxylysine**. Curiously, vitamin C deprivation reduces overall complement activity but does not affect the synthesis of C1q.

Catecholamine synthesis. Ascorbate serves as an electron donor for **dopamine β-monooxygenase**,[81] a copper enzyme located in the chromaffin vesicles[82] of the adrenal medulla and in adrenergic synapses. The enzyme exists in both membrane-bound and soluble forms; both use O_2 and ascorbate to hydroxylate dopamine to form the neurotransmitter norepinephrine (Fig. 10.11).

Peptide hormone processing. Ascorbate is a cosubstrate copper enzyme, **peptidylglycine α-amidating monooxygenase**, which catalyzes the posttranslational processing of peptides by α-amidation. This process consists of adding an amide group to the C-terminals of physiologically active

75. For example, reduced glutathione, cysteine, tetrahydrofolate, dithiothreitol, 2-mercaptoethanol.

76. Studies with apparently vitamin C-adequate burn patients have shown their plasma ascorbic acid levels to drop to nearly zero after the trauma; this is presumed to reflect the movement of the vitamin to the sites of wound repair.

77. **Collagens**, secreted by fibroblasts and chondrocytes, are the major components of skin, tendons, ligaments, cartilage, the organic substances of bones and teeth, the cornea, and the ground substance between cells. Some 19 types of collagen have been characterized; collectively, they comprise the most abundant type of animal protein, accounting for 25–30% of total body protein.

78. One-half of the O_2 molecule is incorporated into the peptidyl prolyl (or peptidyl lysyl) residue; the other half is incorporated into succinate.

79. Accumulated procollagen also inhibits its own synthesis and mRNA translation.

80. About 1% of the prolyl residues in elastin are hydroxylated. This amount can apparently be increased by vitamin C, suggesting that normal elastin may be underhydroxylated.

81. The specific activity of this enzyme has been found to be abnormally low in schizophrenics with anatomical changes in the brain, suggesting impaired norepinephrine and dopamine neurotransmission in those patients.

82. These vesicles accumulate and store catecholamines in the adrenal medulla; they also contain very high concentrations of ascorbic acid, e.g., 20 mM.

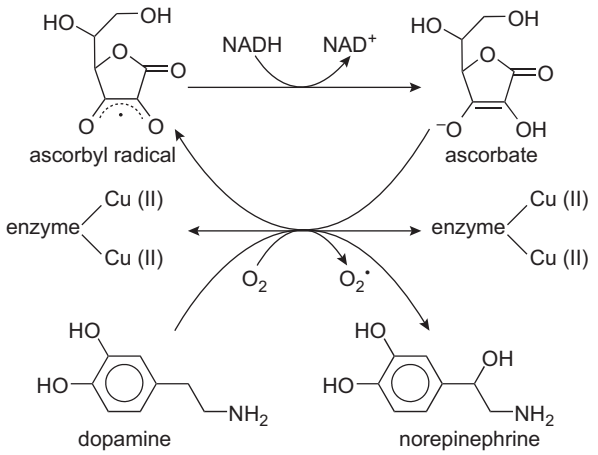


FIGURE 10.11 Role of vitamin C in the conversion of dopamine to norepinephrine by dopamine-β-monooxygenase.

peptides.[83] The enzyme is bifunctional, having two domains that catalyze both of the two steps in the peptide amidation process:

- **peptidylglycine α-hydroxylating monooxygenase**[84] catalyzes the hydroxylation of C-terminal glycine; it is rate limiting to the overall process; that this activity is inhibited by catalase in vitro, which suggests that H_2O_2 is an intermediate in the reaction.
- **peptidyl-α-hydroxyglycine α-amidating lyase** catalyzes the cleavage of the peptidylhydroxyglycine into glyoxylate and the amidated peptide.

Carnitine[85] **synthesis**. Ascorbic acid is a cosubstrate of two Fe^{2+}-containing hydroxylases involved in the synthesis of carnitine (Fig. 10.12): **ε-N-trimethyllysine hydroxylase** and **γ-butyrobetaine hydroxylase**. While ascorbate supports the greatest activities of these enzymes, is it not essential per se, as each can be driven by excess substrate loads.[86] Scorbutic guinea pigs show low carnitine levels in muscle and heart. Impaired carnitine synthesis is the likely basis of the fatigue, lassitude, and hypertriglyceridemia, which are early signs of vitamin C deficiency.

Tyrosine metabolism. Ascorbate is a cosubstrate of two mixed-function oxidases involved in the oxidative degradation of tyrosine:

- **4-hydroxyphenylpyruvate dioxygenase** catalyzes the oxidation and decarboxylation of the intermediate of tyrosine degradation, 4-hydroxyphenylpyruvic acid to homogentisic acid.
- **homogentisate 1,2-dioxygenase** catalyzes the next step in tyrosine degradation.

By impairing both reactions, vitamin C deficiency can result in tyrosinemia[87] and the excretion of tyrosine metabolites in the urine; both conditions respond to vitamin C supplements.

Transcriptional responses to hypoxia. A group of ascorbate-dependent prolyl 4-hydroxylases and an asparaginyl hydroxylase are involved in the regulation of transcriptional responses to hypoxic stress. Under normal cellular O_2 tensions, the hypoxia-inducible factor 1α (HIG-1α) is targeted for degradation by hydroxylation of proline and asparagine residues. However, under hypoxic conditions, it is not degraded but, instead, dimerizes with HIF-1β to induce hypoxia-responsive genes related to glycolysis, erythropoiesis, and angiogenesis.[88] Therefore, it is thought that, by this mechanism, vitamin C deficiency evokes the hypoxic response.

Vascular endothelial function. The functions of ascorbic acid in collagen synthesis, endothelial growth and survival, and radical scavenging make it important in supporting the vascular bed. Thus, it is thought to contribute to the prevention of endothelial dysfunction leading to inflammatory vascular disease, e.g., atherosclerosis.

Drug and steroid metabolism. Ascorbic acid is thought to be involved in microsomal hydroxylation reactions of drug and steroid metabolism, i.e., those coupled to the microsomal electron transport chain. In these roles, it is likely that ascorbic acid functions as a reducing agent to promote catalytic activity of iron-centered enzymes. The enzymes affected include **cholesterol 7α-hydroxylase**, the hepatic microsomal enzyme involved in the biosynthesis of bile acids; its activity is diminished in the chronically vitamin C-deficient guinea pig and is corrected by feeding vitamin C.[89] Epidemiologic studies have noted significant positive correlations of circulating levels of ascorbic acid and HDL–cholesterol in free-living humans.[90] Vitamin C deprivation reduces hepatic cytochrome *P*450-dependent drug metabolism in the guinea pig, increasing the half-lives of phenobarbital, acetanilide, aniline, and antipyrine. The activities of adrenal mitochondrial and microsomal

83. e.g., bombesin (human gastrin-releasing peptide), calcitonin, cholecystokinin, corticotropin-releasing factor, gastrin, growth hormone-releasing factor, melanotropins, metorphamide, neuropeptide Y, oxytocin, vasoactive intestinal peptide, and vasopressin.

84. This enzyme shares significant sequence homology with dopamine β-hydroxylase.

85. 3-Hydroxy-4-(trimethylazaniumyl)butanoate, which is required for the transport of fatty acids into mitochondria for oxidation to provide energy for the cell.

86. Rebouche, C.J., 1995. Metabolism 44, 1639–1643.

87. Transient tyrosinemia (serum levels >4 mg/dL) occurs frequently in premature infants and involves reduced 4-hydroxyphenylpyruvate dioxygenase activity. Low doses of ascorbic acid usually normalize the condition.

88. Bardos, J.I., Ashcroft, M., 2005. Biochim. Biophys. Acta 1755, 107–120.

89. Guinea pigs fed a high-vitamin C diet (500 mg/kg) show substantial reductions in plasma (~40%) and liver (~15%) cholesterol concentrations. Results of human studies addressing this point have been inconsistent.

90. A study of a healthy, elderly Japanese population found serum ascorbic acid to account for about 5% and 11% of the variation in serum HDL–cholesterol concentrations in men and women, respectively.

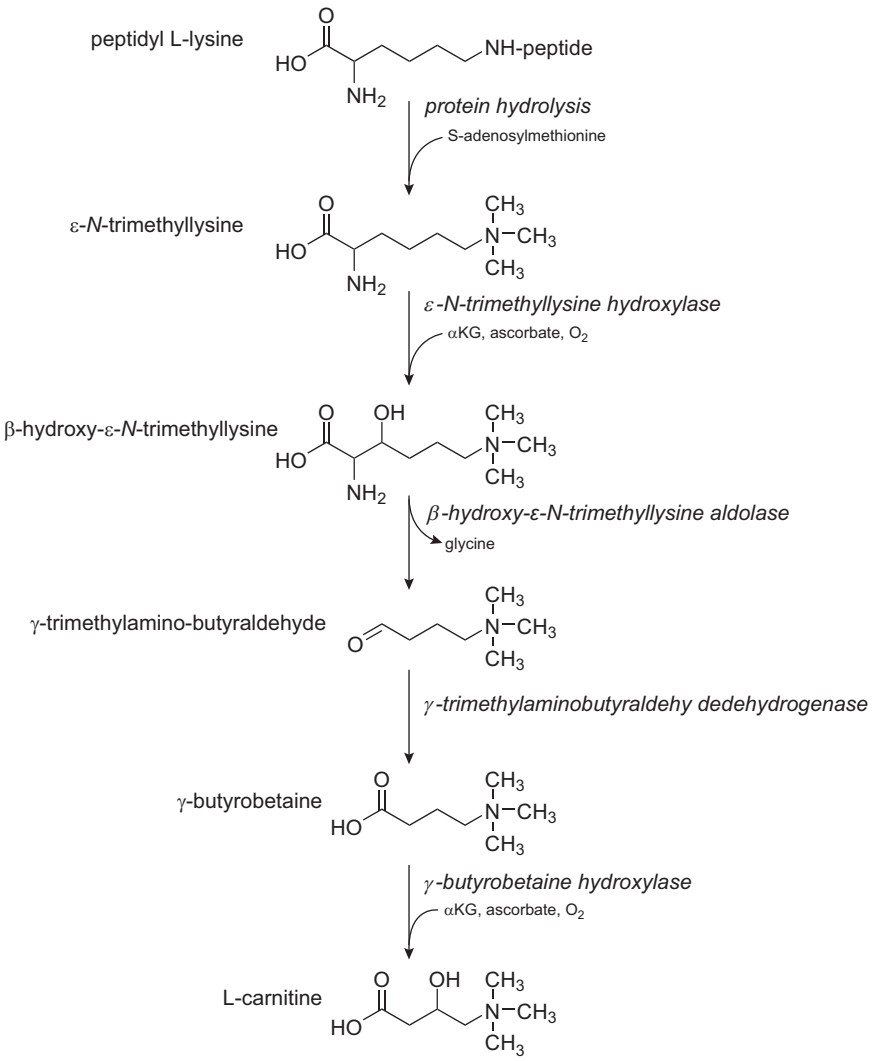


FIGURE 10.12 Ascorbate participates in two steps in the biosynthesis of carnitine from lysine: ε-N-trimethyllysine hydroxylase and γ-butyrobetaine hydroxylase.

steroid hydroxylases are impaired in scorbutic animals and respond to vitamin C therapy.

Immunity and Inflammation

Studies with animal and cell culture models have shown vitamin C to affect immune function in several ways:

- modulation of T cell expression of genes involved in signaling, carbohydrate metabolism, apoptosis, transcription, and immune function;[91]
- support of natural killer cell activity and production of interferons, the proteins that protect cells against viral attack;
- support of positive chemotactic and proliferative responses of neutrophils;
- protection against free radical-mediated protein inactivation associated with the oxidative burst[92] of phagocytic cells;
- support of the synthesis of humoral thymus factor and antibodies of the IgG and IgM classes;
- support of delayed-type hypersensitivity responses.

These functions appear to be affected by vitamin C status: compromised by deprivation of the vitamin; in at least some cases, stimulated by supranutritional doses of vitamin.

91. Grant, M.M., Mistry, N., Lunec, J., et al., 2007. Br. J. Nutr. 97, 19–26.

92. Neutrophils, when stimulated, take up O_2 and generate ROS, which, along with other reactive molecules, kill bacterial pathogens. This **oxidative burst** can be observed in vitro as a rapid consumption of O_2; it also involves the enzymatic generation of bactericidal halogenated molecules via myeloperoxidase. These killing processes are usually localized in intracellular vacuoles containing the phagocytized bacteria.

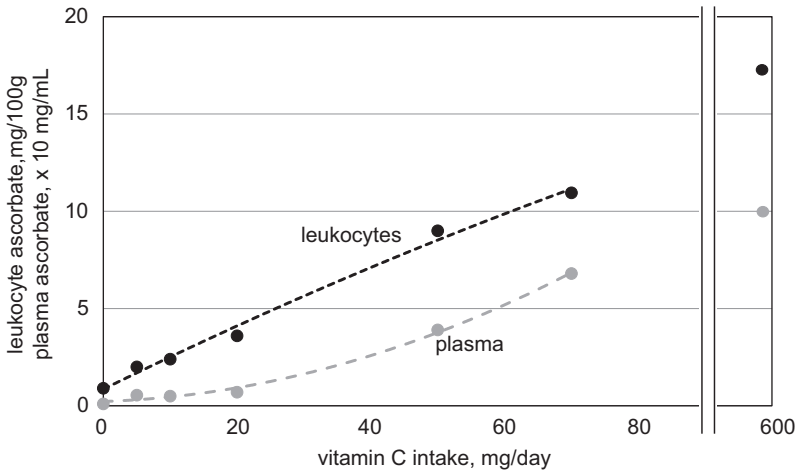


FIGURE 10.13 Relationships of leukocyte and plasma ascorbate concentrations with vitamin C intake. *Note:* leukocyte ascorbate concentrations are an order of magnitude greater than those of plasma. *After Bartley, W., Krebs, H.A., O'Brien, J.R.P., 1953. Special Rep. Series Med. Res. Council 280, 1–179.*

8. BIOMARKERS OF VITAMIN C STATUS

Vitamin C status can be assessed in two ways:

- **Leukocyte ascorbate**—Blood cells contain a substantial fraction of the ascorbic acid in the blood; of these, leukocytes (white cells) have the greatest diagnostic value, as their ascorbic acid concentrations are indicative of dietary intake and tissue levels of the vitamin (Fig. 10.13).[93] White cell ascorbic acid levels increase directly with increasing vitamin C intake, plateauing at vitamin C doses of about 2 g/day. The ascorbic acid contents of other blood cells also increase with vitamin C dose, with lymphocytes, platelets, monocytes, and neutrophils showing lower plateau in that order. Therefore, the use of this biomarker calls for careful preparation to ensure that the analysis is conducted using a uniform cell population.
- **Plasma/serum ascorbate**—These specimens offer the advantage of ease of preparation. Serum ascorbate concentrations of ~60 μM indicate tissue saturation, which requires regular vitamin C intakes of ~100 mg/day (Fig. 10.13). Higher intakes indicate elevated concentrations of the vitamin in extracellular fluids. The common interpretations of plasma ascorbate concentrations are shown in Table 10.10.

Dietary assessment is not recommended for assessment of vitamin C status, particularly for individuals. This approach suffers from the errors inherent in the methodologies for food intake/frequency recalls as well as losses of vitamin C from foods in storage, processing, and cooking.

93. Leukocyte ascorbate concentrations are usually greater in women than men and decrease in both sexes with age.

TABLE 10.10 Physiologic Significance of Plasma Ascorbate Concentration

Vitamin C Status	Plasma Ascorbate, mg/dL (μM)
Tissue ascorbate saturation	0.71–1.5 (40–85)
Adequacy	0.41–0.7 (23–40)
Hypovitaminosis	0.2–0.4 (11–22)
Clinical deficiency	<0.2 (<11)

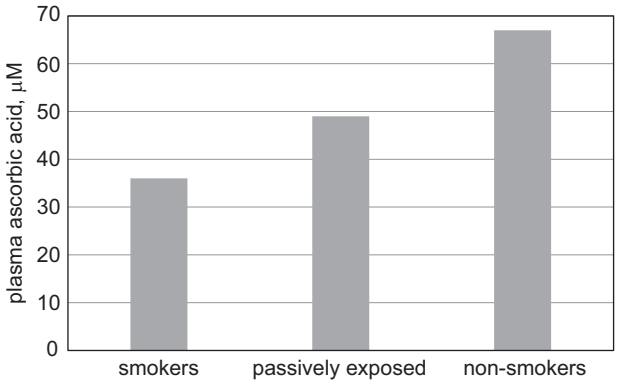


FIGURE 10.14 Effect of smoking on plasma ascorbic acid level. *After Tribble, D.L., Giuliano, L.J., Fortmann, S.P., 1993. Am. J. Clin. Nutr. 58, 886–890.*

Assessment of vitamin C status can be affected by factors unrelated to intake of the vitamin. These include smoking; even passive exposure to tobacco smoke can reduce plasma ascorbic acid concentrations (Fig. 10.14; Table 10.11). Glutathione-S-transferase (GST) genotype has also been found to affect plasma ascorbate level: individuals with the

TABLE 10.11 Effect of Passive Exposure to Tobacco Smoke on Vitamin C Status of Children

Age, Years	Plasma Ascorbic Acid, μM[a]	
	Unexposed	Exposed[b]
2–4	53.0 (50.2–55.8)	47.9 (44.4–51.5)
5–8	53.6 (51.4–55.8)	51.0 (48.5–53.5)
9–12	49.7 (47.4–52.0)	47.7 (45.5–49.9)

[a]*Mean (95% C.I.).*
[b]*A multifactorial ANOVA, with plasma ascorbic acid level adjusted for dietary vitamin C intake, showed the effect of passive exposure to be significant across all age groups, P = .002.*
Adapted from Preston, A.M., Rodriguez, C., Rivera, C.E., et al., 2003. Am. J. Clin. Nutr. 77, 167–172.

GST null genotype are more likely to be clinically deficient if their vitamin C intakes did not meet the Recommended Daily Allowance (RDA).[94]

9. VITAMIN C DEFICIENCY

Determinants of Risk

Vitamin C deficiency can be caused by low dietary intakes, as well as by conditions in which the metabolic demands for ascorbic acid may exceed the rate of its endogenous biosynthesis, thus, increasing the turnover of the vitamin in the body. Such conditions include smoking,[95] environmental/physical stress, chronic disease, and diabetes.

General Signs of Deficiency

In individuals unable to synthesize the vitamin, acute dietary C deficiency is manifested as various signs in the syndrome called scurvy (Table 10.12). The dominant clinical sign is prolonged wound healing time due to diminished rates of collagen synthesis as well as their increased susceptibility to infections. Dietary guidelines for nutritionally adequate intakes of vitamin C have been established (Table 10.13).

TABLE 10.12 General Signs of Vitamin C Deficiency

Organ System	Signs
General	Poor appetite, growth
	Impaired wound healing
	Painful, swollen gums with subsequent tooth loss
	Impaired immunity
	Ill-defined pain in extremities
Muscular	Skeletal muscle atrophy
Vascular	Increased capillary fragility, cutaneous hemorrhages
Nervous	Tenderness; impaired heat resistance

TABLE 10.13 Recommended Vitamin C Intakes

The United States		FAO/WHO	
Age–Sex	RDA[a], mg/day	Age–Sex	RNI[b], mg/day
0–6 months	25	0–6 months	–[c]
7–11 months	30	7–11 months	–[c]
1–6 years	30	1–6 years	30
7–9 years	35	7–9 years	35
10–18 years	40	10–18 years	40
>18 years	45	>18 years	45
Pregnancy	55	Pregnancy	55
Lactation	70	Lactation	70

[a]*Recommended Dietary Allowances; Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, 506 pp.*
[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. WHO, Rome, 286 pp.*
[c]*No value established.*

Deficiency Signs in Humans

Classic scurvy is manifest in adults after 60–90 days of stopping vitamin C consumption, although other signs can be seen by 30 days.[96] Scurvy presents when the total body pool of vitamin C is reduced to <300 mg from its normal level of ~1500 mg. At that low level, patients show plasma vitamin C levels of 0.13–0.25 mg/dL (normal levels are 0.8–1.4 mg/dL). Signs of the disease occur primarily in mesenchymal tissues. Defects in collagen formation are manifested as hemorrhage (due to deficient formation of intercellular substance) in the skin, mucous membranes, internal organs, and muscles; edema; impaired wound healing; and weakening of collagenous structures in bone, cartilage, teeth,

94. Cahill, L.E., Fontaine-Bisson, B., El-Sohemy, A., 2009. Am. J. Clin. Nutr. 90, 1411–1417.
95. Ascorbic acid concentrations of serum and urine of smokers tend to be about 0.2 mg/dL less than those of nonsmokers; these effects have been observed even after correcting for vitamin C intake, which was found to be about 53 mg/day less in smokers. Further, smokers have higher rates of vitamin C turnover (~100 mg/day) than nonsmokers (~60 mg/day). It is estimated that smokers require 52–68 mg *more* vitamin C per day than nonsmokers to attain comparable plasma ascorbic acid levels.
96. Levine, M., Wang, Y., Padayatty, S.J., et al., 2001. Proc. Nat. Acad. Sci. U.S.A. 98, 9842–9846.

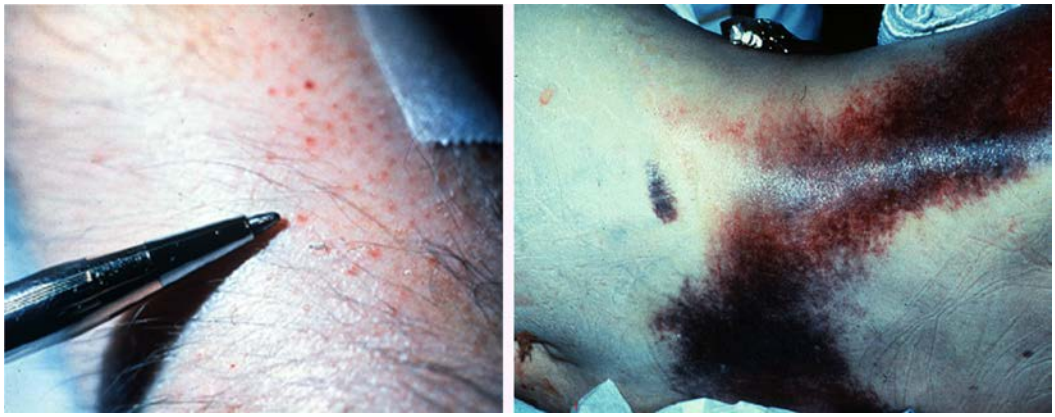


FIGURE 10.15 Hemorrhages in scorbutic patients: petechiae (left), ecchymoses (right). *Courtesy G.F. Combs, Sr.*

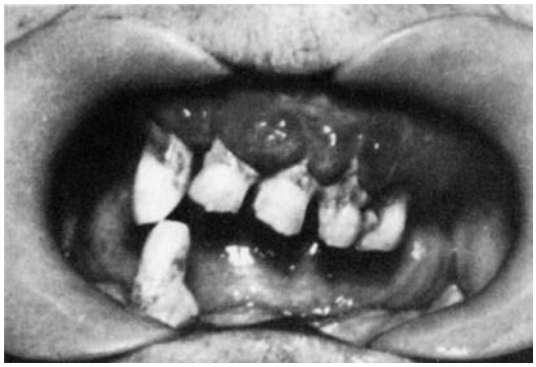


FIGURE 10.16 Scurvy in a middle-aged man. Note: swollen, bleeding gums, and tooth loss. *Courtesy J. Marks, Cambridge University.*

and connective tissues. Scorbutic adults may present with swollen, bleeding gums with tooth loss; that condition may signify accompanying periodontal disease. They also show lethargy, fatigue, rheumatic pains in the legs, muscular atrophy, skin lesions, and hemorrhages in many organs (e.g., skin, gums Fig. 10.16, intestines, subperiosteal tissues, eyes). Cutaneous hemorrhages start as pinpoint, perifollicular **petechiae**, which may coalesce to form large **ecchymoses** (Fig. 10.15). These features are frequently accompanied by psychological changes: hysteria, hypochondria, and depression. Experimental vitamin C deprivation studies conducted in the 1950s and 1960s indicated that the minimum daily dose of vitamin C that would prevent these signs was ≤10 mg.[97] In practice, scurvy is typically observed in subjects who are generally malnourished and show subclinical/clinical signs of thiamin, riboflavin, niacin, and/or pyridoxine hypovitaminoses.

Clinical scurvy was described in an Aboriginal infant who developed the signs at 7 months of age after having been breast-fed by her malnourished mother whose plasma and milk contained only 0.19 mg and 2 mg ascorbic acid per 100 mL, respectively.[98] Pediatric scurvy has also been reported as **Moller–Barlow disease** in nonbreast-fed infants usually at about 6 months of age (when maternally derived stores of vitamin C have been exhausted).[99] That disease is characterized by widening of bone–cartilage boundaries, particularly of the rib cage, stressed epiphyseal cartilage of the extremities, by severe joint pain and, frequently, by anemia and fever. Scorbutic children may present with limp or inability to walk, tenderness of the lower limbs, bleeding of the gums, and petechial hemorrhages.

Hypovitaminosis C. Subclinical deficiency is characterized by plasma ascorbate level <0.75 mg/dL and a total body vitamin C pool <600 mg. It often occurs in the elderly as the product of diminished enteric absorption and increased turnover. This can result in several nonspecific, prescorbutic signs and symptoms: lassitude, fatigue, anorexia, muscular weakness, and increased susceptibility to infection. Epidemiologic data indicate significant associations of low-plasma ascorbic acid concentration with increased risk of ischemic heart disease or hypertension.[100] Low-vitamin C status has been shown to be associated with increased risks of gestational diabetes and premature delivery due to premature rupture of chorioamniotic membranes. This responded to vitamin C supplementation.[101]

97. Bartley, W., Krebs, H.A., O'Brien, J.R.P., 1953. Special Rept. Series, Medical Res. Council. 280, 1–179; Hodges, R.E., Baker, E.M., Hood, J., et al., 1969. Am. J. Clin. Nutr. 22, 535–548; Baker, E.M., Hodges, R.E., Hood, J. et al., 1969. Am. J. Clin. Nutr. 22, 549–556.

98. Kamien, M., Nobile, S., Cameron, P., et al., 1974. Aust. N.Z. J. Med. 4, 126–133.

99. A retrospective study of the 28 cases diagnosed in at the Queen Sirikit National Institute of Child Health from 1995 to 2002, 93% were 1–4 years. of age (Ratanachu-Ek, S., Sukswai, P., Jeerathanyasakun, Y., et al., 2003. J. Med. Assoc. Thai. 86, S734–S740).

100. Patients with each disease are typically of relatively low status with respect to vitamin E and/or copper. In the case of hypertension, a placebo-controlled, double-blind study showed that vitamin C supplements (1 g/day for 3 mos.) significantly reduced systolic and diastolic pressures in borderline hypertensive subjects with normal serum ascorbic acid levels.

101. Casaneuva, E., Ripoll, C., Tolentino, M., et al., 2005. Am. J. Clin. Nutr. 81, 859–863; Borna, S., Borna, H., Daneshbodie, B., 2005. Int. J. Gynecol. Obstet. 90, 16–20.

Hypovitaminosis C appears to compromise the activity of cholesterol 7-α-hydroxylase, the rate-limiting step in the conversion of cholesterol to bile acids. In the guinea pig, this can result in the overproduction of the glycoprotein mucin, apparently as a result of oxidative stress, and the formation of gallstones. Analyses of NHANES data have associated relatively low-serum ascorbic acid concentrations with increased risk of forming gallstones in women (but, curiously, not men). That relationship was U-shaped with the highest prevalence of self-reported gallbladder disease for women with serum ascorbic acid levels in the range of 0.7–1.5 mg/dL.[102] In contrast, an analysis of data from NHANES III by the same group showed a consistent inverse relationship of serum ascorbic acid and gallstone incidence, with vitamin C supplement use being independently associated with a 34% lower prevalence of disease.[103]

Responses to Vitamin C Treatment

Response to vitamin C is dramatic; clinical improvements are seen within a week of supranutritional vitamin C therapy. A repletion study with hypovitaminotic C adults found their serum ascorbate levels to return from nondetectable to tissue saturation levels (~60 μM) within 3 days when given a daily supplement of 1 g of the vitamin; this was after 3 days of a 300 mg/day supplement, which had failed to register a plasma response.[104] Topical application of ascorbic acid has been found useful in treating photodamaged skin, as well as inflammatory conditions of the skin such as acne and eczema.

Deficiency Signs in Animals

In guinea pigs, ascorbic acid deficiency is characterized by intermittent reductions in growth, hematomas (especially of the hind limbs), extremely brittle bones, and abnormalities of epiphyseal bone growth with calcification of bone–cartilage boundaries. Guinea pigs that are deprived of vitamin C also show reduced feed intake and growth, anemia, hemorrhages, altered dentin, and gingivitis. Continued deficiency results in disrupted protein folding and apoptosis in the liver. If not corrected, death usually occurs within 25–30 days. Ascorbic acid deficiency in at least some species of fishes (salmonids and carp) results in spinal curvature (scoliosis[105] and lordosis[106] Fig. 10.17), reduced survival, reduced growth rate, anemia, and hemorrhaging, especially in the fins, tail, muscles, and eyes. Similar signs have been reported in vitamin C-deficient shrimp and eels.

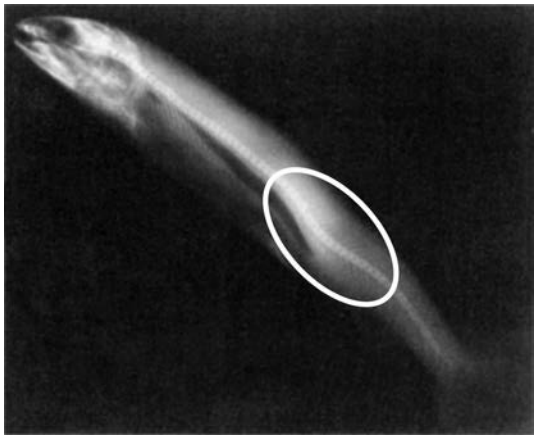


FIGURE 10.17 Radiograph of a vitamin C-deficient trout. Note: lordosis. *Courtesy G.L. Rumsey, Tunison Laboratory of Fish Nutrition, USDI.*

Hypovitaminosis C in animals. Subclinical deficiency in the guinea pig can result in lassitude, anorexia, muscular weakness, loss of reduced glutathione in lymphocytes, loss of α-tocopherol with accumulation of lipid peroxidation products in retinal tissues, hypertriglyceridemia, hypercholesterolemia, and decreased vitamin E concentrations in liver and lungs. These signs respond to vitamin C supplementation.

10. VITAMIN C IN HEALTH AND DISEASE

Vitamin C intakes greater than 100–200 mg/day result in elevated concentrations of the vitamin in extracellular fluids (plasma, connective tissue fluid, humors of the eye). Under such conditions, pharmacologic actions of this antioxidant vitamin may be possible. Clinical studies have found such supranutritional doses of vitamin C to be of some benefits; however, many such studies have compared treated subjects with controls who did not have tissue saturation with the vitamin. Thus, while there appear to be benefits associated with increasing vitamin C intakes to levels that support tissue saturation, evidence supporting benefits of vitamin C intakes above that level is less clear.

Antioxidant Effects

High doses of vitamin C can reduce markers of oxidative stress, which has been implicated as a central mechanism in the development of obesity-related diseases (i.e., cardiovascular disease and type 2 diabetes), and a cause of chronic obstructive pulmonary disease and Alzheimer's disease. Clinical interventions with supranutritional doses

102. Simon, J.A., Hudes, E.S., 1998. Am. J. Pub. Health 88, 1208.
103. Simon, J.A., Hudes, E.S., 2000. Arch. Int. Med. 160, 931.
104. Nobile, S., Woodhill, J.M., 1981. Vitamin C: The mysterious redox-system, a trigger of life?. MTD Press, Lancaster, p. 78.
105. Lateral curvature of the spine.
106. Anteroposterior curvature of the spine.

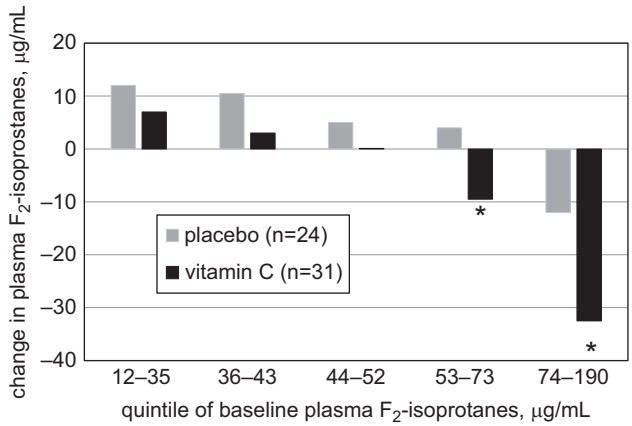


FIGURE 10.18 High-dose vitamin C (1000 mg/day) reduced of plasma F2-isoprostane concentrations in adults showing the highest baseline values. *After Block, G., Jensen, C.D., Morrow, J.D., et al., 2008. Free Rad. Biol. Med. 45, 377–384.*

of vitamin C have been found to reduce plasma concentrations of F_2-isoprostanes, prostaglandin-like products of nonenzymatic free radical attack on PUFAs. These effects are greatest for individuals with relatively high indicators of oxidative stress (Fig. 10.18) and have been found to be affected by factors that contribute to that oxidative stress, e.g., high body mass index (BMI) and smoking.[107]

Antihistamine Effects

High doses of vitamin C can reduce circulating histamine concentrations. On this basis, vitamin C has been used to protect against histamine-induced anaphylactic shock. Ascorbic acid inhibits histamine release and enhances its degradation. It does so by undergoing oxidation to dehydroascorbic acid with the concomitant rupture of the histamine imidazole ring. In cultured cells, this reduces endogenous histamine levels as well as histidine decarboxylase activities, a measure of histamine synthetic capacity. Ascorbic acid can also enhance the synthesis of the prostaglandin E series, members of which mediate histamine sensitivity. Blood histamine concentrations are elevated in several complications of pregnancy associated with marginal ascorbic acid status: preeclampsia,[108] abruption,[109] and prematurity. A large, cross-sectional study indicated that plasma ascorbic acid was inversely associated with biomarkers of inflammation (C-reactive protein) and endothelial dysfunction (tissue plasminogen activator), suggesting that the vitamin has antiinflammatory effects associated with reduced levels of endothelial dysfunction (Table 10.14). Other studies have not observed antiinflammatory effects of vitamin C given to patients.

Common cold. The most widely publicized uses of "megadoses" of vitamin C are in prophylaxis and treatment of the common cold. Large doses (≥1 g) of vitamin C have been advocated for prophylaxis and treatment of the common cold, a use that was first proposed some 25 years ago by Irwin Stone and the Nobel laureate Dr Linus Pauling.[110] Since that time, many controlled clinical studies have been conducted to test that hypothesis (Table 10.15). Whereas many of these have yielded positive results, until recently few have been appropriately designed with respect to blinding, controls, treatment randomization, and statistical power, to make such conclusions unequivocal. In general, most results of well-controlled trials have indicated only small positive effects in reducing the incidence, shortening the duration, and ameliorating the symptoms of the common cold.[111] A meta-analysis of six large clinical trials (including more than 5000 episodes) showed no detectable effects of gram doses of vitamin C on cold incidence, but some evidence of small, protective effects in some subgroups of subjects.[112] A later meta-analysis of 29 randomized, controlled trials noted a consistent benefit of vitamin C supplementation (≥200 mg/day) in reductions of cold duration by 8% in adults and 13.5% in children.[113]

Other Infections

The results of clinical intervention trials with vitamin C on other infections have been inconsistent. Some studies with scorbutic guinea pigs, fishes, and rhesus monkeys have shown vitamin C deficiency to decrease resistance to infections,[114] but several studies have yielded negative results. Studies of ascorbic acid supplementation of species that do not require the vitamin (rodents, birds) have generally shown improved resistance to infection as indicated by increased survival of infected animals, depressed parasitemia, enhanced bacterial clearance, and reduced duration of infection. Several randomized trials

107. Dietrich, M., Block, G., Hudes, M., et al., 2002. Cancer Epidemiol. Biomar. Prev. 11, 7–13; Dietrich, M., Block, G., Benowitz, N.L., et al., 2003. Nutr. Cancer 45, 176–184; Block, G., Jensen, C.D., Morrow, J.D., et al., 2008. Free Rad. Biol. Med. 45, 377–384.
108. The nonconvulsive stage of an acute hypertensive disease of pregnant and puerperal (after childbirth) women.
109. Premature detachment of the placenta.
110. Pauling received two Nobel Prizes: Chemistry, 1954; Peace, 1962.
111. Chalmers, T.C., 1975. Am. J. Med. 58, 532–536.
112. Hemilä, H., 1997. Br. J. Nutr. 77, 59–72.
113. Douglas, R.M. Hemila, H., Chalker, E., et al., 2007 Cochrane Database Syst. Rev. CD000980.
114. e.g., *Mycobacterium tuberculosis*, *Rickettsiae* spp., *Entamoeba histolytica*, and other bacteria, as well as *Candida albicans*.

TABLE 10.14 Relationship of Vitamin C Status and Biomarkers of Inflammation and Endothelial Function

	Quartile[a] of Plasma Ascorbic Acid, μM				
Biomarker	**<14.44**	**14.44–27.11**	**27.11–40.25**	**>40.25**	***p* Value**
CRP[b], mg/L	1.88 (1.73–2.03)[c]	1.73 (1.60–1.80)	1.52 (1.40–1.63)	1.34 (1.23–1.44)	<0.001
Fibrinogen, g/L	3.30 (3.26–3.36)	3.29 (3.24–3.34)	3.18 (3.13–3.23)	3.12 (3.07–3.17)	<0.001
t-PA[d], ng/mL	10.92 (10.63–11.21)	10.66 (10.38–10.93)	10.70 (10.42–10.99)	10.31 (10.03–10.60)	0.01
Blood viscosity, mPa	3.41 (3.39–3.44)	3.41 (3.39–3.43)	3.40 (3.38–3.43)	3.35 (3.33–3.37)	<0.001

[a]*3019 Subjects.*
[b]*C-reactive protein.*
[c]*Mean (95% confidence interval).*
[d]*Tissue plasminogen activator.*
Adapted from Wannamethee, S.G., Lowe, G.D., Rumley, A., et al., 2006. Am. J. Clin. Nutr. 83, 567–574.

TABLE 10.15 Large-Scale, Placebo-Controlled Clinical Trials Do Not Show Vitamin C Protection From Colds

	Vitamin C, g/day months		Vitamin C Group		Placebo Group		
Study			**n**	**Colds/p/year**	**N**	**Colds/p/year**	**RR (95% CI)**
1	1	3	407	5.5	411	5.9	0.93 (0.83–1.04)
2	1	3	339	6.7	349	7.2	0.93 (0.84–1.04)
3	3	9	101	1.7	89	1.8	0.93 (0.73–1.20)
4	2	2	331	11.8	343	11.8	1.00 (0.90–1.12)
5	1	3–6	265	1.2	263	1.2	1.03 (0.80–1.32)
6	1	3	304	8.6	311	8.0	1.08 (0.97–1.21)

Adapted from Hemilä, H., 1997. Br. J. Nutr. 77, 59–72.

have found vitamin C administered to nondeficient individuals to reduce incidence[115] and/or severity[116] of infections other than colds (Table 10.16):

- **Heliobacter pylori**. Randomized trials have shown that vitamin C supplementation can reduce seropositivity for *H. pylori*[117] and protect against the progression of gastric atrophy in seropositive patients.[118] This may be associated with reduced gastric cancer risk for which *H. pylori* is a risk factor.
- **Herpes**. Topical application of ascorbic acid reduced the duration of lesions as well as viral shedding in patients with *Herpes simplex* infections.[119]

Cardiovascular Health

The antioxidant characteristics of ascorbic acid allow it to have an antiatherogenic function in reducing the oxidation of LDLs, a key early event leading to atherosclerosis.[120] Being rich in both cholesterol and PUFAs, LDLs are susceptible to lipid peroxidation by the oxidative attack of ROS. Research has shown that oxidized LDLs stimulate the recruitment, in the subendothelial space of the vessel wall, of monocyte–macrophages that can take up the oxidized particles via scavenger receptors[121] to form the lipid-containing foam

115. e.g., Posttransfusion hepatitis, pneumonia, tuberculosis, pharyngitis, laryngitis, tonsillitis, secondary bacterial infections after a common cold episode, and rheumatic fever.
116. e.g., *Herpes labialis*, bronchitis, tonsillitis, rubella, and tuberculosis.
117. Simon, J.A., Hudes, E.S., Perez–Perez, G., et al., 2003. J. Am. Coll. Nutr. 22, 283–289.
118. Sasazuki, S., Sasaki, S., Tsubono, Y., et al., 2003. Cancer Sci. 94, 378–382.
119. Hamuy, R., Berman, B., 1998. Eur. J. Dermatol. 8, 310–319.

120. **Atherosclerosis** is the focal accumulation of acellular, lipid plaques in the intima of the arteries. The subsequent infiltration by fatty substances (**arteriosclerosis**) and calcific plaques and the consequent reduction in the vessel's lumenal cross-sectional area reduce blood flow to the organs served by the affected vessel, causing such symptoms as angina, cerebrovascular insufficiency, and intermittent claudication.
121. Monocyte–macrophages have few LDL receptors, which are downregulated. When incubated with nonoxidized LDLs they do not form foam cells, as the accumulation of cholesterol further reduces LDL receptor activity. On the other hand, these cells have a specific **scavenger receptor** for modified LDLs. LDL lipid peroxidation products may react with amino acid side chains of apoB to form epitopes with affinities for the scavenger receptor.

TABLE 10.16 Results of Placebo-Controlled, Double-Blinded Studies of Vitamin C and Noncold Infections

Study	Infection	Vitamin C, g/day	Cases/Total Subjects		OR (95% CI)
			Vitamin C	Placebo	
Studies of Infection Incidence					
1[a]	Hepatitis	3.2	6/90	8/85	0.69 (0.26–1.80)
2[b]	Pneumonia	2	1/331	7/343	0.15 (0.01–0.74)
3[c]	Bronchitis	1	8/139	13/140	0.60 (0.27–1.30)
4[c]	Pharyngitis laryngitis and tonsillitis	1	7/139	14/140	0.48 (0.21–1.10)

Study	Infection	Vitamin C, g/day	Outcome	Value (n)	
				Vitamin C	Placebo
Studies of Infection Severity					
5[d]	*Herpes labialis*	0.6	Days healing	4.21 ± 7[f] (19)	9.72 ± 8 (10)
		1.0	Days healing	4.4 ± 3.9 (19)	9.72 ± 8 (10)
6[e]	Bronchitis	0.2	Decreased score	3.4 ± 1.8 (28)	2.3 ± 2.5 (29)
7[a]	Hepatitis	3.2	SGOT[g] units	474 ± 386 (6)	759 ± 907 (8)

[a]*Kodell, R.G., et al., 1981. Am. J. Clin. Nutr. 34, 20–23.*
[b]*Pitt, H.A., Costrini, A.M., 1996. J. Am. Med. Assoc. 241, 908–911.*
[c]*Ritzel, G., 1961. Helv. Med. Acta 28, 63–68.*
[d]*Terezhalmy, T., et al., 1978. Oral Surg. 45, 56–62.*
[e]*Hunt, C. et al., 1994. Int. J. Vit. Nutr. Res. 64, 212–219.*
[f]*Significantly different from control value,* $p < .05$.
[g]*Serum glutamic–oxaloacetic transaminase; now referred to as aspartate aminotransferase (AST).*

cells found in the early stages of atherogenesis. According to this view, atherogenesis can be reduced by protecting LDLs from free-radical attack, which would appear also to involve both vitamin E in quenching radicals produced in the hydrophobic interior of the LDL particle and ascorbate in recycling vitamin E.

Epidemiologic studies have found cardiovascular disease risk to be inversely associated with vitamin C status. Plasma ascorbic acid concentrations were negatively correlated with plasma malonyldialdehyde concentration and several cardiovascular risk factors: blood pressure (Fig. 10.19), total serum cholesterol, and LDL–cholesterol.[122] An intervention trial found vitamin C supplementation to reduce blood pressure and improve arterial stiffness in patients with noninsulin-dependent diabetes. It has been suggested that ascorbic acid may serve to protect cell membrane pumps from oxidative damage in such ways as to promote ion flux and enhance the vasoactive characteristics of blood vessels.

An analysis of the cohort examined in NHANES II showed that individuals with serum ascorbic acid levels associated with tissue saturation (≥1 mg/dL or ≥60 μM) had risk

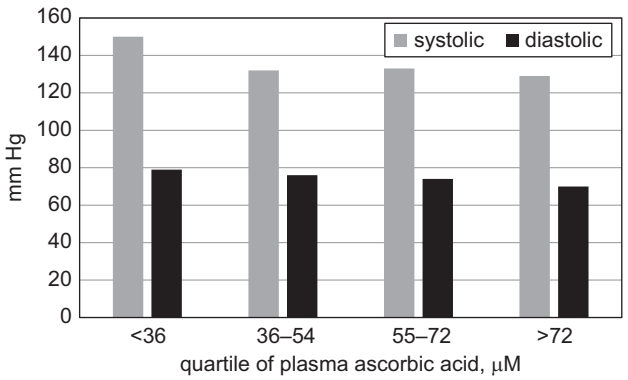


FIGURE 10.19 Relationship between blood pressure and plasma ascorbic acid. *After Choi, E.S.K., Jacques, P.F., Dallai, G.E., et al., 1991. Nutr. Res. 11, 1377–1385.*

reductions of 21–27% for coronary heart disease incidence, stroke incidence, and cardiovascular deaths compared to individuals with lower levels of the vitamin. Serum ascorbic acid levels were associated with a 52% reduction in risk to angina without reductions in myocardial infarction or stroke.[123] Two large prospective studies in Japan and Finland found

122. Toohey, L., Harris, M.A., Allen, K.G., et al., 1996. J. Nutr. 126, 121–128.

123. Simon, J.A., Hudes, E.S., Browner, W.S., 1998. Epidemiology 9, 316–321; Simon, J.A., Hudes, E.S., Tice, J.A., 2001. J. Am. Coll. Nutr. 3, 255–263.

TABLE 10.17 Relationship of Vitamin C Intake and Relative Risk of Coronary Heart Disease: Pooled Results of Nine Studies

Vitamin C Intake, mg/day	Relative Risk[a]
0	1.00
<100	0.97 (0.80–1.18)[b]
100–399	0.82 (0.57–1.18)
400–699	0.94 (0.68–1.32)
≥700	0.75 (0.58–0.98)

[a]*Incidence and mortality.*
[b]*95% C.I.*
Adapted from Knekt, P., Ritz, J., Pereira, M.A., et al., 2004. Am. J. Clin. Nutr. 80, 1508–1520.

serum ascorbic acid levels >0.8 mg/dL to be associated with 30–50% reductions in risks to cerebral infarction and hemorrhagic stroke; these effects were not associated with supplement use, suggesting a role of vitamin C-containing foods, perhaps unrelated to the vitamin per se.[124] Still, protective effects of relatively high vitamin C intakes and, particularly, vitamin C supplement use, are apparent. Individuals in the NHANES I cohort with the highest vitamin C intakes showed 34% less fatal cardiovascular disease compared to subjects with lower estimated vitamin C intakes.[125] A pooled analysis of nine cohorts found fatal coronary heart disease to be reduced by 25% among individuals with vitamin C intakes ≥700 mg/day (Table 10.17).[126] A large, prospective study found that Japanese adults with serum ascorbate concentrations >45 μM had 30–50% less risk of stroke compared to subjects with lower serum levels.[127]

Pulmonary Function

Whether supplemental vitamin C can benefit pulmonary function is not clear. Population studies have found high vitamin C intake to be associated with improved pulmonary function.[128,129] The results of five trials have suggested that vitamin C intake may be inversely related to susceptibility to pneumonia. Three controlled trials have found vitamin C supplements effective in preventing pneumonia; two found that treatment effective in reducing the symptoms of that condition.[130] Half of the dozen clinical intervention trials of vitamin C to date have found improvements in parameters of respiratory function of asthma patients; but these studies have been very small (fewer than 160 patients total). A meta-analysis of randomized, controlled trials revealed no evidence to support the use of vitamin C in the management of asthma.[131]

Anticarcinogenesis

Ascorbic acid has been observed to reduce the binding of polycyclic aromatic carcinogens to DNA and to reduce/delay tumor formation in several animal models. This effect is thought to involve quenching of radical intermediates of carcinogen metabolism. Ascorbic acid is also a potent inhibitor of nitrosamine-induced carcinogenesis, functioning as a nitrite scavenger. This action results from ascorbate reduction of nitrate (the actual nitrosylating agent of free amines) to NO, blocking the formation of nitrosamines.[132] Evidence indicates that ascorbic acid, normally secreted in relatively high concentrations in gastric juice,[133] is a limiting factor in nitrosation reactions, particularly in individuals with gastric pathologies affecting secretion.

Some malignant cells appear to be particularly sensitive to ascorbic acid, namely, those that overexpress GLUT1. Studies have demonstrated this phenomenon for cancer cells with KRAS or BRAF[134] mutations, rapidly take up dehydroascorbic acid.[135] That form can be oxidized to ascorbate to increase ROS and deplete GSH and NADPH. It can also react directly with homocysteine thiolactone, overproduced by cancer cells, converting the latter to mercaptopropionaldehyde, which is lethal to cancer cells.[136]

Protective effects of dietary vitamin C have been detected in two-thirds of the epidemiologic studies in which a dietary vitamin C index has been calculated. In several cases, high vitamin C intake was associated with half the cancer risk associated with low intake. Protective effects have also been detected in a similarly high proportion of studies in which the intake of fruit, but not vitamin C, was assessed. Prediagnostic plasma ascorbate has been found to be inversely associated with risk of gastric adenocarcinoma in a Chinese cohort.[137] Clinical reports have indicated that high doses (10–60 g) can be useful in raising plasma ascorbic acid to levels (*c.*20 mM) capable

124. Yokoyama, T., Date, C., Kokubo, Y., et al., 2000. Stroke 31, 2287–2294; Kurl, S., Tuomainen, T.P., Laukkanen, J.A., et al., 2002. Stroke 33, 1568–1573.
125. Enstrom, J.E., Kanim, L.E., Klein, M.A., 1992. Epidemiology 5, 194–202.
126. Knekt, P., Ritz, J., Pereira, M.A., et al., 2004. Am. J. Clin. Nutr. 80, 1508–1520.
127. Yokoyama, T., Date, C., Kokubo, Y., et al., 2000. Stroke 31, 2287–2294.
128. i.e., Improved forced expiratory volume and improved forced vital capacity.
129. Hu, G., Cassano, P.A., 2000. Am. J. Epidemiol. 151, 975–981.
130. Hemila, H., Louhiala, P., 2007. J. Roy. Soc. Med. 100, 495–498.
131. Ram, F.S., Rowe, B.H., Kaur, B., 2004. Cochrane Database Syst. Rev. 3, CD00993.
132. Vitamin E also has this effect.
133. The concentration of ascorbic acid in gastric juice has often been found to exceed those in the plasma.
134. The *KRAS* and *BRaf* genes encode proteins that function in signaling and directing cell growth.
135. Yun, J., Mullarky, E., Lu, C., et al., 2015. Science 350, 1391–1396.
136. Toohey, J.I., 2008. Cancer Lett. 263, 164–169.
137. Lam, T.K., Freedman, N.D., Fan, J.H., et al., 2013. Am. J. Clin. Nutr. 98, 1289–1297.

TABLE 10.18 Adrenal Depletion of Ascorbic Acid in Laying Hens Under Simulated Adrenal Stress

Treatment	Renal Ascorbate µg/g	Adrenal Ascorbate µg/g	Adrenal Cholesterol mg/g	Adrenal Corticosterone µg/g	Serum Corticosterone µg/L
Control	1.41 ± 0.10	1.02 ± 0.05	6.93 ± 0.25	18 ± 2	4.8 ± 0.4
ACTH[a]	1.14 ± 0.14*[c]	0.77 ± 0.04*	2.57 ± 0.36*	32 ± 3*	4.4 ± 0.4
Dex[b]	1.30 ± 0.06*	0.82 ± 0.08*	8.02 ± 0.83*	17 ± 2	2.4 ± 0.9*

[a]*ACTH, adrenocorticotropic hormone, 2.5 IU/day.*
[b]*Dex, dexamethasone (a suppressor of adrenal corticosterone production), 50 µg/day.*
[c]*Significantly different from control,* $p < .05$.
Adapted from Rumsey, G.L., 1969. Studies of the Effects of Simulated Stress and Ascorbic Acid upon Avian Adrenocortical Function and Egg Shell Metabolism. (Ph.D. thesis). Cornell University, Ithaca, New York.

of killing cancer cells in vitro and in improving outcomes of three small series of cancer patients.[138] A meta-analysis of 10 studies with a total of almost 18,000 subjects found that postdiagnostic supplementation with vitamin C increased survival of breast cancer patients by 15%. For a 100 mg per day increase in vitamin C intake, all-cause mortality was reduced by 27%, and breast cancer mortality was reduced by 22%.[139]

Oxidative Stress

Ischemia–reperfusion injury. Tissues sustain injury upon reperfusion after a period of ischemia, a phenomenon with particular relevance to the preservation of organs for transplantation. ROS are thought to contribute to milder forms of tissue injury at the time of reperfusion (e.g., myocardial stunning, reperfusion arrhythmias); however, it is not clear the extent to which free radicals may also be responsible for the acute tissue damage seen under those circumstances. Vitamin C has been shown to be protective against ischemia–reperfusion injury in animal models,[140] and a randomized trial with men with peripheral artery disease found that intra-arterial administration of ascorbic acid (24 mg/min) completely prevented experimental ischemia–reperfusion-induced endothelial dysfunction.[141]

Exercise. Vigorous physical activity increases ventilation rates and produces oxidative stress,[142] which is thought to affect endothelial function. Studies have shown that antioxidant supplementation can alleviate muscle damage and protein oxidation induced by exercise. Vitamin C treatment prevented acute endothelial dysfunction induced by exercise in patients with intermittent claudication[143] (calf pain during walking). This effect is likely due to the protection of nitric oxide (NO), which mediates endothelium-dependent vasodilation.

Metabolically produced ROS also appear to have essential metabolic functions as signaling molecules for the adaptation of skeletal muscle to accommodate the stresses presented by exercise training or periods of disuse. This signaling involves redox-sensitive kinases, phosphatases, and NF-κB, which affect the rate of mitochondrial biogenesis, as well as the induction of genes related to insulin sensitivity and ROS defense. This system of adaptive responses to oxidative stress facilitates the ultimate development of long-term resistance to that stress. This system, which has been called mitochondrial **hormesis**,[144] can be impaired by high-level antioxidant treatment. Combined supplements of vitamin C (500 mg/day ascorbic acid) and E (400 IU/day α-tocopheryl acetate) blocked the upregulation of muscle glucose uptake otherwise induced by exercise in untrained subjects.[145] This finding raises questions as to what level of "redox tone" may be beneficial.

Environmental stress. Vitamin C deficiency can have benefits under conditions of environmental stress in which the metabolic demands for ascorbic acid may exceed the rate of its endogenous biosynthesis. This is the case in commercial poultry production. Although the chicken does not require the vitamin in the classic sense, under practical conditions of poultry management, the species frequently benefits from ascorbic acid supplements[146] under stressful environmental conditions (e.g., extreme temperature, prevalent disease, crowding, inadequate ventilation) that stimulate adrenal depletion of ascorbic acid (Table 10.18). Controlled experiments with laying hens have shown that supplemental ascorbic acid can improve egg production and eggshell characteristics in laying hens subjected to heat stress.

138. Cameron, E., Pauling, L., 1978. Proc. Natl. Acad. Sci. U.S.A. 75, 4538–4342; Drisko, J., Chapman, J., Hunter, V., 2003. J. Am. Coll. Nutr. 22, 118–123; Padayatty, S., Riordan, H. Hewitt, S., et al., 2006. CMAJ 174, 937–942.
139. Harris, H.R., Orsini, N., Wolk, A., 2014. Eur. J. Cancer 50, 1223–1231.
140. Bailey, D.M., Raman, S., McEneny, J., et al., 2006. Free Rad. Biol. Med. 40, 591–600.
141. Pleiner, J., Schaller, G., Mittermayer, F., et al., 2008. Atherosclerosis 197, 383–391.
142. O_2 utilization increases 10- to 15-fold during exercise.
143. Silvestro, A., Scopacasa, F., Oliva, G., et al., 2002. Atherosclerosis 165, 277–283.
144. Hormesis is the term for a generally favorable biological response to low exposures to stressors/toxins.
145. Ristow, M., Zarse, K., Oberbach, A., et al., 2009. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc. Nat. Acad. Sci. U.S.A. 106, 8665–8670.
146. e.g., 150 mg/kg diet.

TABLE 10.19 Recommended Upper Tolerable Intakes (ULs) of Preformed Vitamin C

Ages, Years	UL, mg/day
<1	–[a]
1–3	400
4–8	650
9–13	1200
14–18	1800
>18 years	2000
Pregnancy	
≤18 years	1800
>18 years	2000
Lactation	
≤18 years	1800
>18 years	2000

[a]*No value established.*
Adapted from Food and Nutrition Board, 2000. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids. National Academy Press, Washington, DC, 506 pp.

11. VITAMIN C TOXICITY

No significant adverse effects of ascorbic acid, its various salts and esters, have been identified.[147] High doses of the vitamin have been used both orally and intravenously without incident. Some subjects taking megadoses of the vitamin have reported gastrointestinal disturbances and diarrhea. Upper tolerable intakes for vitamin C have been established (Table 10.19).

Little information is available on vitamin C toxicity in animals, although acute LD_{50} (50% lethal dose) values for most species and routes of administration appear to be at least several grams per kilogram of body weight. A single study showed mink to be very sensitive to hypervitaminosis C, with daily intakes of 100–200 mg of ascorbic acid, pregnant females developed anemia and had reduced litter sizes.

Questions have been raised as to whether megadoses of vitamin C may have risks. Evidence has shown that most, if not all, of these concerns are not warranted.

- **Urinary oxalates**. In humans, unlike other animals, oxalate is a major metabolite of ascorbic acid, which accounts for 35–50% of the 35–40 mg of oxalate excreted in the urine each day.[148] Metabolic studies have demonstrated that healthy individuals convert <1.5% of ingested ascorbic acid to oxalic acid each day. Even when the vitamin was administered intravenously, <0.5% was excreted as <0.5% as oxalate.[149] Still, a case of urolithiasis in a 9-year-old boy was reported. The subject showed extremely high-urinary oxalate concentrations after having been given high doses of vitamin C since age 3. Prohibition of vitamin C supplements reduced his oxalate excretion to normal levels with no recurrence of symptoms for the duration of follow-up (3 years).[150] Some clinical studies have found multiple gram doses of vitamin C to produce oxaluria of low magnitude and within normal variation.[151] While such effects are unlikely to increase risk of forming urinary calculi, prudence dictates avoiding vitamin C doses >1000 mg for individuals with histories of renal stones.
- **Uricosuria**. Ascorbic acid and uric acid are both reabsorbed by the renal tubules, perhaps by the same SCVT. If so, then high levels of ascorbic acid might competitively inhibit uric acid reabsorption. Evidence does not support that hypothesis. A randomized trial with healthy subjects showed that vitamin C (500 mg/day) significantly reduced serum uric acid concentrations and increased the glomerular filtration rate.[152]
- **Vitamin B_{12} stability**. In the 1970s, it was claimed that high levels of ascorbic acid added to test meals resulted in the destruction of food vitamin B_{12}. Studies have not supported that prospect. In fact, the only cobalamin that is sensitive to reduction by ascorbic acid is aquocobalamin, which is not a major form of the vitamin in foods. Several clinical investigations have found high doses of vitamin C not to affect vitamin B_{12} status.
- **Iron overload?** That ascorbic acid can enhance the enteric absorption of dietary iron has led to questions as to whether megadoses of the vitamin C might promote iron accumulation in iron-replete individuals. Such an effect is not expected, as optimal iron absorption is effected with much lower doses of vitamin (25–50 mg per meal). Studies in mice have found ascorbic acid not to enhance prooxidative effects induced by dietary iron; nor did high parenteral doses of ascorbic acid increase prooxidative biomarkers in human subjects.[153] Nevertheless, patients with

147. Hathcock, J.N., Azzi, A., Blumberg, J., et al., 2005. Am. J. Clin. Nutr. 81, 736–745; Elmore, A.R., 2005. Int. J. Toxicol. 24, 51–111.
148. The balance of urinary oxalate comes mainly from the degradation of glycine (about 40% of the total); but some also can come from the diet (5–10%).
149. Robitaille, L., Mamer, O.A., Miller, Jr., W.H., et al., 2009. Metab. Clin. Exp. 58, 263–269.
150. Chen, X., Shen, L., Gu, X., et al., 2014. Urology 84, 922–924.
151. Forty percentage of subjects given 2 g of ascorbic acid daily showed increases in urinary oxalate excretion by more than 10% (Chai, W., Liebman, M., Kynast-Gales, S., et al., 2004. Am. J. Kidney Dis. 44, 1060–1069).
152. Huang, H.Y., Appel, L.J., Choi, M.J., et al., 2005. Arthritis Rheum. 52, 1843–1847. This finding suggests that vitamin C may be beneficial in the management of gout.
153. Up to 7500 mg (Mühlhöfer, A., Mrosek, S., Schlegel, B., et al., 2004. Eur. J. Clin. Nutr. 58, 1151–1158).

hemochromatosis or other forms of excess iron accumulation are recommended to avoid taking vitamin C supplements with their meals.

- **Systemic conditioning**. That chronic intakes of large amounts of vitamin C might lead to persistent upregulation of ascorbic acid catabolism was once proposed. The concern was that such "systemic conditioning" might precipitate "rebound scurvy" in individuals returning to nutritional intakes of the vitamin. That hypothesis was based on uncontrolled observations of a few individuals and what is now widely regarded as erroneous interpretations of experimental results.[154] A well-controlled study with guinea pigs found transient declines in plasma ascorbic acid levels in some animals removed from high-level vitamin C treatments. Controlled studies have not consistently demonstrated such effects. Studies in humans indicate that high doses of ascorbic acid are mostly degraded to CO_2 by the gut microbiome, with major portions also excreted intact in the urine.
- **Mutagenesis**. Vitamin C is not intrinsically mutagenic; however, mutagenesis can be demonstrated in vitro by treating cells with ascorbate and Cu^{2+}, which produces ROS. No evidence of mutagenic effects in vivo has been produced; doses as great as 5000 mg have not induced mutations in mice.[155]

12. CASE STUDIES

Instructions

Review each of the following case reports, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

Case 1

A 26-year-old man volunteered for a 258-day experiment of ascorbic acid metabolism. He was 184 cm tall and weighed 84.1 kg. His medical history, physical examination, vital signs, and past diet history revealed a healthy individual with no irregularities. During the experiment, his temperature, pulse, and respiration rates were recorded four times daily, and his blood pressure was measured twice daily. He was examined by an internist daily; periodically, he was examined by an ophthalmologist and had chest radiograms and electrocardiograms made. Twenty-four-hour collections of urine and feces were made daily to determine urinary and fecal nitrogen, and for the radioactive assay of ascorbic acid. Samples of expired air were collected for the measurement of radioactivity.

The subject was fed a control diet consisting of soy-based products. The diet provided 2.5 mg of ascorbic acid per day, which was supplemented by a daily capsule containing an additional 75 mg of ascorbic acid. The subject's body vitamin C pool was labeled with L-[1–^{14}C]ascorbate 1 week before initiating vitamin C depletion; it was calculated to be 1500 mg. Beginning on day 14, the diet was changed to a liquid formula containing no vitamin C, as ascertained by actual analysis. This diet, based on vitamin-free casein, provided 3300 kcal and supplied protein, fat, and carbohydrate as 15%, 40%, and 45% of total calories, respectively. It was fed from day 14 to day 104, during which time the subject developed signs of scurvy. Ascorbic acid was not detectable in his urine after 30 days of depletion. He showed petechiae on day 45, when his vitamin C pool was 150 mg and his plasma level was 0.19 mg/dL. Spontaneous ecchymoses occurred over days 36–103; these were followed by coiled hairs, gum changes, *hyperkeratosis*,[156] congested follicles and the Sjögren sicca syndrome,[157] dry mouth, and enlarged parotid salivary glands. The subject developed joint pains on day 68 and joint effusions[158] shortly thereafter, when his vitamin C pool was 100 mg and his plasma ascorbic acid level was less than 0.16 mg/dL. He also had the unusual complication of a bilateral femoral neuropathy, which began on day 71, when his vitamin C pool was 80 mg and his plasma ascorbic acid level was 0.15 mg/dL. This, accompanied by the joint effusions, was attributed to hemorrhage into the sheaths of both femoral nerves. On day 80, he experienced a rapid increase in weight, from 81 to 84 kg, in combination with dyspnea[159] on exertion and swelling of the legs. At this time, his vitamin C pool was 40 mg and his plasma level was 0.15 mg/dL.

Beginning on day 105, the subject was put on a vitamin C-repletion regimen involving daily doses of 4 mg of ascorbic acid. Immediately following this treatment, the edema worsened, urinary output dropped to 340 ml/day, and weight increased to 86.6 kg on day 109. There was no evidence of pulmonary congestion or cardiac failure. The ascorbic acid-repletion dose was increased to 6.5 mg/day on day 111. His edema persisted for 4 days, at which time he had a profound diuresis with complete disappearance of the edema by day 133 at which time his weight was 77.2 kg (he lost 9.4 kg of extracellular fluid). From days 101–133, his body ascorbic acid pool increased from 33 to 128 mg. The subject was given 6.5 mg of ascorbic acid per day from day 133 to day 227. During this time, all his scorbutic manifestations disappeared,

154. For example, enhanced $^{14}CO_2$ excretion from guinea pigs with larger body pools of ascorbic acid was taken as evidence of greater catabolism.
155. Vojdani, A., Bazargan, M., Vojdani, E., et al., 2000. Cancer Detect. Prev. 24, 508–523.
156. A disease of the mouth characterized with variously sized and shaped, grayish white, flat, adherent patches; having diffuse borders, and a smooth surface with no papillary projections, fissures, erosions, or ulcerations.
157. Dry eyes due to reduction in tears, i.e., keratoconjunctivitis.
158. The escape of fluid from the blood vessels or lymphatics into the joint capsule.
159. Subjective difficulty or distress in breathing, frequently rapid breathing.

and his plasma ascorbic acid fluctuated between 0.10 and 0.25 mg/dL. His body pool was restored slowly to an excess of 300 mg. Beginning on day 228, he received 600 mg of ascorbic acid per day, which rapidly repleted his body pool. At the end of the study, his weight was 81 kg and he was discharged from the metabolic ward in excellent health.

Case 2

A 72-year-old man was admitted to the hospital with symptoms of increasing anorexia, epigastric discomfort unrelated to meals, and nonradiating precordial[160] pain. During the year before admission, he had become increasingly weak and easily fatigued, and had lost nearly 13 kg in weight. Six weeks before admission, he began to have sudden attacks of severe substernal pain followed by cough and dyspnea, and 1 month before admission he had a small *hematemesis*[161] and had noted bright red blood in his stools. He had been living alone and his diet during the past year had consisted chiefly of bread and milk with various soups. For a considerable period, he had noted easy bruising of his skin. His past health had been good except for occasional seizures; these began 2 years before admission and involved loss of consciousness, spasmodic twitching of the limbs, and incontinence preceded by abdominal discomfort.

Physical examination on admission revealed a thin, depressed, lethargic man with a rather gray complexion, and numerous petechiae over the arms, legs, and trunk. His blood pressure was 140/80, his pulse was 68, his respiration was 19, and his temperature was 98.8°F. Examination of his head and neck showed an edentulous[162] mouth, foul breath, ulcerated palate, and retracted gums without hemorrhage. He had a large ecchymosis (15 cm in diameter) on his right thigh. Neurological examination was negative.

Laboratory Findings

Parameter	Patient	Normal Range
Hb	13.2 g/dL	15–18 g/dL
WBC	8000/μL	5000–9000/μL
Platelets	140,000/μL	150,000–300,000/μL
Clotting time	5.75 min	5–15 min
Blood urea	48 mg/dL	10–20 mg/dL
Serum protein	7 g/dL	6–8 g/dL
Serum albumin	3.9 g/dL	3.5–5.5 g/dL
Serum ascorbic acid	<0.1 mg/dL	0.4–1.0 mg/dL

160. Relating to the diaphragm and anterior surface of the lower part of the thorax.
161. Vomiting of blood.
162. Toothless.

His heart was not enlarged and there were no heart murmurs; however, his electrocardiogram showed changes typical of an old myocardial infarction.[163] His chest radiograms showed emphysematous[164] and atheromatous[165] changes. His urine contained occasional pus cells with moderate growth of *Escherichia coli*; no abnormal bacilli were seen in the sputum. Sigmoidoscopy revealed no lesions in the distal 25 cm of the bowel. Because of his anorexia, epigastric discomfort, weight loss, and hematemesis, further investigation of the gastrointestinal tract was made using a barium bolus; this revealed a mass and ulcer crater in the prepyloric area of the stomach, suggesting a gastric neoplasm. A laparotomy[166] was planned. The tentative diagnoses were anterior myocardial infarction, suspected cancer of the stomach, epilepsy, and hemorrhagic diathesis[167] (probably scurvy). Accordingly, the patient was given a high-protein diet and ascorbic acid (1 g/day for 2 weeks, then 150 mg/day for a month).

The patient showed marked improvement following ascorbic acid treatment. He no longer showed an air of lassitude; he gained weight and began to relish his meals. His skin hemorrhages rapidly decreased and no new ones appeared. Three weeks after admission, blood disappeared from his feces. At that time, his epilepsy was satisfactorily controlled using phenobarbital, and his liver function tests and blood chemistry were normal.

Laboratory Findings after Vitamin C Treatment

Parameter	Patient
Blood urea	28 mg/dL
Serum protein	6.3 g/dL
Serum albumin	3.9 g/dL
Serum ascorbic acid	1.0 mg/dL

A second radiological examination, conducted 1 month after ascorbic acid treatment, indicated a normal pylorus; this was confirmed by gastroscopy. A biopsy of the previously involved area showed only a natural glandular pattern, with hemorrhage of the superficial layer of the gastric mucosa. The patient was discharged after 8 weeks of hospitalization and was well when seen later in the outpatient

163. Necrotic changes resulting from obstruction of an end artery.
164. Emphysema involves dilation of the pulmonary air vesicles, usually due to atrophy of the septa between the alveoli.
165. Atheroma refers to the focal deposit or degenerative accumulation of soft, pasty, acellular, lipid-containing material frequently found in intimal and subintimal plaques in arteriosclerosis.
166. A surgical procedure involving incision through the abdominal wall.
167. Any of several syndromes showing a tendency to spontaneous hemorrhage, resulting from weakness of the blood vessels and/or a clotting defect.

clinic. The gastric lesion did not recur. It was concluded that what had appeared to be a prepyloric tumor and ulcer had actually been a bleeding site with a hematoma.[168]

Case Study Questions

1. What thresholds are suggested by the results of the first case study for total body ascorbic acid pool size and plasma ascorbic acid concentration associated with freedom from signs of scurvy?
2. Compute the rate of reduction in ascorbic acid body pool size from the observations on the subject of the first case. Was it linear throughout the study?
3. What signs/symptoms did the patient in the second case show that indicated a problem related to vitamin C status?

13. STUDY QUESTIONS AND EXERCISES

1. Construct a concept map illustrating the relationship of the chemical properties and physiological functions of vitamin C.
2. Construct a decision tree for the diagnosis of vitamin C deficiency in humans.
3. What health complications might you expect to be shown by scorbutic individuals?
4. Compare and contrast the antioxidant properties of vitamins C and E.

168. A localized mass of extravasated blood, usually clotted.

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Chapter 11

Thiamin

Chapter Outline

Anchoring Concepts

1. Thiamin is the trivial designation of a specific compound, 3-(4-amino-2-methylpyrimidin-5-ylmethyl)-5-(2-hydroxyethyl)-4-methylthiazolium, which is sometimes also called **vitamin B_1**.
2. Thiamin is hydrophilic and its protonated form has a quaternary nitrogen center in the thiazole ring.
3. Deficiencies of thiamin are manifest chiefly as neuromuscular disorders.

There is present in rice polishing a substance different from protein and salts, which is indispensable to health and the lack of which causes nutritional polyneuritis.

C. Eijkman and G. Grijns[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of thiamin
2. To understand the means of absorption and transport of thiamin
3. To understand the biochemical function of thiamin as a coenzyme and the relationship of that function to the physiological activities of the vitamin
4. To understand the physiological implications of low thiamin status.

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1. Christiaan Eijkman (1858–1930) was a Dutch physician whose demonstration that beriberi in Java, Dutch East Indies (Indonesia) had a dietary etiology. His assistant at the time, Dutch physician Gerrit Grijns (1865–1944), is thought to have interpreted their results as indicating a nutrient deficiency, presaging the "vitamin theory." For seminal work leading to the recognition of the vitamins, Eijkman shared with Frederick Gowland Hopkins (who had demonstrated "accessory food factors") the 1929 Nobel Prize for Physiology or Medicine.

VOCABULARY

Acute pernicious beriberi
Alcohol
Aleurone
γ-Aminobutyric acid (GABA)
Amprolium
Anorexia
Ataxia
ATPase
Beriberi
Bradycardia
Cardiac beriberi
Cardiac hypertrophy
Chastek paralysis
Cocarboxylase
Confabulation
Dry beriberi
Dyspnea
Encephalopathy
Fescue toxicity
Hexose monophosphate shunt
Infantile beriberi
α-Ketoglutarate dehydrogenase
Maple syrup urine disease
Neuropathy
Nystagmus
Ophthalmoplegia
Opisthotonos
Oxythiamin
Pentose phosphate pathway
Perseveration
Phosphorylase
Polioencephalomalacia

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00011-3

Polyneuritis
Pyrimidine ring
Pyrithiamin
Pyruvate decarboxylase
Pyruvate dehydrogenase
Rapsyn
Shoshin beriberi
Stargazing
Sulfate
Sulfite
Tachycardia
Thiamin-binding protein (TBP)
Thiamin disulfide
Thiaminases
Thiamin diphosphate phosphotransferase
Thiamin monophosphate (TMP)
Thiamin monophosphatase
Thiamin pyrophosphate (TPP)
Thiamin pyrophosphatase
Thiamin pyrophosphokinase
Thiamin-responsive megaloblastic anemia (TRMA)
Thiamin transporters (ThTr1, ThTr2)
Thiamin triphosphate (TTP)
Thiazole ring
Thiochrome
Transketolase
TPP–ATP phosphoryltransferase
Vitamin B_1
Wernicke–Korsakoff syndrome
Wet (edematous) beriberi
Wilson disease

1. THE SIGNIFICANCE OF THIAMIN

Thiamin is essential in carbohydrate metabolism and neural function; it is required for the production of ATP, ribose, NAD,[2] and DNA. Severe thiamin deficiency results in the nerve and heart disease **beriberi**. Less severe deficiency results in nonspecific signs: malaise, loss of weight, irritability, and confusion. Thiamin-deficient animals show inanition and poor general performance and, in severe cases, **polyneuritis**, making thiamin status economically important in livestock production.

Historically, thiamin deficiency has been prevalent among peoples dependent on polished rice as a staple food. Demographic trends indicate that for many people dependence on rice is likely to increase in the future. Rice and rice/wheat crop rotations are now the basis of the food systems currently supporting one-fifth of the world's people, i.e., those in East, South, and Southeast Asia where populations are expected to more than double within the next four decades.

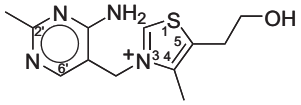
2. Nicotinamide adenine dinucleotide, a metabolically active form of the vitamin niacin (see Chapter 13).

The irony is that whole-grain rice and other cereals are not particularly deficient in thiamin; however, the removal of their thiamin-containing **aleurone** cells[3] renders the polished grains, which consist of little more than the carbohydrate-rich endosperm, nearly devoid of thiamin and other vitamins and essential elements. In fact, thiamin-containing rice polishings are often used to fuel the parboiling of the thiamin-deficient grain. Thus, storage technologies that reduce the need to polish rice such that increased reliance on the new, high-yielding cultivars of rice will not lead to expansions of thiamin deficiency among the poor of south Asia.

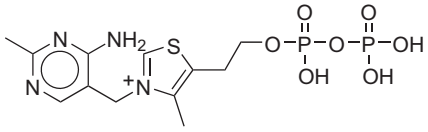
2. PROPERTIES OF THIAMIN

The term **thiamin** designates the compound 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methylthiazolium, formerly known as **vitamin B_1**, **aneurine**, and **thiamine**. The structural features that are essential for its biological activity include an aromatic **pyrimidine ring** with an amino group on C-4 joined to an aromatic **thiazole ring** containing a quaternary N, an open C-2, and a phosphorylatable hydroxyethyl group on C-5.

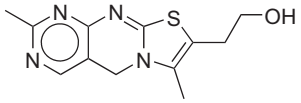
Chemical Structures of Thiamin Vitamers



Thiamin (free base)



Thiamin pyrophosphate



Thiochrome

Thiamin Chemistry

Free thiamin is a white powder with a characteristic "thiazome" odor and bitter taste. It is stable at moderate

3. The aleurone is the outermost layer of the endosperm, surrounding the starchy interior, of grass seeds. In rice and other cultivated cereals, it contains protein-storing vacuoles, and large amounts of oils and minerals, which function in seed development and dormancy. Thus, it is the most nutritious component of most brans. However, rice oil is highly polyunsaturated and, thus, prone to peroxidative rancidity particularly when stored at tropical temperatures. For this reason, milled rice is "polished" by gentle mechanical abrasion to remove the aleurone layer, which greatly increases its useful storage life.

temperatures under slightly acidic conditions but is heat labile at neutral pH and highly unstable under alkaline conditions in which the thiazolium C-2 is vulnerable to ring-opening hydroxyl attack. It is freely soluble in water but practically insoluble in organic solvents. Therefore, the hydrochloride and mononitrate forms are used in commerce. Thiamin hydrochloride (actually, thiamin chloride hydrochloride) is a colorless crystal that is very soluble in water (1 g/mL, thus making it a very suitable form for parenteral administration), soluble in methanol and glycerol, but practically insoluble in acetone, ether, chloroform, and benzene. The protonated salt has two positive charges: one associated with the pyrimidine ring and one associated with the thiazole ring. The mononitrate form is more stable than the hydrochloride form, but it is less soluble in water (27 mg/mL). It is used in food/feed supplementation and in dry pharmaceutical preparations.

Free thiamin is easily oxidized to thiamin disulfide and other derivatives including thiochrome, a yellow biologically inactive product with strong blue fluorescence that can be used for the quantitative determination of thiamin. The thiazole hydroxyethyl group can be phosphorylated in vivo to form thiamin mono-, di-, and triphosphates. Thiamin diphosphate, also called **thiamin pyrophosphate (TPP)**, is the metabolically active form sometimes referred to as **cocarboxylase**. Thiamin antagonists of experimental significance include **pyrithiamin** (the analog consisting of a pyridine moiety replacing the thiazole ring) and **oxythiamin** (the analog consisting of a hydroxyl group replacing the C-4 amino group on the pyrimidine ring).

3. SOURCES OF THIAMIN

Hindgut Microbial Synthesis

Thiamin is produced by bacteria and fungi, including those in the hindgut microbiome. A genomic analysis of 256 representative organisms of the human gut microbiota found more than half capable of de novo synthesis of the vitamin.[4] Those findings suggested that hindgut microbial synthesis may produce some 2.3% of the daily human need for thiamin. It is not clear whether the colon is capable of carrier-mediated absorption of the vitamin, although a high-affinity thiamin transporter (THTR2) has been identified in the colonic mucosa. Therefore, it is likely that thiamin produced in the hindgut may be useful to noncoprophagous animals but that has not been demonstrated. It is clear that rumen microbiome is an important source of thiamin for ruminants. However, because rumen microbes can reduce sulfate to sulfite, high dietary levels of sulfate can have antithiamin antagonistic effects for ruminants.

4. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.

Distribution in Foods

Thiamin is widely distributed in foods but most contain only low concentrations of the vitamin (Table 11.1). The richest sources are yeasts (e.g., dried brewer's and baker's yeasts) and liver (especially pork liver); however, cereal grains comprise the most important sources of the vitamin in most human diets. Thiamin in foods is considered to be readily available to healthy subjects except in cases of exposure to certain antagonists.

TABLE 11.1 Thiamin Contents of Foods

Food	Thiamin (mg/100 g)
Grains	
Cornmeal	0.39
Oats	0.76
Rice	
Brown	0.18
White, cooked	0.02–0.20
Rye flour	0.29
Wheat	
Whole grain	0.52
White	0
Vegetables	
Asparagus	0.16
Beans, green	0.07
Broccoli	0.07
Cabbage	0.06
Carrots	0.07
Cauliflower	0.05
Kale	0.05
Peas, green	0.27
Potatoes	0.11
Tomatoes	0.04
Fruits	
Apples	0.02
Apricots	0.03
Bananas	0.03
Grapes	0.09
Oranges	0.07
Pears	0.01
Pineapples	0.08

Continued

TABLE 11.1 Thiamin Contents of Foods—cont'd

Food	Thiamin (mg/100 g)
Meats	
Beef	0.02–0.10
Duck	0.26
Pork	0.41–0.92
Cured ham	0.82
Trout	0.15
Salmon	0.02–0.16
Liver beef	0.19
Pork	0.28
Dairy Products and Eggs	
Cheese	0.01–0.15
Milk	0.04–0.05
Eggs	0.04
Other	
Baker's yeast	1.89
Human milk	0.01

From USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

Whole grains are typically rich in thiamin; however, the vitamin is distributed unevenly in grain tissues. The greatest concentrations of thiamin in grains are typically found in the scutellum (the thin layer between the germ and the endosperm) and the germ. The endosperm (the starchy interior) is quite low in the vitamin. Therefore, milling to degerminate grain, which, because it removes the highly unsaturated oils associated with the germ, yields a product that will not rancidify and, thus, has a longer storage life, also has very low thiamin content. It is estimated that more than one-third of thiamin in the US food supply is provided by grains and grain products, with meats providing about a quarter. In foods derived from plants thiamin occurs predominantly as free thiamin and thiamin monophosphate (TMP); in animal tissues it is found almost entirely (95–98%) in phosphorylated forms, predominantly (80–85%) TPP. TTP comprises only a small portion of total tissue thiamin in most foods, with the notable exceptions of chicken breast muscle and pork skeletal muscle, in which >70% of the vitamin is present at TPP.[5]

Stability in Foods

The stability of food thiamin depends largely on the form and pH. Thiamin is stable in dry preparations and at room temperatures under slightly acidic conditions. However, under conditions of neutral pH, it is susceptible to destruction by heating,[6] and at high temperatures it can undergo Maillard reactions.[7] Accordingly, thiamin is partially lost in cooking. Thiamin is very sensitive to sulfites, which cleave its two ring systems;[8] this reaction can result in the loss of thiamin from sulfite-treated foods, even at low storage temperatures. Thiamin is also sensitive to oxidation by ROS generated by UV light and ionizing radiation (Table 11.2). Protein-bound thiamin, found in animal tissues, is more stable to such losses. Thiamin is stable during frozen storage; substantial losses occur during thawing, however, mainly due to removal via drip fluid.

TABLE 11.2 Thiamin Losses in Food Processing

Procedure	Food	Loss (%)
Convection cooking	Meats	25–85
Baking	Bread	5–35
Heating with water	Vegetables	0–60
Pasteurization	Milk	9–20
Spray drying	Milk	10
Canning	Milk	40
Room temperature storage	Fruits, vegetables	0–20

Thiamin Antagonists

Thiamin in foods can be destroyed or antagonized by several compounds that may occur naturally (Table 11.3). Cases of thiamin deficiency have been found to be related to the ingestion of food containing such antagonists (Table 11.4).[9] They include the following:

- **Thiaminases**—Bacterial thiaminases are exoenzymes, i.e., they are bound to the cell surface; their activities depend on their release from the cell surface, which can occur under acidotic conditions in the rumen. Thiaminases are heat labile and can be rendered ineffective by heat treatment.

5. These tissues also have appreciable amounts of adenylate kinase, which can produce it from TPP using ADP as the phosphate donor.

6. Therefore, the practice of adding sodium bicarbonate to peas or beans for retention of their color in cooking or canning results in large losses of thiamin.

7. Reactions of amino acids and reducing sugars in food matrices; a form of nonenzymatic browning.

8. To yield (6-amino-2-methylprimid-5-yl)methanesulfonic acid and 5-β-hydroxyethyl-4-methylthiazole.

9. Perhaps the best known of these is the condition "**Chastek paralysis**," a neurological disorder described in commercial foxes fed a diet containing raw carp. The syndrome, named for the fox producer, was found to be a manifestation of thiamin deficiency brought on by a microbial thiaminase present in fish gut tissue. Cooking the fish before feeding them to Mr Chastek's foxes prevented the syndrome, apparently by heat denaturing the thiaminase.

TABLE 11.3 Types of Thiaminases

Type	Present in	Mechanism
I	Fresh fish, shellfish, ferns, some bacteria	Displaces pyrimidine methylene group with a nitrogenous base or SH compound to eliminate the thiazole ring
II	Certain bacteria	Hydrolytic cleavage of methylene–thiazole–nitrogen bond to yield the pyrimidine and thiazole moieties

TABLE 11.4 Thiaminase Activities in Seafoods

Seafood	Thiamin Destroyed (mg/100 g/h)
Yellowfin tuna	265
Red snapper	265
Skipjack tuna	1000
Mahi mahi	120
Clam	2640

From Hilker, D.M., Peter, G.F., 1966. J. Nutr. 89, 419–426.

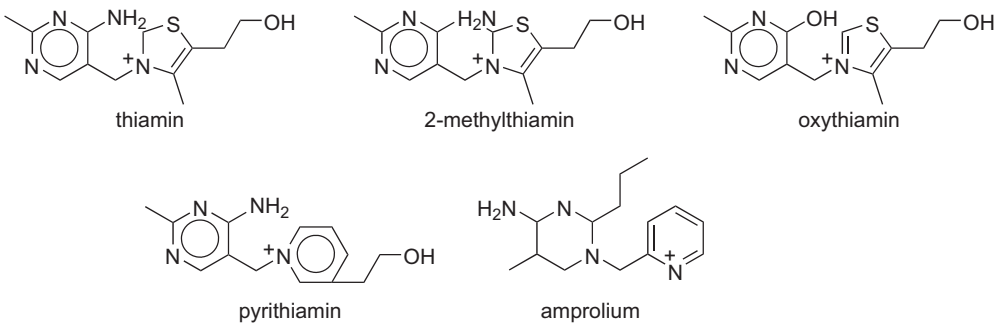
- **Thiamin antagonists**
  - *o*- and *p*-Hydroxypolyphenols (e.g., caffeic acid, chlorogenic acid, tannic acid)[10] in ferns, tea, and betel nut react with thiamin to oxidize the thiazole ring yielding the nonabsorbable form **thiamin disulfide**;
  - Some plant flavonoids (e.g., quercetin, rutin) have been reported to antagonize thiamin;
  - Hemin[11] in animal tissues is thought to bind the vitamin.
- **Thiamin analogs**—Several analogs are effective thiamin antagonists, each involving a substitution on either the pyrimidine or thiazole ring[12]. These include the following:
  - **Oxythiamin**—lacks the pyrimidine 4′-amino group essential for the release of aldehyde adducts from the C-2 of the thiazole ring; does not cross the blood–brain barrier; and, therefore, does not affect thiamin-dependent enzymes in the central nervous system.
  - **2-Methylthiamin—**has a methyl group at the 2-position of the thiazole ring; forms an enzymatically inactive complex with TPP enzymes.
  - **Pyrithiamin**—has a pyridine ring structure in place of the thiazole ring; is taken into cells by the thiamin transporter, and competitively inhibits the conversion of thiamin to TPP, increasing the urinary excretion of thiamin.
  - **Amprolium**—has a thiamin-like pyrimidine ring combined through a methylene bridge to a quaternary nitrogen of the pyridine ring. The absence of a hydroxyethyl side chain on that ring prevents the analog from forming diphosphate derivatives, making it a weak thiamin antagonist in animals. An efficient inhibitor of thiamin uptake by bacteria, it is used as an anticoccidial drug for poultry.[13]

4. ABSORPTION OF THIAMIN

Free thiamin must be released from phosphorylated dietary forms to be absorbed across the gut. That dephosphorylation is accomplished by phosphatases and pyrophosphatases in the upper region of the small intestine. The free (nonesterified) vitamin is absorbed by two mechanisms:

- **Active transport** at low luminal concentrations (<2 μM). This saturable, carrier-dependent mechanism is located in the apical brush border of the mucosal epithelium, with greatest activity in the duodenum. Because adrenalectomized rats absorb thiamin poorly,

10. These and related compounds are found in blueberries, red currents, red beets, Brussels sprouts, red cabbage, betel nuts, coffee, and tea.
11. Ferriprotoporphyrin, the nonprotein, Fe^{3+}-containing portion of hemoglobin.
12. The structures of these thiamin antagonists:



13. These compounds are valuable in protecting young poultry from coccidia (i.e., protozoans that can cause intestinal diseases in humans and many animal species). At low doses, they inhibit thiamin transport by the parasite; at higher doses, they can also inhibit thiamin absorption by the host to produce clinical thiamin deficiency.

it is thought that enteric absorption of the vitamin may also be subjected to control by corticosteroid hormones. These transporters can be inhibited by alcohol and pyrithiamine. Involved in this process are two cation carriers, THTR1 and THTR2, the gene products of *slc19a2* and *slc19a3*, respectively.[14,15] Both are thiamin-specific members of the SLC19 (solute carrier proteins) superfamily of transport proteins.[16] Both facilitate the exchange of the thiamin cation for protons, driven by an extracellularly directed proton gradient. These transporters have different functions in the enterocyte.[17] The principle uptake of thiamin across the brush boarder is facilitated by THTR2, which has a relatively high capacity, saturable at thiamin concentrations in the μM range. THTR2 is expressed along the length of the gut with highest expression in the duodenum and jejunum. That it is expressed in colonocytes raises the possibility that humans and other monogastrics may be able to absorb thiamin produced by the hindgut microbiome, although that has not been demonstrated. Located in the basolateral membrane is THTR1, which has a lower transport capacity, being saturated at thiamin concentrations in the nM range.

- **Passive diffusion**—at higher concentrations (e.g., a 2.5 mg dose for a human).

Movement of thiamin across the enterocyte basolateral membrane into the blood is Na^+-dependent, being coupled to the hydrolysis of ATP by a Na^+/K^+ ATPase. While most of the thiamin present in the intestinal mucosa is in phosphorylated form, thiamin arriving on the serosal side of the intestine is largely in the free (nonphosphorylated) monovalent cation. Therefore, the movement of thiamin through the mucosal cell is coupled to its phosphorylation/dephosphorylation.

5. TRANSPORT OF THIAMIN

Thiamin Bound to Serum Proteins

Most of the thiamin in serum is bound nonspecifically to protein, chiefly albumin. About 90% of the total thiamin in blood (typically, 5–12 μg/dL) is contained in erythrocytes.[18] A specific binding protein, **thiamin-binding protein** (**TBP**), has been identified in rat serum.[19] With a molecular mass of 38 kDa, TBP binds free thiamin and forms a complex with the riboflavin-binding protein. Like the latter, TBP appears to be regulated by estrogens, i.e., it is inducible in male or ovariectomized rats by parenterally administered estrogen.

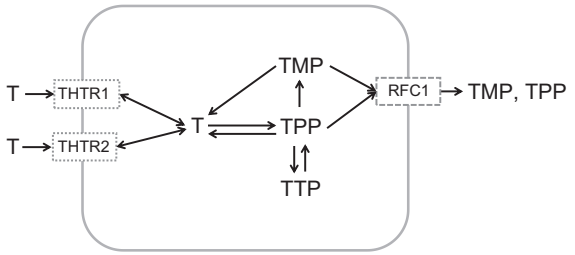


FIGURE 11.1 Membrane transporters involved in thiamin entry and export from cells (see text for abbreviations).

Cellular Uptake

Being hydrophilic and positively charged in the plasma, thiamin does not readily cross the plasma membrane. Cellular entry and export of the positively charged thiamin is facilitated by THTR1 and THTR2 (Fig. 11.1). That THTR1 is particularly important in this regard is indicated by the fact that individuals born with genetic defects in that transporter show **thiamin-responsive megaloblastic anemia (TRMA)**, also characterized by deafness due to nerve damage.[20] A variant of THTR2 was identified in siblings with Wernicke–Korsakoff syndrome and persistent seizures, which signs responded to high doses of thiamin.[21] Another SLC protein, the reduced folate carrier (RFC1, the gene product of *scl25a19*) is involved in the export of negatively charged phosphorylated forms of thiamin. Thiamin is rapidly phosphorylated upon entry into the cell. Hence, TPP comprises most (70–90%) of intracellular thiamin, some 90% of which is bound to proteins. Almost one-third of intracellular TPP enters mitochondria via high-affinity transporters thought to be ThTr1/2 and SLC25A19,[22] which is thought to facilitate entry of TPP by exchange with ATP. Most mitochondrial TPP quickly becomes bound to two proteins (α-ketoglutarate dehydrogenase (α-KGDH) and

14. We are using the convention of citing the gene in lower case italics, and the gene product in all caps except in cases in which the product was named before the gene was identified, e.g., ThTr1.
15. Mutations in the *slc19a2* gene are associated with thiamin-responsive megaloblastic anemia, insulin-dependent diabetes, and sensory-neural hearing loss (TRMA, or Roger's disease). Studies have shown that thiamin deprivation results in the upregulation of *slc19a3* in the mouse. Curiously, a genetic defect in the *slc19a3* gene has been found to cause biotin-responsive basal ganglia disease (Zhang, W.G., Al-Yamani, E., Arcierno, Jr., J.S., et al., 2005. Am. J. Hum. Genet. 77, 16–26).
16. This family also includes the folate transporter, SLC19A1.
17. This is evidenced by the fact that patients with thiamin-responsive megaloblastic anemia (TRMA) due to a mutation in the *slc19a2* gene show normal plasma thiamin levels (Neufeld, E.J., Fleming, J.C., Tartaglini, E., et al., 2001. Blood Cells Mol. Dis. 27, 135–138).
18. Several children who died of SIDS (sudden infant death syndrome) have been found to have very high plasma thiamin concentrations, e.g., fivefold those of infants who died of other diseases. The physiological basis of this effect is unknown, although thiamin deficiency is not thought to be a cause of death in SIDS.
19. TBP has also been identified in rat liver and hens' eggs (yolk and albumen).
20. Some patients respond to high doses (50 mg/day) of thiamin.
21. Kono, S., Miyajima, H., Yoshida, K., et al., 2009. N. Eng. J. Med. 360, 1792–1794.
22. A rare mutation in *slc25a19* causes a fatal microencephaly.

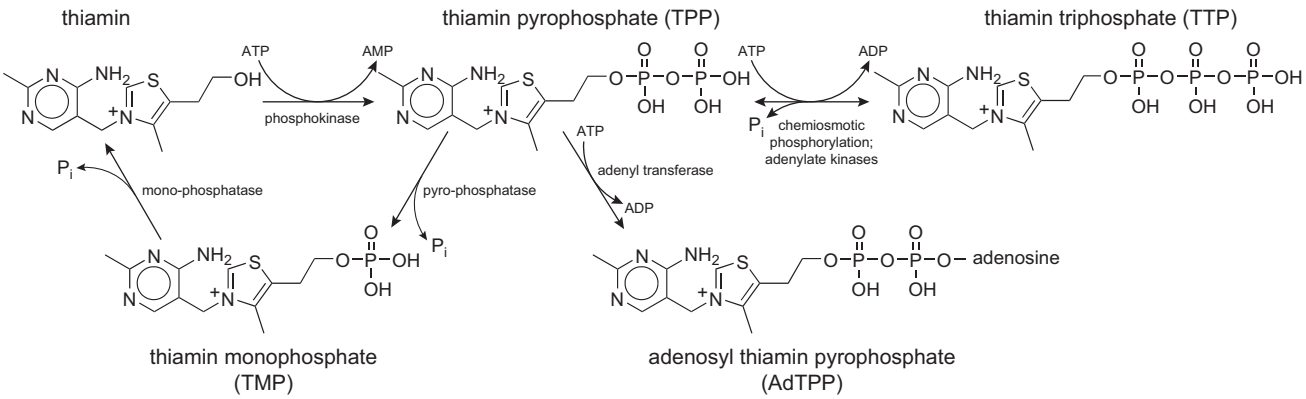


FIGURE 11.2 Metabolic activation of thiamin.

pyruvate dehydrogenase).[23] It can also be dephosphorylated to form TMP, which can move to the cytosol where it can be converted back to thiamin.

Tissue Distribution

That adult human stores only 30–50 mg thiamin, most of which is in skeletal muscle, heart, brain, liver, and kidneys as TPP. Plasma, milk, and cerebrospinal fluid and probably all extracellular fluids contain free thiamin and TMP which, unlike the more highly phosphorylated forms (TPP, TTP), appear capable of crossing cell membranes. Tissue levels of thiamin vary within and between species, with no appreciable storage in any tissue.[24] In infants, blood thiamin levels decline after birth, owing initially to a decrease in free thiamin followed by a decrease in phosphorylated forms. In thiamin-deficient chickens fed the vitamin, heart tissues take up thiamin at much greater rates than liver or brain. In general, the thiamin contents of human tissue tend to be less than those of analogous tissues in other species, particularly the pig, which has relatively high tissue thiamin stores.

While the brain is vulnerable to thiamin deprivation, it does not store substantial amounts of the vitamin. Studies have shown that, during periods of low thiamin intake, the brain is depleted of thiamin more slowly than other tissues; and when given therapeutic amounts of thiamin, it is not repleted as rapidly as other tissues such as liver. Such observations suggest a relatively limited transport of the vitamin across the blood–brain barrier compared to other tissues; unlike the liver, in which thiamine uptake is driven by a Na^+ gradient, crossing the blood–brain barrier appears to be a nonenergy-dependent process driven by intracellular phosphorylation in neural tissues.

6. METABOLISM OF THIAMIN

Phosphorylation–Dephosphorylation

Thiamin is phosphorylated in peripheral tissues to form three products (Fig. 11.2):

- **TMP** is produced from thiamin by non-specific phosphatases using ATP.
- **Thiamin diphosphate (TPP)** is formed by **thiamin diphosphokinase (TPK)**[25] using ATP. TPK is a soluble, Mg^{2+}–dependent, cytosolic enzyme with a high affinity for thiamin (K_m 0.1–1 μM) and 10-fold lower affinity for ATP; it functions as a 46–56 kDa homodimer each subunit of which binds a thiamin molecule. The equilibrium of the phosphorylation reaction is driven in favor of TPP by the binding of that product by TK and/or its transport into the mitochondria.[26] TPK is present in liver and brain (particularly, cerebellum and pons) and is inhibitable by pyrithiamine.
- **TTP can be** formed from TPP in two ways:
  - By **adenylate kinases**, particularly in the cytosol of skeletal muscle; this may provide significant amounts of TTP only when tissue concentrations of TTP and AMP are high.
  - By a **chemiosmostic mechanism**[27] similar to that of ATP synthesis in the process of mitochondrial oxidative phosphorylation driven by the protomotive force.[28]
- **Adenosylthiamin pyrophosphate (AdTPP)** and **adenosylthiamin triphosphate (AdTTP)** are thought to be produced by a Mg^{2+} (of Mn^{2+})-dependent adenyltransferase using ATP and ADP.

23. It is estimated that of the approximate 30 μM thiamin in mitochondria, only 2 μM is not enzyme bound (Bettendorf, L., Mastrogiacomo, J., LaMarch, J., et al., 1996. Mov. Disord. 11, 437–439.
24. Thiamin concentrations are generally greatest in the heart (0.28–0.79 mg/100 g), kidneys (0.24–0.58 mg/100 g), liver (0.20–0.76 mg/100 g), and brain (0.14–0.44 mg/100 g), and are retained longest in the brain.
25. Also, thiamin pyrophosphokinase
26. Accordingly, free cytosolic TPP comprises <10% of total thiamin in the rat brain.
27. Gangolf, M., Wins, P., Thiry, B., et al., 2010. J. Biol. Chem. 285, 583–594.
28. The potential energy associated with proton and voltage gradients across the mitochondrial inner membrane.

The phosphate esters of thiamin can be hydrolyzed by nonspecific phosphatases, including alkaline phosphatase and acid phosphatase, as well as several specific phosphatases:

- **TTP—triphosphatases**. Two specific TTP-phosphatases (membrane-associated and soluble) which hydrolyze TTP to yield TPP have been identified. The soluble enzyme, a 25 kDa monomer, appears to be found in most mammalian tissues with highest expression in neurons and glial cells. Mutations prevent it from being active as a TTPase in the pig, and it has not been found in birds.
- **TPP—diphosphatases**. Several TPPases that can hydrolyze TPP to yield TMP have been identified in association with the endoplasmic reticulum. Two microsomal nucleoside diphosphatases, one activated by ATP, another inhibited by ATP and thought to be a Golgi uridine diphosphatase.
- **TMP hydrolysis**. It is to yield free thiamin appears to be accomplished by nonspecific phosphorhydrolases found in many tissues. No specific TMPase has been reported.

The net result of these phosphorylation/dephosphorylation processes is a large body TPP pool (80% of total) with relatively small pools of TMP and TTP (apparently only in mitochondria). Adenylated forms of TPP and TTP are found at an order of magnitude less than other nucleotides (AMP, ATP, NAD). Thiazole ring analogs with hydroxyethyl groupings (e.g., oxythiamin, pyrithiamin, 2-methylthiamin) compete with the vitamin for phosphorylation.

Catabolism

The turnover of thiamin varies between tissues but is generally high.[29] Thiamin in excess of that which binds in tissues is rapidly excreted. With an estimated half-life of 10–20 days in humans, thiamin deficiency states can deplete tissue stores within a couple of weeks. Studies with fasting and undernourished soldiers have shown that food restriction increases the rate of thiamin excretion.[30] Declines in tissue thiamin levels are thought to involve enhanced degradation of TPP-dependent enzymes in the absence of the vitamin. Numerous metabolites of thiamin have been identified (Table 11.5).

Excretion

Thiamin is excreted in the urine, chiefly as free thiamin and TMP, but also in smaller amounts as TPP, the oxidation product **thiochrome**,[31] more than 20 other metabolites and a 25 kDa thiamin-containing peptide. Metabolites retaining the pyrimidine–thiazole ring linkage account for increasing proportions of total thiamin excretion as thiamin status declines. Urinary losses of thiamin metabolites vary with plasma thiamin levels but increase markedly when renal tubular reabsorption is saturated, which occurs in healthy adults at intakes of 0.3–0.4 mg thiamin per 1000 kcal.[32] Above that threshold, excretion of the vitamin exceeds 100 µg/day, whereas urinary excretion in deficient individuals is < 25 µg/day. Small amounts of the vitamin have also been reported to be lost in sweat.[33]

TABLE 11.5 Urinary Metabolites of Thiamin

Free thiamin
Thiamin disulfide thiamin monophosphate (TMP)
Thiamin diphosphate (TPP)
Thiochrome
Thiamin acetic acid
2-Methyl-4-amino-5-pyrimidine carboxylic acid
4-Methylthiazole-5-acetic acid
2-Methyl-4-aminopyrimidine-5-carboxylic acid
2-Methyl-4-amino-5-hydroxymethylpyrimidine
5-(2-Hydroxyethyl)-4-methylthiazole
3-(2′-Methyl-4-amino-5′-pyrimidinylmethyl)-4-methylthiazole-5-acetic acid
2-Methyl-4-amino-5-formylaminomethylpyrimidine

7. METABOLIC FUNCTIONS OF THIAMIN

Cosubstrate Functions of Thiamin Phosphate Esters

TTP can serve as a phosphate donor and does so for the phosphorylation of certain proteins including the neuromuscular synapse protein, **rapsyn**.[34] It is not clear whether TMP or adenylated derivatives have direct metabolic functions.

Coenzyme Functions of Thiamin Diphosphate

TPP (cocarboxylase) is an essential cofactor for five enzyme complexes (Fig. 11.3). Transketolase catalyzes the

29. Thiamin turnover in rat brain was 0.16–0.055 µg/g/h, depending on the region (Rindi, G., Patrini, C., Comincioli, V., et al., 1980. Brain Res. 181, 369–376).
30. Consolazio, C.F., Johnson, H.L, Krzywicki, J., et al., 1971. Am. J. Clin. Nutr. 24, 1060–1066.
31. The strong fluorescence of thiochrome has been used in the determination of thiamine, which can be oxidized to thiochrome using potassium ferricyanide or cyanogen bromide (Fujiwara, M., Matsui, K., 1953. Anal. Chem. 25, 810–816).
32. Interdepartmental Committee on Nutrition for National Defense, 1963. Manual for Nutrition Surveys, second ed. US Government Printing Office, Washington, DC.
33. Pearson, W.H., 1967. Am. J. Clin. Nutr. 20, 514–521; Sauberlich, H.E., Herman, Y.F., Stevens, C.O., et al., 1979. Am. J. Clin. Nutr. 32, 2237–2244.
34. This receptor-associated protein of the synapse is a 43 kDa protein believed to play a role in anchoring the acetylcholine receptor at sites in synapses.

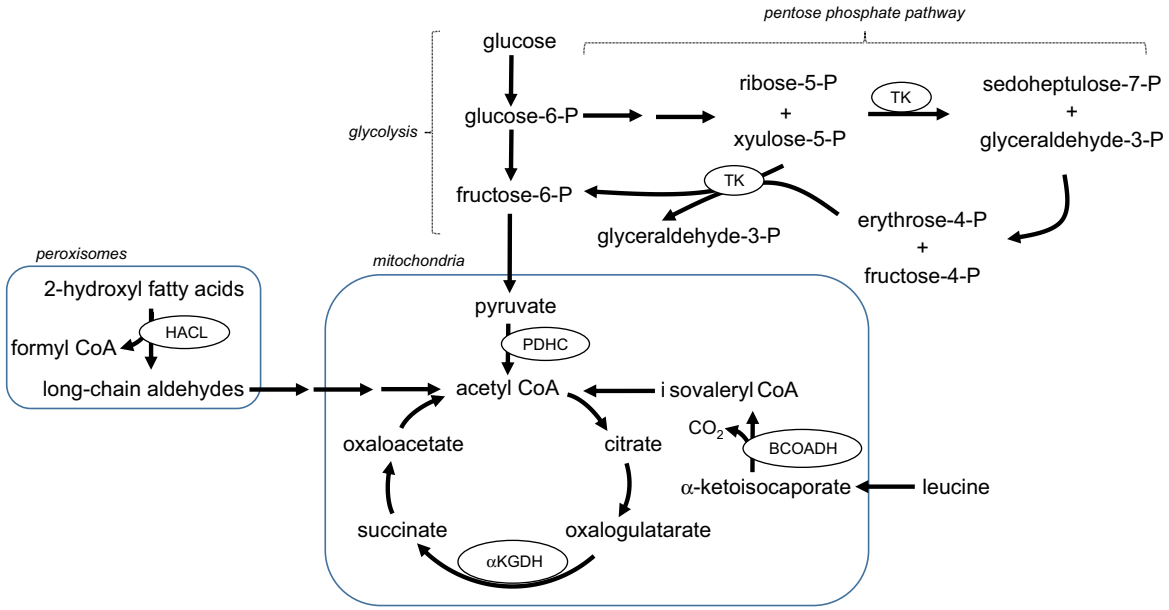


FIGURE 11.3 Roles of TTP-dependent enzymes (ovals) in metabolism. *HACL*, 2-hydroxyacyl CoA lyase; *PDHC*, pyruvate dehydrogenase complex; *TK*, transketolase; *α-KGDH*, α-ketoglutarate dehydrogenase.

transfer of a glycoaldehyde moiety between sugars. Three α-keto acid dehydrogenases catalyze oxidative decarboxylation reactions. A lyase catalyzes the cleavage of 2-hydroxy straight chain fatty acids and 3-methyl branched chain fatty acids after the α-oxidation of the latter. In each of these reactions, TPP serves as a classic coenzyme, binding covalently to the respective holoenzyme, which recognizes both its substituted pyrimidyl and thiazole moieties. Binding is facilitated by Mg^{2+} or some other divalent cation, which is required for enzyme activity. TPP functions as an energy-rich phosphoanhydride with high potential for phosphate group transfer. It is deprotonated to form a carbanion at C-2 of the thiazole ring, which reacts with the polarized 2-carbonyl group of the substrate (an α-keto acid or α-keto sugar) and labializes certain C–C bonds to release CO_2. The remaining adduct reacts by the following:

- protonation to give an active aldehyde addition product (e.g., decarboxylases);
- direct oxidation with suitable electron acceptors—to yield a high-energy, 2-acyl product;
- reacting with oxidized lipoic acid—to yield an acyldihydrolipoate product (e.g., oxidases or dehydrogenases); or
- addition to an aldehyde carbonyl to yield a new ketol.

In higher animals, the decarboxylation is oxidative, producing a carboxylic acid. This involves transfer of the aldehyde from TPP to lipoic acid (forming a 6-*S*-acylated dihydrolipoic acid and free TPP) and then to coenzyme A.[35]

Transketolase (TK). TK functions at two points in the pentose pathway, which generates pentoses and NADPH from the oxidation of glucose.[36] Both points involve the reversible transfer of 2-C "active glycoaldehyde" fragment from a ketose donor (xylulose-5-phosphate) to an aldose acceptor (ribose-5-phosphate or erythrose-5-phosphate). This is done by the TPP thiazole ring complexing with ketose substrate, releasing glyceraldehyde-3-phosphate and forming a complex with glycoaldehyde, which is transferred to the aldose. By these reactions, thiamin directs glucose either to glycolysis or to the pentose pathway, which generates ribose (required for DNA) while reducing the production of glucose metabolites, a feature of potential import in mitigating diabetic pathology.[37] TK is found in the cytosol of most tissues. It is present in remarkably high amounts in the cornea, where it has been reported to comprise some 10% of total soluble protein. It is present in high amounts in adipose, mammary gland, adrenal cortex, and erythrocytes, all of which rely on carbohydrate metabolism.[38] TK activity depends on its binding to TPP; therefore, responses to thiamin may involve activation of the apoenzyme, That thiamin may also have a direct effect on the genetic expression of the enzyme is suggested by the finding that thiamin deprivation increases the expression of TK mRNA. In thiamin-adequate subjects, TPP binding is at least 85% of

35. Coenzyme A is the metabolically active form of the vitamin **pantothenic acid**.

36. This pathway, also called the **hexose monophosphate shunt**, is an important alternate to the glycolysis–Krebs cycle pathway, especially for the production of pentoses for RNA and DNA synthesis and NADPH for the biosynthesis of fatty acids, etc.

37. Thiamin has been found useful in mitigating diabetic nephropathy (Rabbani, N., Alam, S., Riaz, S., et al., 2009. Diabetology 52, 208–211).

38. TK is also highly expressed in pancreatic tumors.

saturation, whereas in thiamin deficiency the percentage of TK bound to TPP is much less. TK isolated from patients with Wernicke–Korsakoff syndrome[39] has been found to have an abnormally low binding affinity for TPP.

α-Keto acid dehydrogenases. TPP is an essential cofactor in multienzyme complexes that catalyze the oxidative decarboxylation of α-keto acids. Each complex is composed of a decarboxylase that binds TPP, a core enzyme that binds lipoic acid, a flavoprotein dihydrolipoamide dehydrogenase that regenerates lipoamide, and one or more regulatory components. There are three classes of this type of TPP-dependent enzyme:

- **Pyruvate dehydrogenase complex (PDHC)**. It converts pyruvate produced from glycolysis to acetyl CoA, a key intermediate in the synthesis of fatty acids and steroids and an acyl donor for numerous acetylation reactions. Its TPP-dependent component is pyruvate dehydrogenase (E1). It also has two non-TPP enzymes, dihydrolipoyl acetyltransferase and dihydrolipoyl dehydrogenase,[40] a kinase and a phosphatase. The latter components regulate enzymatic activity by interconverting the dehydrogenase between active (nonphosphorylated) and inactive (phosphorylated) forms involving three specific serine residues that participate in TPP binding. Regulation also occurs via end product inhibition (by acetyl CoA and NADH). Thiamin status regulates E1 expression; deprivation of thiamin increases the mRNA for the β-subunit of the enzyme. A genetic defect in the E1-α polypeptide produces chronic neurological dysfunction with central nervous system degeneration and, generally, lactic acidosis; most signs respond to high doses of thiamin.
- **α-Ketoglutaric dehydrogenase complex (α-KGDH)**. It converts α-ketoglutarate to succinyl CoA. Its TPP-dependent component is α-ketoglutaric dehydrogenase, which appears to be regulated through stimulation by Ca^{+2} and inhibition by ATP, GTP, NADH, and succinyl CoA.
- **Branched chain α-keto acid dehydrogenase complex (BCKDH)**. It converts branched chain α-keto acids (produced by the transaminations of valine, leucine, and isoleucine) to the corresponding acyl CoAs (isobutyryl-, isovaleryl- and α-methylbutyryl-, respectively), which are subsequently oxidized to yield acetyl and propionyl CoAs. The dehydrogenase is regulated by phosphorylation–dephosphorylation involving a single serine residue. Deprivation of thiamin increases the proportion of dephosphorylated (active) enzyme, thus, serving to mitigate against the metabolic consequences of thiamin deficiency. Genetic defects in subunits of this enzyme complex result in the condition called **maple syrup urine disease (MSUD)**. Signs are manifest in infancy: lethargy, seizures, and, ultimately, mental retardation and a maple syrup odor of the urine due to the presence of the keto acid leucine. Five types of MSUD have been identified. One involves the enzyme's loss of affinity for TPP, reducing its BCKDH activities by 30–40%; this type of MSUD responds to high doses (10–200 mg/day) of thiamin.

2-Hydroxyacyl CoA lyase (HACL). TPP is required by this component of the peroxisomal enzyme complex involved in fatty acid catabolism.[41] This enzyme catalyzes the TPP-dependent cleavage of 2-hydroxy fatty acids (e.g., 2-hydroxyoctadecanoic acid[42]) to yield formate and a 1C-shortened aldehyde.

Neurologic Function

Thiamin has a vital role in nerve function, as the signs of thiamin deficiency are mainly neurologic. Thiamin is found in the brain, synaptosomal membranes, and cholinergic nerves. Nervous stimulation by either electrical or chemical means results in the release of thiamin (free thiamin, TMP) associated with the dephosphorylation of its higher phosphate esters. The antagonist pyrithiamin can displace thiamin from nervous tissue and change the electrical activity of the tissue. Irradiation with ultraviolet light at wavelengths absorbed by thiamin destroys the electrical potential of nerve fibers in a manner corrected by thiamin treatment. TTP, which appears to serve as a phosphate donor for phosphorylating synaptic proteins, has been shown to stimulate chloride transport.[43] Brain TTP concentrations tend to be resistant to changes with thiamin deprivation or parenteral thiamin administration, suggesting some degree of homeostatic control in that organ. Thiamin deprivation has been shown to cause oxidative stress, alter neurotransmitter metabolism, and cause dysfunction of the blood–brain barrier in experimental animals.

Thiamin has an essential role in the metabolism of glucose, on which the brain depends as its energy source.[44] TPP is required for the oxidative decarboxylations of pyruvate and α-ketoglutarate, essential steps in energy production via the tricarboxylic acid cycle. However, several

39. Thiamin-responsive encephalopathy characterized by neurological symptoms including ophthalmoplegia (weakness in muscles responsible for eye movements), ataxia, and confusion. Also called Korsakoff's psychosis and alcoholic encephalopathy.

40. The complex in eukaryotes consist of a multiunit structure containing 20–30 heterotetramers of PDHC, each with two α and two β subunits, associated with multiple units of the acyltransferase, a dihydrolipoyl dehydrogenase-binding protein, and homodimers of the dihydrolipoyl dehydrogenase.

41. The complex also catalyzes the chain shortening of these fatty acids to facilitate their β-oxidation.

42. This fatty acid is found in cerebrosides and sulfatides in brain.

43. Bettendorf, L., Kolb, H.A., Schoffeniels, E., 1993. J. Membr. Biol. 136, 281–288.

44. Unlike other tissues, the brain cannot use fatty acids as a source of energy.

TABLE 11.6 Effects of Thiamin Deficiency on Brain Metabolism in the Rat

Parameter	Thiamin Fed	Thiamin Deficient
Body weight (g)	135 ± 4	96 ± 5
Erythrocyte Transketolase (nmol/min/mg)		
Basal activity	6.4 ± 0.6	2.9 ± 0.4[a]
+ TPP	6.9 ± 1.4	4.7 ± 0.8
Activation coefficient	1.08 ± 0.08	1.63 ± 0.12[a]
Liver thiamin (nmol/g)	132.0 ± 8.2	6.0 ± 0.7[a]
Brain Analytes		
Thiamin (nmol/g)	12.6 ± 0.6	6.2 ± 0.3[a]
ATP (μmol/g)	2.8 ± 0.1	2.9 ± 0.1
Glutamate (μmol/g)	13.8 ± 0.3	11.3 ± 0.2[a]
α-Ketoglutarate (μmol/g)	0.14 ± 0.01	0.09 ± 0.0[a]
GABA (μmol/g)	1.67 ± 0.05	1.58 ± 0.03[a]

[a]*P* < .05.
From Page, M.G., Ankoma-Say, V., Coulson, W.F., et al., 1989. Br. J. Nutr. 62, 245–253.

experiments have indicated that the depressions of pyruvate and α-KGDH activities that occur in thiamin-deficient animals (Table 11.6) are not of sufficient magnitude to produce the neurological dysfunction associated with the deficiency. That brain ATP levels are unaffected by thiamin deprivation suggests that the metabolic flux though the alternative pathway, the **γ-aminobutyric acid** (**GABA**) shunt,[45] may be considerably increased in the brains of thiamin-deficient individuals. This suggests that, in addition to its role in the synthesis of that neurotransmitter, the GABA shunt may also yield energy under conditions of thiamin deprivation (Fig. 11.4). Such a phenomenon may explain the anorexia characteristic of thiamin deficiency, as increased GABA flux through the hypothalamus has been shown to inhibit feeding in animals.

It has been suggested that thiamin may be involved the synthesis of myelin. However, the turnover of myelin is much slower ($T_{½}$ 4–5 days) than the response of thiamin therapy (full recovery within 24 h). Therefore, thiamin/TPP would appear to have other functions related to nerve transmission, e.g., roles in regulating Na^+ permeability and maintaining the fixed negative charge on the inner surface of the plasma membrane.

45. *GABA* is synthesized by decarboxylation of glutamate, which is produced by transamination of α-ketoglutarate. GABA can be transaminated to form succinic semialdehyde, which is oxidized to succinate and enters the TCA cycle.

Three conditions indicate key roles of the vitamin in neurologic function:

- **Wernicke–Korsakoff syndrome**. This syndrome consists of Wernicke's encephalopathy and Korsakoff psychosis with signs ranging from mild confusion to coma. Pathology is limited to the central nervous system, with lesions limited to the submedial thalamic nucleus and parts of the cerebellum, particularly the superior cerebellar vermis. Patients frequently have a TK isoform with abnormally low binding affinity for TPP; in some cases this can be overcome with high doses of thiamin. A quarter of patients can be cured by thiamin treatment; it is thought that the balance may have another aberrant TK (or other TPP-dependent enzyme) incapable of binding TPP.
- **Alzheimer's disease**. That thiamin has a role in protecting against Alzheimer's disease is suggested by observations that patients have lower brain activities of TPP-dependent enzymes: 55% less α-KGDH, 70% less PDH, and markedly reduced TK.[46] Patients with frontal lobe degeneration of the non-Alzheimer's type have been found to have only half the cortical TPP levels of unaffected individuals. In an Alzheimer's disease mouse model, thiamin deprivation increased the accumulation of plaques.[47] In another mouse model, the synthetic thiamin precursor benfotiamine[48] was found to both halt the progression of amyloid plaques and promote their regression.[49]
- **Parkinson's disease (PD)**. That thiamin may have a role in protecting against PD is suggested by findings that patients have relatively low cerebrospinal fluid levels of free thiamin (although, normal levels of TPP and TMP) and reduced activities of α-KGDH,[50] which is known to be inhibited by the dopamine oxidation products that are elevated in the disease.[51] PD patients given L-dopa show increased cerebrospinal fluid concentrations of thiamin.[52] Mice given TPP or TTP intrastriatally showed dopamine release.[53] A PD-associated polymorphism in α-KGDH has been suggested.

46. Gibson, G.E., Zhang, H., Sheu, K.F., et al., 1998. Ann. Neurol. 44, 676–681; Gibson, G.E., Pulsinelli, W., Blass, J.P., et al., 1981. Am. J. Med. 70, 1247–1254; Butterworth, R.F., Besnard, A.M., 1990. Metab. Brain Dis. 5, 179–184.
47. Karuppagoundeer, S.S., Xu, H., Shi, Q., et al., 2009. Neurobiol. Aging 30, 1587–1600.
48. *S*-benzoylthiamin O-monophosphate.
49. Pan, X., Gong, N., Zhao, J., et al., 2010. Brain 133, 1342–1351.
50. Mizuno, Y., 1995. Biochim. Biophys. Acta 1271, 265.
51. Cohen, G., Farooqui, R., Kesler, N., 1997. Proc. Nat. Acad. Sci. U.S.A. 94, 4890–4894.
52. Jimenéz- Jimenéz, F.J., Mlina, J.A., Hermánz, A., et al., 1999. Neurosci. Lett. 271, 33–36.
53. Yamashita, H., Zhang, Y.X., Nakamura, S., 1993. Neurosci. Lett. 158, 229–231.

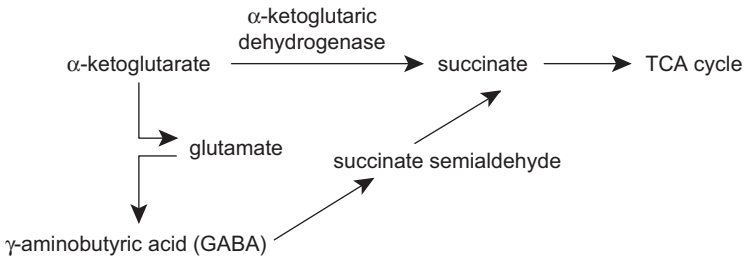


FIGURE 11.4 Flux through the GABA shunt is increased to maintain brain ATP levels in thiamin deficiency.

Vascular Function

Diabetic vascular complications appear to involve insufficiencies of thiamin/TK. TTP has an antidiabetic type role by virtue of its function in TK, which diverts cellular excesses of fructose-6-phosphate and glyceraldehyde-3-phosphate from glycolysis to the hexose monophosphate shunt. This serves to downregulate intracellular glucose levels, thus, avoiding cellular damage. Diabetic subjects have been found to have lower circulating thiamin levels and lower erythrocyte TK activities than healthy controls. Both thiamin and the lipophilic thiamin-derivative benfotiamine[54] have been shown to reduce the accumulation of glycation products and prevent apoptosis in vascular cells cultured under hyperglycemic conditions. Supplementation of either of these compounds has been shown to prevent diabetic cardiomyopathy and neuropathy in the streptozotocin-induced diabetic rat model with moderate insulin treatment.[55] Both have been found effective in preventing vascular dysfunction, oxidative stress, and proteinuria in subjects with type 2 diabetes.[56] Therefore, it has been suggested that diabetes may appropriately be considered a thiamin-deficient state.

Antioxidant Function

The ability to transfer protons from its pyrimidine amino group to its thiazole ring allows thiamin to serve in redox control of cellular pH and to quench prooxidant species. This has been demonstrated in vitro by thiamin prevention of peroxidation in oleic acid or microsomal lipids, with thiamin being oxidized to thiochrome and thiamin disulfide.[57] The physiological relevance of this antioxidant effect is indicated by the fact that thiamin can prevent hepatocyte cytotoxicity and formation of ROS induced by mitochondrial respiratory inhibitors.[58] Oxidative stress of thiamin deficiency is thought to affect immune cell function and to contribute to thiamin-responsive neurologic disorders.

8. BIOMARKERS OF THIAMIN STATUS

Thiamin status has been assessed in two ways:

- **Degree of TPP saturation of thiamin-dependent enzymes**. This is the most useful means of assessing thiamin status. It takes advantage of the in vitro binding of TPP by erythrocyte transketolase (eTK) from hemolysates. Because thiamin-adequate subjects typically have >85% of eTK bound to TPP, the addition of exogenous TPP should stimulate their eTK activities by no more than 15%. Any stimulation above that level indicates that less eTK was bound to TPP. Therefore, the eTK response to added TPP measures eTK saturation and, thus, thiamin status. This is measured as an activity coefficient:

  eTK activity coefficient = baseline eTK activity/eTK activity with added TPP

  Subjects with eTK activity coefficients <1.15 are considered to be at low risk of thiamin deficiency; those with activity coefficients of 1.15–1.25 or >1.25 are considered to be at moderate and high risks, respectively. Symptoms of beriberi are associated with eTK activity coefficients >1.4.
- **Blood and urinary metabolites**. Thiamin deficiency increases concentrations of both pyruvate and α-ketoglutarate in whole blood[59] or plasma and of methylglyoxal[60] in the urine and cerebrospinal fluid.

54. *S*-benzoylthiamine O-monophoshate, a synthetic S-acyl derivative of thiamin that is dephosphorylated to yield the lipid-soluble S-benzoylthiamin.
55. Thornally, P.J., Jahan. I., Ng, R., 2001. J. Biochem. 129, 543; Kohda, Y., Shirakawa, H., Yamane, K., et al., 2008. J. Toxicol. Sci. 33, 459–472.
56. Stirban, A., Negrean, M., Mueller-Roesel, M., et al., 2006. Diabetes Care 29, 2064–2071; Arora, S. Lidor, A., Abularrage, C.J., et al., 2006. Ann. Vasc. Surg. 20, 653–658; Riaz, S., Skinner, V., Srai, S.K., 2011. J. Pharmaceut. Biomed. Anal. 54, 817–825.
57. Lukienko, P.I., Mel'nichenko, N.G., Zverinski, I.V., et al., 2000. Bull. Exp. Biol. Med. 130, 874–876.
58. Mehta, R., Shangari, N., O'Brien, P.J., 2008. Mol. Nutr. Food Res. 52, 379–385.
59. Normal ranges (fasting): pyruvate, 260–450 μg/dL; α-ketoglutarate, 75–170 mg/dL.
60. This appears to result from decreases in reduced glutathione, a cofactor of glyoxylase.

TABLE 11.7 Recommended Thiamin Intakes

US		FAO/WHO	
Age–Sex	RDA[a] (mg/day)	Age–Sex	RNI[b] (μg/day)
0–6 months	[0.2][c]	0–6 months	0.2
7–11 months	[0.3][c]	7–11 months	0.3
1–3 years	0.5	1–3 years	0.5
4–8 years	0.6		
9–13 year females	0.9	4–6 years	0.6
14–18 year females	1.0	7–9 years	0.9
>18 year females	1.1	10–>65 year females	1.1
Males	1.2	Males	1.2
Pregnancy	1.4		1.4
Lactation	1.4		1.5

[a]*Recommended Dietary Requirements; Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.*
[b]*Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*
[c]*RDAs have not been set; AIs are given instead.*

9. THIAMIN DEFICIENCY

Thiamin deficiency can have primary (privational) and secondary (nonprivational) causes. Because thiamin is widely distributed in foods, the latter causes are more common. Thiamin needs (Table 11.7) are directly related to the level of carbohydrate intake.[61] Probably, because overweight/obese individuals tend to have diets high in simple sugars, such individuals tend to have lower circulating thiamin levels than nonobese individuals. A prominent secondary cause of thiamin deficiency is chronically high alcohol consumption, which impairs thiamin absorption and is frequently associated with insufficient dietary intake.

Groups at Risk to Thiamin Deficiency
- Older adults
- Alcoholics
- HIV+/AIDS patients
- Subjects with malaria
- Pregnant women with prolonged hyperemesis gravidarum.

61. Elmadfa, I., Majchrzak, D., Rust, P., et al., 2001. Int. J. Vitam. Nutr. Res. 71, 217–224.

TABLE 11.8 General Signs of Thiamin Deficiency

Organ system	Signs
General appetite	Severe decrease
Growth	Decrease
Dermatologic	Edema
Muscular	Cardiomyopathy, bradycardia, heart failure, weakness
Gastrointestinal	Inflammation, ulcer
Vital organs	Hepatic steatosis
Nervous	Peripheral neuropathy, opisthotonos

Three ways chronic, excess alcohol consumption can lead to thiamin deficiency:
- **by reducing thiamin intake**—the displacement of thiamin-containing foods
- **by impairing thiamin utilization**—inhibition of thiamin absorption
- **by increasing thiamin need—**high associated carbohydrate intake.

General Signs

Thiamin deficiency in humans and animals is characterized by a predictable range of signs/symptoms including the loss of appetite (**anorexia**), cardiac and neurologic signs (Table 11.8). Many of these signs, particularly in the early phases of deficiency, are nonspecific, common, and frequently overlooked: mental fatigue, emotional lability, paresthesias, generalized weakness, myalgias, back pain, nausea, and reduced physical work capacity. Observational studies have indicated suboptimal thiamin status in nearly one-third of elderly, hospitalized patients, 16–29% of preoperative bariatric surgery patients[62] in the United States, and 45% of HIV+ patients in Switzerland.[63]

Underlying these symptoms are a number of metabolic effects including increased plasma concentrations of pyruvate, lactate, and to a lesser extent α-ketoglutarate (especially after a glucose meal), as well as decreased activities of eTK. These effects result from diminished activities of TPP-dependent enzymes. Increased production of ROS and RNS[64] has been reported in the brains of thiamin-deficient animals. This and the finding that antioxidants

62. Kerns, J.S., Arundel, C., Chawla, L.S., 2015. Adv. Nutr. 6, 147–153.
63. Müri, R.M., von Overbeck, J., Furrer, J., et al., 1999. Clin. Nutr. 18, 375–378.
64. Reactive oxygen species and reactive nitrogen species, respectively.

can attenuate the neurologic effects of thiamin deficiency[65] suggests that oxidative stress plays contributes to the clinical manifestations of the nutritional deficiency. The presentation of thiamin deficiency is variable, affected by such factors as age, caloric (especially carbohydrate) intake, and presence/absence of other micronutrient deficiencies.[66]

Deficiency Signs in Humans

Beriberi. The classic syndrome resulting from thiamin deficiency in humans is beriberi. This disease is prevalent in Southeast Asia, where polished rice is the dietary staple. It appears to be associated with the consumption of diets high in highly digestible carbohydrates but marginal or low in micronutrients. The general symptoms of beriberi are anorexia, cardiac enlargement, lassitude, muscular weakness (with resulting ataxia), paresthesia,[67] loss of knee and ankle jerk responses (with subsequent foot and wrist droop), and dyspnea on exertion. Beriberi occurs in three clinical types:

- **Dry** (neuritic) **beriberi** (Fig. 11.5) occurs primarily in adults; it is characterized by peripheral neuropathy consisting of symmetrical impairment of sensory and motor nerve conduction affecting the distal (more than proximal) parts of the arms and legs. It usually does not have cardiac involvement.
- **Wet** (edematous) **beriberi** (also called **cardiac beriberi**) involves as its prominent signs edema, tachycardia,[68] cardiomegaly, and congestive heart failure; in severe cases, heart failure is the outcome. The onset of this form of beriberi can vary from chronic to acute, in which case it is called **shoshin beriberi** (also called **acute pernicious beriberi)** and is characterized by greatly elevated circulating lactic acid levels.
- **Infantile** (or acute) **beriberi** occurs in breast-fed infants of thiamin-deficient mothers, most frequently at 2–6 months of age. It has a rapid onset and may have both neurologic and cardiac signs with death due to heart failure usually within a few hours. Affected infants are anorectic and regurgitate ingested milk; they may experience vomiting, diarrhea, cyanosis, tachycardia, and convulsions. Their mothers typically show no signs of thiamin deficiency.

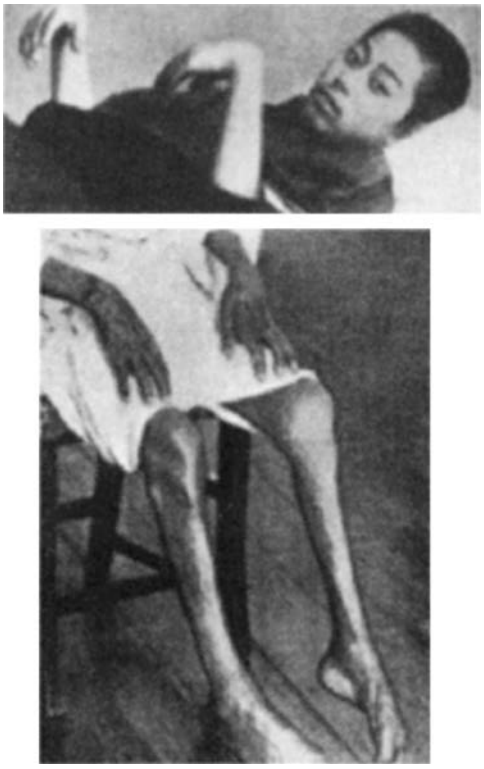


FIGURE 11.5 Neurologic signs of beriberi. *Courtesy Cambridge University Press.*

Wernicke–Korsakoff syndrome.[69] This syndrome condition can be triggered by excessive alcohol consumption in subjects of marginal to deficient thiamin status. It consists of Wernicke's encephalopathy, which includes ophthalmoplegia[70] with lateral or vertical involuntary eye movements (nystagmus[71]) and cerebellar ataxia, and Korsakoff psychosis, which includes severely impaired retentive memory and cognitive function, apathy and confabulation.[72] Pathology is seen in the submedial thalamic nucleus and parts of the cerebellum, particularly the superior cerebellar vermis. The syndrome appears to have a genetic basis, but the affected gene(s) has not been determined. The thiamin-responsive syndrome has been diagnosed in nonalcoholic patients with hyperemesis gravidarum[73] or undergoing dialysis. Patients with the syndrome frequently have a form of TK with low binding affinity for TPP, which can be cured in a quarter of patients by treatment with high oral or intramuscular doses of thiamin. It has been suggested that patients who

65. Pannunzio, P., Hazell, A.S., Pannunzio, M., et al., 2000. J. Neurosci. Res. 62, 286–292.
66. For example, deprivation of magnesium was shown to aggravate the signs of thiamin deficiency in the rat (Dyckner, T., Ek, B., Nyhlin, H., et al., 1985. Acta Med. Scand. 218, 129–131).
67. An abnormal spontaneous sensation, such as burning, pricking, numbness, etc.
68. In contrast to thiamin-deficient animals, which show **bradycardia** (slow heart beat), beriberi patients show **tachycardia** (rapid heart rate, >100 beats/min).
69. This condition has been underdiagnosed by as much as 80% (Harper, C.G., 1979. J. Neurol. Neurosurg. Psychiatry 42, 226–231).
70. Paralysis of one or more of the motor nerves of the eye.
71. Rhythmical oscillation of the eyeballs, either horizontally, rotary, or vertically.
72. Readiness to answer any question fluently with no regard whatever to facts.
73. **Hyperemesis gravidarum** is a severe and intractable form of nausea and vomiting in pregnancy.

fail to respond may have another aberrant TK (or other TPP-dependent enzyme) incapable of binding TPP.

Thiamin-responsive megaloblastic anemia (TRMA). TRMA with diabetes and deafness is a rare, autosomal recessive disorder reported in fewer than three-dozen families. The disorder presents early in childhood with any of the above signs, plus optic atrophy, cardiomyopathy, and stroke-like episodes. The anemia responds to high doses of thiamin[74]. Defects in thiamin transport were reported in TRMA patients.[75] These have been found to be due to mutations of the *slc19a2* gene encoding the high-affinity thiamin transporter.

Other conditions. In women with gestational diabetes, maternal thiamin deficiency correlates with macrosomia (abnormally high infant body weight).[76] Thiamin deficiency has been implicated in cases of sleep apnea and sudden infant death syndrome (SIDS). While this relationship has not been elucidated, it would appear reasonable to expect thiamin to have a role in maintaining the brainstem function governing automatic respiration. In children admitted to a pediatric intensive care unit in Brazil, low blood thiamin level was associated with inflammation.[77] Widespread thiamin depletion in Cuba was reported in 1992–93 during an epidemic of optic and peripheral neuropathy that affected some 50,000 people in a population of 11 million.[78] A large portion (30–70%) of both the cases and the apparently unaffected population showed signs of low thiamin status. The incidence of new cases subsided with the institution of multivitamin supplementation. Still, it is not clear that thiamin deficiency, while widespread in that population, was the cause of the **epidemic neuropathy**.

Thiamin dependency. A few cases of apparent thiamin dependency have been reported. These involved cases of with intermittent episodes of cerebral ataxia, pyruvate dehydrogenase deficiency, branched chain ketoaciduria, and low TK activity coefficients—all of which responded to high doses of thiamin.[79]

Deficiency Signs in Animals

Polyneuritis. The most remarkable sign of thiamin deficiency in most species is anorexia, which is so severe and more specific than any associated with other nutrient deficiencies (apart from that of sodium) that it is a useful diagnostic indicator for thiamin deficiency. Other signs include the secondary effects of reduced total feed intake: weight loss, impaired efficiency of feed utilization, weakness, and hypothermia. The appearance of anorexia correlates with the loss of transketolase activity and precedes changes in pyruvate or α-KGDH activities.

Animals also show neurologic dysfunction due to thiamin deficiency; birds, in particular, show a tetanic retraction of the head called **opisthotonos**, also **stargazing** (Figs. 11.6 and 11.7).[80] Other species generally show **ataxia** and incoordination, which progresses to convulsions and death.

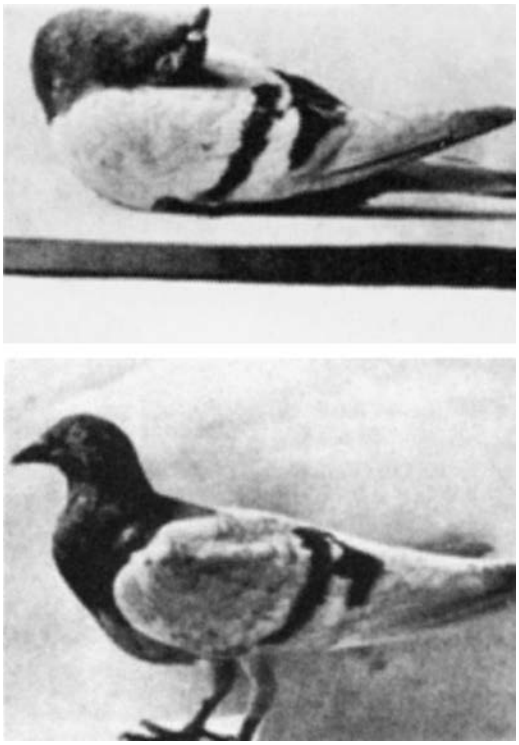


FIGURE 11.6 Opisthotonus in a thiamin-deficient pigeon before (*top*) and after (*bottom*) thiamin treatment. *Courtesy Cambridge University Press.*

FIGURE 11.7 Opisthotonos in a thiamin-deficient sheep (unaffected sheep in foreground). *Courtesy of M. Hidiroglou, Agricultural Canada, Ottawa, Ontario, Canada.*

74. 20–60 times higher than RDA levels.
75. Poggi, V., Longo, G., DeVizia, et al., 1984. J. Inherit. Metab. Dis. 7, 153–154.
76. Baker, H., Hockstein, S., DeAngelis, B., et al., 2000. Int. J. Vit. Nutr. Res. 70, 317–320.
77. Lima, L.F.P., Leite, H.P., Taddei, J.A., 2011. Am. J. Clin. Nutr. 93, 57–61.
78. Macias-Matos, C., Rodriguez-Ojea, A., Chi, N., et al., 1996. Am. J. Clin. Nutr. 64, 347–353.
79. Lonsdale, D., 2006. Evid. Based Complement. Alternat. Med. 3, 49–59.
80. This sign occurs in young mammals, but it is not usual.

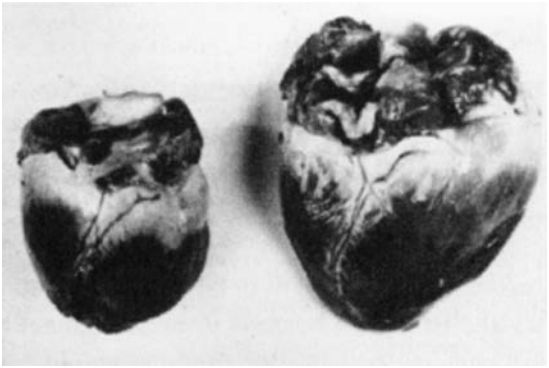


FIGURE 11.8 Hearts from normal (*left*) and thiamin-deficient (*right*) pigs. *Courtesy of T. Cunha, University of Florida.*

These conditions are generally referred to as **polyneuritis**. Most species, but especially dogs and pigs, show **cardiac hypertrophy**[81] (Fig. 11.8), with slowing of the heart rate (**bradycardia**) and signs of congestive heart failure, including labored breathing and edema. Some species also show diarrhea and achlorhydria (rodents), gastrointestinal hemorrhage (pigs), infertility (chickens[82]), high neonatal mortality (pigs), and impaired learning (cats).

The clinical manifestation of thiamin deficiency in young ruminants is the neurologic syndrome called **polioencephalomalacia**,[83] a potentially fatal condition involving inflammation of brain gray matter and presenting as opisthotonos; it readily responds to thiamin treatment.[84] Thiamin deficiency can occur if microbial production of the vitamin is impaired. Such cases have occurred due to the following:

- **Depressed ruminal thiamin synthesis**—as a result of a change in diet that disturbs rumen fermentation;
- **Increased thiamin degradation**—due to an alteration in the microbial population that increases total thiaminase activity. The most important organisms in this regard are those *Bacteroides thiaminolyticus*, *Clostridium sporogenes*, *Megasphaera elsdenii*, *Streptococcus bovis*, other *Clostridium* spp., *Bacillus* spp., and gram-negative cocci, which have thiaminases bound to their cell surfaces as exoenzymes.[85] These can cause significant thiamin losses when they are released into the rumen fluid, which can happen under conditions of sharply declining rumen pH. Accordingly, signs of thiamin deficiency have been observed in animals fed high-concentrate diets that tend to acidify the rumen.
- **Thiamin antagonists**—excess **sulfate** (which rumen microbes reduce to **sulfite**), excess amprolium, or factors contained in bracken fern or endophyte-infected fescue. Thiamin treatment has been found to reduce the signs of summer **fescue toxicity** (reduced performance, elevated body temperature, and rough hair coat) in grazing beef cattle.

81. Enlargement of the heart.
82. Thiamin deficiency impairs the fertility of both roosters (via testicular degeneration) and hens (via impaired oviductal atrophy).
83. Cerebrocortical necrosis.
84. Polioencephalomalacia is thought to be a disease of thiamin deficiency induced by thiaminases synthesized by rumen microbiota or present in certain plants. Affected animals are listless and have uncoordinated movements; they develop progressive blindness and convulsions. The disease is ultimately fatal but responds dramatically to thiamin.
85. Of these, *Bacteroides thiaminolyticus* appears to be of greatest pathogenic importance, as it appears to occur routinely in the ruminal contents and feces of all cases of polioencephalomalacia.

Response to Treatment

Thiamin-deficient humans are typically treated with several days of relatively high doses of the vitamin. Several days of doses are 10–200 mg/day. Cases of cardiac ("wet") beriberi have been treated with parenteral doses of 100 mg for several days.

10. ROLE OF THIAMIN IN HEALTH AND DISEASE

Two thiamin-responsive disorders have been reported:

- **DIDMOAD**. Genetic defects in the calcium channel protein wolframin (encoded by the *wfs1* gene) produce symptoms similar to TRMA syndrome: diabetes insipidus, insulin-dependent diabetes, bilateral progressive optic atrophy and deafness (DIDMOAD), depression, and psychosis. Thiamin supplementation can correct the glucose abnormalities and hematological symptoms.
- **Wilson disease**, due to a mutation of the *atp7b* gene that encodes an ATPase-Cu^{2+} transport protein, leads to toxic accumulation of Cu, which ultimately inhibits two TPP enzymes, α-KGDH and PDH. Supplements of thiamin and lipoate can correct this condition.

Dietary thiamin may also have a role in managing some neurologic diseases:

- **Alzheimer's disease**. The lower brain activities of α-KGDH, PDH, and TK in Alzheimer's disease patients compared to controls may reflect genetic variations in genes encoding portions of those enzymes. Animal studies have demonstrated the potential of thiamin supplementation to arrest and, perhaps, regress the formation of amyloid plaques. Only a few small trials have tested the therapeutic value of thiamin for treating Alzheimer's disease; those have yielded inconsistent results.
- **Parkinson's disease (PD)**. That PD patients given L-dopa showed increased cerebrospinal fluid concentrations of thiamin[86] suggests that supplemental thiamin may have value in managing PD. That hypothesis has not been tested.

86. Jimenéz- Jimenéz, F.J., Mlina, J.A., Hermánz, A., et al., 1999. Neurosci. Lett. 271, 33–36.

11. THIAMIN TOXICITY

Thiamin is generally well tolerated. Therapeutic doses as great as 300 mg/day are used therapeutically (e.g., to treat beriberi, Wernicke–Korsakoff syndrome, etc.) in humans without adverse reactions; however, greater doses have produced allergic reactions, headache, convulsions, weakness, paralysis, and cardiac arrhythmia. In the dog, very high doses (e.g., 1000-fold required levels) of thiamin hydrochloride have been found to be fatal, suppressing respiration and producing curare[87]-like signs suggestive of blocked nerve transmission: restlessness, epileptiform convulsions, cyanosis, and dyspnea.[88] Upper tolerable limits of exposure have not been set for thiamin.

12. CASE STUDIES

Instructions

Review each of the following case reports, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

Case 1

A 35-year-old man with a history of high alcohol intake for 18 years was admitted to the hospital complaining of massive swelling and shortness of breath on exertion. For several months, he had subsisted almost entirely on beer and whiskey, taking no solid food. He was grossly edematous, slightly jaundiced, and showed transient cyanosis[89] of the lips and nail beds. His heart showed gallop rhythm.[90] His left pleural cavity contained fluid. His liver was enlarged with notable ascites.[91] He had a coarse tremor of the hands and reduced tendon reflexes. His electrocardiogram showed sinus **tachycardia**.[92] His radiogram showed pulmonary edema and cardiac enlargement. He was evaluated by cardiac catheterization.

87. Curare is an extract of various plants (e.g., *Strychnos toxifera, Strychnos castelraei, Strychnos crevauxii, Chondrodendron tomentosum*). Practically inert when administered orally, it is a powerful muscle relaxant when administered intravenously or intramuscularly, exerting its effect by blocking nerve impulses at the myoneural junction. Curare is used experimentally and clinically to produce muscular relaxation during surgery. It was used originally as an arrow poison by indigenous hunters of South America to kill prey by inducing paralysis of the respiratory muscles.
88. Davis, R.E., Icke, G.C., 1983. Adv. Clin. Chem. 23, 93–140.
89. Dark bluish discoloration of the skin resulting from deficient oxygenation of the blood in the lungs or abnormally reduced flow of blood through the capillaries.
90. Triple cadence to the heart sounds at rates of >100 beats/min, indicative of serious myocardial disease.
91. Accumulation of serous fluid.
92. Rapid beating of the heart (>100 beats/min), originating in the sinus node.

Results

Parameter	Patient	Normal Value
Systemic arterial pressure (mm Hg)	100/55	120/80
Systemic venous pressure (cm H_2O)	300	<140
Pulmonary artery pressure (mm Hg)	64/36	<30/<13
Right ventricular pressure (mm Hg)	65/17	<30/<5
O_2 consumption (mL/min)	259	200–250
Peripheral blood O_2 (mL/L)	148	170–210
Pulmonary arterial blood O_2 (mL/L)	126	100–160
Cardiac output (L/min)	11.8	5–7
Blood hemoglobin (g/dL)	11.0	14–19
Cyanide circulation time (s)	12	20
Femoral arterial pyruvate (mg/dL)	1.5	0.8
Femoral arterial lactate (mg/dL)	14.1	4.7
Femoral arterial glucose (mg/dL)	86	74
Femoral arterial lactate (mg/dL)	14.1	4.7

The patient was given thiamin intravenously (10 mg every 6 h) for several days. Improvement was evident by 48 h and continued for 2 weeks. Thirty days later, he or she was free of edema, dyspnea, and cardiomegaly. Cardiac catheterization at that time showed that his or her blood, systemic venous, and all intracardiac pressures, as well as cardiac output, had all returned to normal.

Case 2

Fibroblasts were cultured from skin biopsies from four patients with Wernicke–Korsakoff syndrome and from four healthy control subjects. The properties of transketolase were studied (Table 11.9). The first patient was a 50-year-old woman with a history of chronic alcoholism. She had been admitted to the hospital with disorientation, nystagmus, sixth nerve weakness,[93] ataxia, and malnutrition. Treatment with intravenous thiamin and large oral

TABLE 11.9 Characteristics of Transketolase From Wernicke–Korsakoff Patients

Parameter	Patients	Controls
V_{max} (nmol/min/mg protein)	27 ± 3	17 ± 1
K_m (μM) TPP	195 ± 3	116 ± 2

93. The sixth cranial nerve is the *nervus abducens*, the small motor nerve to the lateral rectus muscle of the eye.

doses of multivitamins had improved her neurologic signs over a few months, but her mental state had deteriorated. She was readmitted with disorientation in both place and time, impaired short-term memory, nystagmus, ataxia, and signs of peripheral neuropathy. She was treated with parenteral thiamin and enteral B vitamins with thiamin; this had improved her general health but had not affected her mental status. The second patient, a 48-year-old man with a 20-year history of chronic alcoholism, was admitted in a severe confusional state. He was disoriented and had severe impairment of recent memory, confabulation, **perseveration**,[94] delusions, nystagmus, and ataxia. Treatment with thiamin and B vitamins had improved his behavior, without affecting his memory.

These results show that the affinity of transketolase for its coenzyme (TPP) in Wernicke–Korsakoff patients was less, by an order of magnitude, than that of controls. Further, this biochemical abnormality persisted in fibroblasts cultured for >20 generations in medium containing excess thiamin and no ethanol. The characteristics of pyruvate and α-KGDHs were similar in fibroblasts from patients and controls.

Case Questions

1. What factors would appear to have contributed to the thiamin deficiencies of these patients?
2. What defect in cardiac energy metabolism would appear to be the basis of the high-output cardiac failure observed in the first case?
3. What evidence suggests that the transketolase abnormality of these patients was hereditary? Would you expect such patients to be more or less susceptible to thiamin deprivation? Explain.

94. The constant repetition of a meaningless word or phrase.

13. STUDY QUESTIONS AND EXERCISES

1. Construct a schematic map of intermediary metabolism showing the enzymatic steps in which TPP is known to function as a coenzyme.
2. Construct a decision tree for the diagnosis of thiamin deficiency in humans or animals.
3. How does the chemical structure of thiamin relate to its biochemical function?
4. What parameters might you measure to assess the thiamin status of a human or animal?
5. Construct a concept map illustrating the possible interrelationships of excessive alcohol intake and thiamin status.

RECOMMENDED READING

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Chapter 12

Riboflavin

Chapter Outline

Anchoring Concepts

1. Riboflavin is the trivial designation of a specific compound, 7,8-dimethyl-10-(1′-D-ribityl)-isoalloxazine, sometimes also called **vitamin B_2**.
2. Riboflavin is a yellow, hydrophilic, and tricyclic molecule that is usually phosphorylated (to FMN and FAD) in biological systems.
3. Deficiencies of riboflavin are manifested chiefly as dermal and neural disorders.

In retrospect—the discovery of riboflavin may be considered a scientific windfall. It opened the way to the unraveling of the truly complex vitamin B_2 complex. Perhaps even more significantly, it bridged the gap between an essential constituent and cell enzymes and cellular metabolism. Today, with the general acceptance of this idea, it is not considered surprising that water-soluble vitamins represent essential parts of enzyme systems.

P. György[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of riboflavin
2. To understand the means of enteric absorption and transport of riboflavin
3. To understand the biochemical function of riboflavin as a component of key redox coenzymes and the relationship of that function to the physiological activities of the vitamin
4. To understand the physiologic implications of low riboflavin status

VOCABULARY

Acyl-CoA dehydrogenase
Adrenodoxin reductase
Alkaline phosphatase
Amino acid oxidases
Cheilosis
Curled toe paralysis
Dehydrogenase
Electron transfer flavoprotein (ETF)
Erythrocyte glutathione reductase
FAD
FAD pyrophosphatase
FAD synthase
Flavin
Flavin adenine dinucleotide (FAD)
Flavin exchange protein (FLX1)
Flavin mononucleotide (FMN)
Flavoenzyme
Flavokinase
Flavoprotein
Flavoproteome
FMN
FMN phosphatase
Geographical tongue
Glossitis
L-Gulonolactone oxidase

1. Paul Gyorgy (1893–1976) was a Hungarian-born American pediatrician and nutritionist known for his discoveries of riboflavin, vitamin B_6, and biotin. In 1975, he was awarded the U.S. National Medal of Science (Biology).

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00012-5

Hypoplastic anemia
Isoalloxazine nucleus
Leukopenia
Lumichrome
Lumiflavin
Monoamine oxidase
Multiple acyl-CoA dehydrogenase deficiency (MADD)
NADH–cytochrome *P*450 reductase
NADH dehydrogenase
Normocytic hypochromic anemia
Ovoflavin
Oxidase
Reticulocytopenia
Riboflavin-binding proteins (RfBPs)
Riboflavin adenine diphosphate
Riboflavin monophosphate
Riboflavin-5′-phosphate
Riboflavin transporters (RFTs)
Riboflavinuria
Riboflavinyl radical
Ribotyl side chain
Stomatitis
Subclinical riboflavin deficiency
Succinate dehydrogenase
Thrombocytopenia
Thyroxine
Ubiquinone reductase
Vitamin B_2

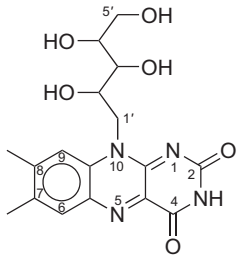
1. THE SIGNIFICANCE OF RIBOFLAVIN

Riboflavin is essential for the intermediary metabolism of carbohydrates, amino acids, and lipids and also supports cellular antioxidant protection. The vitamin discharges these functions in the form of coenzymes that undergo reduction through two sequential single-electron transfer steps. This allows the reactions catalyzed by **flavoproteins** (i.e., **flavoenzymes**) to involve single- as well as dual-electron transfers. This versatility means that flavoproteins serve as switching sites between obligate two electron donors such as the pyridine nucleotides and various obligate one electron acceptors. Because of these fundamental roles of riboflavin in metabolism, a deficiency of the vitamin first manifests itself in tissues with rapid cellular turnover, such as skin and epithelium.

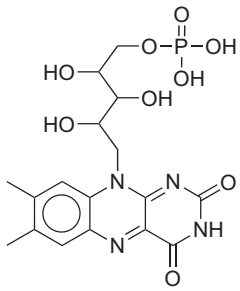
2. PROPERTIES OF RIBOFLAVIN

Riboflavin is among the substituted isoalloxazines synthesized by bacteria, yeasts, and plants. The term riboflavin refers to the compound 7,8-dimethyl-10-(1′-d-ribityl)isoalloxazine[2], which consists of a substituted **isoalloxazine nucleus** with a d-ribityl side chain and reducible nitrogen atoms in nucleus. It is metabolically functional as **flavin mononucleotide** (**FMN**) and **flavin adenine dinucleotide** (**FAD**).[3]

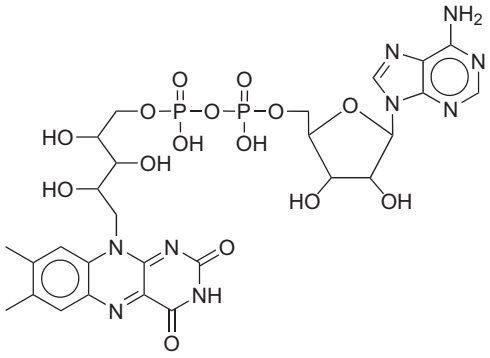
Chemical structures of riboflavin and its metabolically functional forms are as follows:



Riboflavin



Flavin mononucleotide (FMN)



Flavin adenine dinucleotide (FAD)

Riboflavin Chemistry

Riboflavin is a yellow tricyclic molecule that is usually phosphorylated (to FMN and FAD) in biological systems. In FAD, the isoalloxazine and adenine nuclear systems are arranged one above the other and are nearly coplanar. The flavins are light-sensitive, undergoing photochemical degradation of the ribityl side chain, which results in the formation of such breakdown products as **lumiflavin** and lumichrome.[4] Therefore, the handling of riboflavin must be done in the dark or under subdued red light.

2. Formerly known as vitamin B_2, vitamin G, lactoflavin or riboflavin.

3. In fact, these compounds are not nucleotides; they are more properly referred to as **riboflavin monophosphate** and **riboflavin adenine diphosphate**, respectively.

4. 7,8-Dimethylalloxazine, an irradiation product of riboflavin believed also to be produced by intestinal microbiota.

Riboflavin is moderately soluble in water (10–13 mg/dL) and ethanol but insoluble in ether, chloroform, and acetone. It is soluble but unstable under alkaline conditions. Because riboflavin cannot be extracted with the usual organic solvents, it is extracted with chloroform as lumiflavin after photochemical cleavage of the ribityl side chain. Flavins show two absorption bands, at <370 nm and <450 nm, with fluorescence emitting at 520 nm.

The catalytic functions of riboflavin are carried out primarily at positions N-1, N-5, and C-4 of the isoalloxazine nucleus. In addition, the methyl group at C-8 participates in covalent bonding with enzyme proteins. The flavin coenzymes are highly versatile redox cofactors because they can participate in either one- or two-electron redox reactions, thus serving as switching sites between obligate two electron donors (e.g., NAD(H), succinate) and obligate one electron acceptors (e.g., iron–sulfur proteins, heme proteins). They serve this function by undergoing reduction through a two-step sequence involving a radical anion intermediate. Because the latter can also react with molecular oxygen, flavins can also serve as cofactors in the two-electron reduction of O_2 to H_2O and in the reductive four-electron activation and cleavage of O_2 in the monooxygenase reactions. In these redox reactions, riboflavin undergoes changes in its molecular shape, i.e., from a planar oxidized form to a folded reduced form. Differences in the affinities of the associated apoprotein for each shape affect the redox potential of the bound flavin.

Riboflavin antagonists include analogs of the isoalloxazine ring (e.g., diethylriboflavin, dichlororiboflavin, phenothiazine drugs) and the ribityl side chain (e.g., d-araboflavin, D-galactoflavin, 7-ethylriboflavin), bind flavin coenzymes (e.g., adriamycin, tetracycline), or bind riboflavin directly (e.g., boric acid).

3. SOURCES OF RIBOFLAVIN

Hindgut Microbial Synthesis

Riboflavin can be produced by the microbiome of the colon. A genomic analysis of 256 representative organisms of the human gut microbiota found more than half (56%) capable of de novo synthesis of the vitamin.[5] Those findings suggested that hindgut microbial synthesis may produce nearly 3% of the daily human need for riboflavin. However, direct evidence is lacking for the absorption of riboflavin across the colon. It is likely that noncoprophagous animals derive no benefit from this source of the vitamin.

Distribution in Foods

Riboflavin is widely distributed in foods (Table 12.1), where it is present almost exclusively bound to proteins, mainly in

5. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.

TABLE 12.1 Riboflavin Contents of Foods

Food	Riboflavin (mg/100 g)
Dairy Products	
Milk	0.17–0.19
Yogurt	0.28
Cheese	
Cheddar	0.43
Cottage	0.17
Meats	
Liver, beef	2.76
Beef	0.09–0.24
Chicken	0.12–0.22
Pork	0.19–0.39
Ham, cured	0.26
Cereals	
Wheat, bran	0.58
Rye flour	0.11
Oats	0.14
Rice	0.01–0.07
Cornmeal	0.20
Vegetables	
Asparagus	0.14
Broccoli	0.12
Cabbage	0.04
Carrots	0.06
Cauliflower	0.06
Lima beans	0.10
Potatoes	0.02
Spinach	0.19
Tomatoes	0.02
Fruits	
Apples	0.03
Bananas	0.07
Oranges	0.05
Peaches	0.03
Strawberries	0.02
Other	
Eggs	0.46

From USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

the form of FMN and FAD.[6,7] Rapidly growing, green, leafy vegetables are rich in the vitamin; however, meats and dairy products are the most important sources. Animal tissues have been found to contain small amounts of riboflavin-5′-a-D-glucoside, which appears to be as well utilized as free riboflavin. It is estimated that milk and milk products contribute about 50% of the riboflavin in the American diet, with meats, eggs, and legumes contributing a total of about 25%, and fruits and vegetables each contributing about 10%.

Stability

Riboflavin is stable to heat; therefore, most means of heat sterilization, canning, and cooking do not affect the riboflavin contents of foods. However, exposure to light (e.g., sun drying, sunlight exposure of milk in glass bottles, cooking in an open pot) can result in substantial losses, as the vitamin is very sensitive to destruction by light. Thus, exposure of milk in glass bottles to sunlight can result in the destruction of more than half of its riboflavin within a day. Irradiation of food results in the production of reactive oxygen species (ROS, e.g., superoxide, hydroxyl radical) that react with riboflavin to destroy it. The short exposure of meat to sterilizing quantities of γ–irradiation destroys 10–15% of its riboflavin content. Riboflavin photodegradation can be exacerbated by sodium bicarbonate, which is used to preserve vegetable colors. Also, because riboflavin is water soluble, it leaches into water used in cooking and into the drippings of meats. As riboflavin in cereal grains is located primarily in the germ and bran, the milling of such materials,[8] which removes those tissues, results in considerable losses in their contents of the vitamin. For example, about half of the riboflavin in whole grain rice, and more than one-third of riboflavin in whole wheat, is lost when these grains are milled. Parboiled ("converted") rice contains most of the riboflavin of the parent grain, as the steam processing of whole brown rice before milling this product drives vitamins originally present in the germ and aleurone layers into the endosperm, where they are retained.

Bioavailability

The apparent bioavailability of riboflavin can depend on the method of assessment. Because the vitamin is largely removed from the liver after entering the circulation, plasma responses to oral dosing underestimate bioavailability. Better estimates are obtained from assays based on the prevention of clinical signs in animals (e.g., curled toe paralysis in the chick) or on monitoring urinary output. Such assays show that the noncovalently bound forms of riboflavin in foods, FMN, FAD, and free riboflavin appear to be well absorbed. In contrast, covalently bound flavin complexes tend to be more stable to digestion and, thus, less bioavailable; some 10–15% of flavins from plant sources have been found not to be utilized by humans.[9] In general, riboflavin in animal products tends to have a greater bioavailability than that in plant products. While foods contain relatively little free riboflavin, that form is widely consumed in multivitamin supplements and vitamin-fortified cereals.

4. ABSORPTION OF RIBOFLAVIN

Hydrolysis of Coenzyme Forms

The enteric absorption of riboflavin depends on its being in the free, nonphosphorylated form. This occurs by the actions of nonspecific proteolytic activities of the intestinal lumen, which release riboflavin coenzymes from their protein complexes, and the subsequent hydrolytic activities of several nonspecific brush border pyrophosphatases that liberate riboflavin in free form. Principal contributors among the latter enzymes are the relatively nonspecific alkaline phosphatase,[10] as well as FAD pyrophosphatase (which converts FAD to FMN) and FMN phosphatase (which converts FMN to free riboflavin).

Enteric Absorption of Free Riboflavin

Riboflavin is absorbed across the enterocyte in the free form by highly specific **riboflavin transporters (RFTs)**. These are located on the enterocyte brush border (RFT-1[11]) and basolateral surface (RFT-2), and in intracellular vesicles (RFT-3) and are expressed in several tissues including the proximal small intestine and colon. A genetic defect in RFT-1 was found to manifest as elevated plasma acylcarnitine levels and organic aciduria, suggestive of **multiple acyl-CoA dehydrogenase deficiency (MADD)**.[12] A defect in RFT-2 has been associated with Brown–Vialetto–Van Laere syndrome, which is manifest as a biochemical profile similar to MADD with progressive neurological dysfunction.[13]

6. Notable exceptions are milk and eggs, which contain appreciable amounts of free riboflavin.
7. It should be noted that, strictly speaking, FMN is not a nucleotide, nor is FAD a dinucleotide, because each is a D-ribotyl derivative; nevertheless, these names have been accepted.
8. It is the practice in many countries to enrich refined wheat products with several vitamins, including riboflavin, which result in their actually containing *more* riboflavin than the parent grains (e.g., 0.20 mg/100 g *versus* 0.11 mg/100 g). However, rice is usually *not* enriched with riboflavin to avoid coloring the product yellow by this intensely colored vitamin.
9. Decker, K.F., 1993. Ann. Rev. Nutr. 13, 17–41.
10. Alkaline phosphatase appears to have the greatest hydrolytic capacity of the brush border phosphatases.
11. RFT-1 has been identified as G-protein-coupled receptor 172B (GPR172B).
12. Ho, G., Yonezawa, A., Masuda, S., et al., 2011. Human?
13. Bosch, A.M., Abeling, N.G., Ijist, L., et al., 2011. J. Inherit. Metab. Dis. 34, 159–164.

The upper limit of intestinal riboflavin absorption has been estimated to be about 27 mg[14]—more than an order of magnitude greater than the dietary requirement.[15] Riboflavin absorption is enhanced by riboflavin deficiency, by bile salts[16], and by factors that stimulate intestinal motility, e.g., the presence of food. Psyllium fiber reduces riboflavin absorption; but wheat bran has no effect. Alcohol impairs both the digestion of food flavins and the absorption of the free vitamin. Riboflavin absorption is downregulated by high doses of the vitamin, apparently through reduced activity of the riboflavin carrier induced by increased intracellular concentrations of cyclic AMP.

Much of the riboflavin transported into the enterocyte is quickly phosphorylated to FMN. This is accomplished by an ATP-dependent **flavokinase**. Thus, riboflavin enters the portal circulation as both the free vitamin and FMN.

5. TRANSPORT OF RIBOFLAVIN

Protein Carriers

Nonspecific carriers. Riboflavin is transported in the plasma as both free riboflavin and FMN, both of which are mostly bound to plasma proteins. This includes albumin and several immunoglobulins (IgA, IgG, and IgM); collectively, these bind about half of the free riboflavin and 80% of FMN in plasma. This involves hydrogen bonding, the strength of which varies among these proteins as well as among the various species of the vitamin,[17] thus, influencing the distribution of those species to the tissues as well as their clearance across the kidney. The vitamin can be displaced readily from these nonspecific carriers by boric acid,[18] several drugs,[19] lumiflavin, and lumichrome to inhibit its transport to the tissues.

Specific riboflavin-binding proteins (RfBPs). RfBPs have been identified in the plasma of the laying hen and pregnant cows, mice, rats, monkeys, and humans. Of these, the avian RfBP is the best characterized. It is not found in the immature female but is synthesized in the liver under the stimulus of estrogen with the onset of sexual maturity or with induction by estrogen treatment. The avian plasma RfBP is a 37-kDa phosphoglycoprotein with a single binding site for riboflavin. It appears to be one of three products of a single gene, which are variously modified posttranslationally. RfBP synthesized by the liver is N-linked to oligosaccharides and phosphorylated at six serinyl residues prior to being exported to the plasma. This RfBP is antigenically similar in the laying hen and pregnant mice, rats, cows, and humans. The RfBP in avian eggs (albumen and yolk) is synthesized by the oviduct.[20] Male mice produce RfBP in their testicular Leydig cells; it is secreted in response to luteinizing hormone via G-protein-induced production of cyclic AMP.[21] RfBP localizes in the sperm head. Pregnant women show low-circulating plasma levels of RfBP until about 4 months of gestation at which time those levels increase. Placental trophoblasts can also synthesize RfBP. These increases corresponding to two- to threefold increases in the RfBP contents of amniotic fluid. Thus, in each species, RfBP has vital functions in the transplacental/transovarian movement of riboflavin[22] and in the uptake of riboflavin by spermatozoa, as immunoneutralization of the protein terminates pregnancy in females and reduced sperm fertility in males.[23]

Cellular Uptake

Riboflavin uptake into cells occurs by the transfer of the vitamin by RfBP in plasma to RFTs on the plasma membrane. This process is mediated by a Ca^{2+}-dependent RfBP receptor located in clathrin[24]-coated pits on the plasma membrane, which facilitates endocytosis and release of the vitamin, with recycling of the receptor and RfBP being catabolized within endosomes. Receptor-mediated endocytosis has also been implicated in the transport of riboflavin across the placental barrier.[25] RFTs are expressed in the small intestine, colon, placenta, embryonic kidney, testes, prostate, and brain. They show varying sequence homologies and different functionalities. RFT-1 is not sensitive to pH; but RFT-2 has a pH optimum around 6.0. Their apparent K_m values vary in a way suggestive of intracellular riboflavin homeostasis being controlled by

14. Zempleni, J., Galloway, J.R., McCormick, D.B., 1996. Am. J. Clin. Nutr. 63, 54–66.
15. Greater amounts of the vitamin, e.g., from massive doses pass to the hindgut where they are degraded by the microbiota and excreted.
16. Children with biliary atresia (a congenital condition involving the absence or pathological closure of the bile duct) show reduced riboflavin absorption.
17. The binding affinities have been estimated: albumin–riboflavin (one binding site), $K_d = 3.8–10.4$ mM; Ig-riboflavin (two binding sites), $K_d = 2.43$ and 0.068 nM; Ig-FAD (two binding sites), $K_d = 1.73$ and 0.078 nM.
18. Boric acid can produce riboflavinuria and precipitate riboflavin deficiency; some effects of boric acid toxicity can be overcome by feeding riboflavin.
19. For example, ouabain, theophylline, penicillin.
20. It is cotransported into the yolk with the glycoprotein vitellogenin.
21. Subramanian, S., Adiga, P.R., 1996. Mol. Cell Endocrinol. 120, 41–50.
22. The astute observation that a particular hen that produced eggs lacking the normal faint yellow tinge of its otherwise clear albumen led to the discovery of RfBP (which that hen failed to express) as being essential for the transfer of riboflavin to the egg (Winter, W.P., Buss, E.G., Claget, C.O., et al., 1967. Comp. Biochem. Physiol. 22, 889–896).
23. Plasma RfBP or fragments has been suggested as having potential as a vaccine to regulate fertility in both sexes (Adiga, P.R., 1997. Human Reprod. Update 3, 325–332).
24. Named for the Latin "*clatratus*," meaning "like a lattice", this protein plays a major role in the formation of coated vesicles that facilitate endocytosis and exocytosis to allow cells to communicate.
25. Foraker, A.M., Knantwell, C.M., Swan, P.W., 2002. Adv. Drug Deliv. Rev. 55, 1467.

its trafficking into acidic vesicular compartments (RFT-1, 1.38 μM; RFT-2, 0.98 μM; RFT-3, 0.33 μM). A genetic defect in RFT-1 was found to manifest as elevated plasma acylcarnitine levels and organic aciduria, suggestive of MADD.[26] A defect in RFT-2 has been associated with Brown–Vialetto–Van Laere syndrome, which is manifest as a biochemical profile similar to MADD with progressive neurological dysfunction.[27]

Tissue Distribution

Riboflavin is transported into cells in its free form. However, in the tissues, riboflavin is converted to the coenzyme form, predominantly as FMN (60–95% of total flavins) but also as FAD (5–22% of total flavins in most tissues but about 37% in kidney), both of which are found almost exclusively bound to specific flavoproteins. The greatest concentrations of the vitamin are found in the liver, kidney, and heart. In most tissues, free riboflavin comprises <2% of the total flavins. Significant amounts of free riboflavin are found only in retina, urine, and cow's milk,[28] where it is weakly bound to casein. Although the riboflavin content of the brain is not great, the turnover of the vitamin in that tissue is high and the concentration of the vitamin is relatively resistant to gross changes in riboflavin nutriture. These findings suggest a homeostatic mechanism for regulating the riboflavin content of the brain; such a mechanism has been proposed for the choroid plexus,[29] in which riboflavin transport has been found to be inhibited by several of its catabolic products and analogues. It has been estimated that the total body reserve of riboflavin in the adult human is equivalent to the metabolic demands for 2–6 weeks. Riboflavin is found in much lower concentrations in maternal plasma than in cord plasma (in humans, this ratio has been found to be 1:4.7), suggesting the presence of a transplacental transport mechanism.

Tissue RfBPs have been identified in the liver, egg albumen,[30] and egg yolk of the laying hen. Each is similar to the plasma RfBP[31] in that species, differing only in the nature of their carbohydrate[32] contents.[33] A hereditary abnormality in the chicken results in the production of defective RfBPs (in plasma as well as liver and egg). Affected hens show **riboflavinuria** and produce eggs with about half the normal amount of riboflavin and embryos that fail to develop.[34]

6. METABOLISM OF RIBOFLAVIN

Conversion to Coenzyme Forms

After it is taken up by the cell, free riboflavin is converted to its coenzyme forms (Fig. 12.1) in two steps, both of which appear to be regulated by thyroid hormones:

1. **Conversion to FMN** occurs by ATP-dependent phosphorylation to yield **riboflavin-5′-phosphate**, i.e., FMN, in the cytoplasm of most cells. This step is catalyzed by flavokinase[35] (also called riboflavin kinase), which uses ATP or dATP as the phosphate donor and Zn^{2+} as an activator. Flavokinase expression is stimulated by thyroxine. It is downregulated under conditions of dietary riboflavin deficiency and inflammation. The latter effect may be related to overexpression of tumor necrosis factor α (TNFα). Flavokinase is known to bind a TNFα-receptor 1 binding protein that binds a subunit of NADPH oxidase; this multicomponent linking may be important for the activation of NADPH oxidase. Flavokinase has catalytically important sulfhydryl domains, as reducing agents (e.g., reduced glutathione) protect it from inhibition. FMN can be complexed with specific FMN-requiring apoproteins to form functional flavoproteins (Table 12.2).
2. **Conversion to FAD**[36] occurs by the further metabolism of the major portion of FMN to the other coenzyme form, **FAD,** by a second ATP-dependent enzyme, **FAD synthase**.[37] There are two forms of this enzyme, a mitochondrial form (FAD synthase 1) and a cytosolic form (FAD synthase 2). This multicompartment distribution

26. Ho, G., Yonezawa, A., Masuda, S., et al., 2011. Hum. Mutat. 31, E1976–E1984.
27. Bosch, A.M., Abeling, N.G., Ijlst, L., et al., 2011. J. Inherit. Metab. Dis. 34, 159–164.
28. It should be noted that cow's milk differs from human milk in both the amount and form of riboflavin. Cow's milk typically contains 1160–2020 μg of riboflavin per liter, which (like the milk of most other mammals studied) is present mostly as the free vitamin. In contrast, human milk typically contains 120–485 μg of riboflavin per liter (depending on the riboflavin intakes of the mother), which is present mainly as FAD and FMN.
29. The anatomical site of the blood–cerebrospinal fluid barrier.
30. This is the flavoprotein formerly called **ovoflavin**. Comprising nearly 1% of the total protein in egg white, it is the most abundant of any vitamin-binding protein. Unlike the plasma RfBP, which is normally saturated with its ligand, the egg white RfBP is normally less than half-saturated with riboflavin, even when hens are fed diets high in the vitamin. Its bound riboflavin is responsible for the faint yellow tinge of egg albumen.
31. It appears that plasma RfBP, produced and secreted by the liver in response to estrogens, is the precursor to these other binding proteins found in tissues.
32. Primarily in their contents of sialic acid, which occurs in many polysaccharides.
33. It is interesting to note that egg white RfBP forms a 1:1 complex with the thiamin-binding protein (TBP) from the same source.
34. Embryos from hens homozygous for the mutant *rd* allele die of riboflavin deficiency on day 13–14 of incubation but can be rescued by injecting riboflavin or FMN into the eggs.
35. Therefore, hypothyroidism is associated with low flavokinase activity and, accordingly, low tissue levels of FMN and FAD. Hyperthyroidism, in contrast, results in increased flavokinase activity, although tissue levels of FMN and FAD, which appear to be regulated via degradation, are unaffected.
36. Small amounts of FAD derivatives have also been identified: 8α-S-cysteinyl-FAD, 8α-N^1-histidinyl-FAD, 8α-N^3-histidinyl-FAD.
37. This activity is also increased by thyroxine.

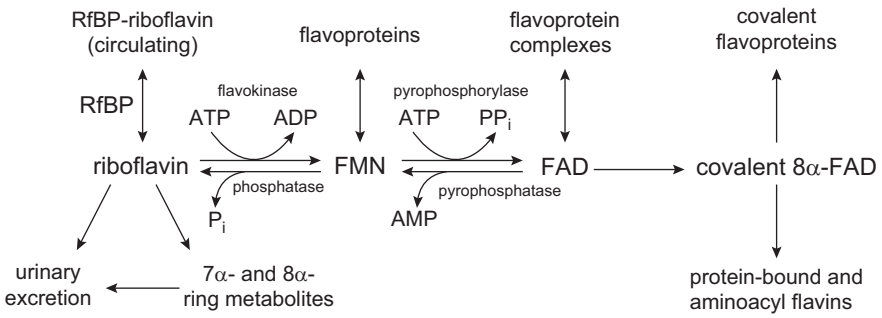


FIGURE 12.1 Riboflavin metabolism.

TABLE 12.2 Covalent Flavoproteins in Animals

Linkage	Enzyme
Histidinyl(N^3)-8α-FAD	Succinate dehydrogenase Dimethylglycine dehyrogenase Sarcosine dehydrogenase
Histidinyl(N^1)-8α-FAD	L-gulonolactone oxidase
Cysteinyl(s)-8α-FAD	Monoamine oxidase

FAD, flavin adenine dinucleotide.

allows FAD to complex, mostly through noncovalent associations with dinucleotide-binding domains, with different dehydrogenases and oxidases within the mitochondria. Noncomplexed FAD is exported from mitochondria into the cytosol by a **flavin exchange protein, FLX1**. FAD synthesis is feedback inhibited by FAD, which is complexed in tissues apparently facilitated by a protein factor. This includes associations via hydrogen bonding with purines,[38] phenols, and indoles (e.g., to peptidyl tryptophan in RfBPs). Less than 10% of FAD is covalently attached to apoenzymes. Linkages of this type involve the riboflavin 8-methyl group, which can form a methylene bridge to the peptide histidyl imidazole function (e.g., in succinic dehydrogenase and sarcosine oxidase) or to the thioether function of a former cysteinyl residue (e.g., in monoamine oxidase.[39]

Glycosylation

The capacity to glycosylate riboflavin has been demonstrated in rat liver. Riboflavin 5′-α-D-glucoside appears to be a metabolically significant metabolite. It is found in the urine of riboflavin-fed rats and has been shown to be comparable to riboflavin as a cellular source of the vitamin.

Catabolism

Flavins that are bound to proteins are resistant to degradation. However, unbound forms are subject to catabolism. Both FAD and FMN are catabolized by intracellular enzymes with different isoforms in mitochondria and cytosol. Thus, FAD is converted to FMN by FAD pyrophosphatase (releasing AMP), and FMN is degraded to free riboflavin by FMN phosphohydrolase. Both FAD and FMN are split to yield free riboflavin by alkaline phosphatase, which is recognized by RFT-2 for export from the cell. Unlike their synthetic analogs, neither FAD pyrophosphatase or FMN phosphohydrolase is not affected by thyroid hormones or riboflavin status.

The degradation of riboflavin per se involves initially its hydroxylation at the 7α- and 8α-positions of the isoalloxazine ring by hepatic microsomal cytochrome *P*450-dependent processes. It is thought that catabolism proceeds by the oxidation and then removal of the methyl groups. The liver, in at least some species, has the ability to form riboflavin α-glycosides. As a result of this metabolism, human blood plasma contains FAD and FMN as the major riboflavin metabolites, as well as small amounts of 7α-hydroxyriboflavin.[40] Side chain oxidation has been observed in bacterial systems but not in higher animals.

Excretion

Riboflavin is rapidly excreted, primarily in the urine. Therefore, dietary needs for the vitamin are determined by its rate of excretion, not metabolism. In a riboflavin-adequate human adult nearly all of a large oral dose of the vitamin will be excreted, with peak concentrations showing in the urine within a couple of hours. Studies in the rat have shown riboflavin to be turned over with a half-life of about 16 days in adequately nourished animals and much longer

38. In FAD, the riboflavin and adenine moieties are predominantly (85%) hydrogen bonded in an intramolecular complex.

39. Another type of linkage involving the 8-methyl group, i.e., a thiohemiacetal linkage, is found in a microbial FAD-containing cytochrome.

40. This compound is also called 7-hydroxymethylriboflavin.

in riboflavin-deficient animals. In normal human adults, the urinary excretion of riboflavin is about 200 μg/24 h; whereas, riboflavin-deficient individuals may excrete only 40–70 μg/24 h. Studies with a diabetic rat model[41] have shown riboflavin excretion to be significantly greater in diabetic individuals than in controls. Riboflavin excretion responds to the level of riboflavin intake; excretion of <27 μg/mg creatinine is generally considered to indicate riboflavin deficiency in adults; however, this parameter tends to reflect current intake of the vitamin rather than total flavin stores.

The vitamin is excreted mainly (60–70%) as the free riboflavin, with smaller amounts of 7α- and 8α-hydroxyr iboflavin,[42] 8α-sulfonylriboflavin, 5′-riboflavinylpeptide, 10-hydroxyethylflavin, riboflavin 5′-α-D-glucoside, lumichrome, and 10-formylmethylflavin. Small amounts of riboflavin degradation products are found in the feces (<5% of an oral dose). As only about 1% of an oral dose of the vitamin is excreted in the bile by humans, most fecal metabolites are thought to be mostly of gut microbial origin. Little, if any, riboflavin is oxidized to CO_2.[43] Ingestion of boric acid, which binds to the riboflavin side chain, increases the urinary excretion of the vitamin.

Riboflavin is secreted into milk mostly as free riboflavin and FAD and the antagonistic metabolite 10-(2′-hydroxyethyl)flavin; amounts depend on the riboflavin intake of the mother. Milk also contains small amounts of other metabolites including 7- and 8-hydroxymethylriboflavins, 10-formylmethylflavin, and lumichrome.

7. METABOLIC FUNCTIONS OF RIBOFLAVIN

Riboflavin functions metabolically as the essential component of the coenzymes FMN and FAD, which act as intermediaries in transfers of electrons in biological oxidation–reduction reactions. More than 100 enzymes are known to bind FAD or FMN in animal and microbial systems. These are encoded by 90 genes.[44] Ten flavoproteins occur in multiple isoforms; allelic variants in two-thirds have been associated with clinical disorders.

Coenzyme Functions

FAD is the cofactor for some 84% of the flavoenzymes; FMN is the cofactor for 16% of them. In most cases, the flavinyl cofactor is bound tightly but noncovalently; a few flavoenzymes[45] bind FAD covalently via histidinyl or cysteinyl linkages to the 8α-position of the isoalloxazine ring. These flavoenzymes include oxidases, which function aerobically, and dehydrogenases, which function anaerobically. Some involve one electron transfers, whereas others involve two electron transfers. This versatility allows flavoproteins to serve as switching sites between obligate two electron donors (e.g., NADH, succinate) and obligate one electron acceptors (e.g., iron–sulfur proteins, heme proteins). Flavoproteins serve this function by undergoing reduction through two single-electron transfer steps (Fig. 12.2) involving a riboflavinyl radical or semiquinone intermediate (with the unpaired electron localized at N-5). Because the radical intermediate can react with molecular oxygen, flavoproteins can also serve as cofactors in the two-electron reduction of O_2 to H_2O, and in the four-electron activation and cleavage of O_2 in monooxygenase reactions.

Collectively, the flavoproteins show great versatility in accepting and transferring one or two electrons with a range of potentials. This feature owes to the variation in the angle between the two planes of the isoalloxazine ring system (intersecting at N-5 and N-10), which is modified by specific protein binding. The flavin-containing dehydrogenases or reductases (their reduced forms) react slowly with molecular oxygen, in contrast to the fast reactions of the flavin-containing oxidases and monooxygenases. In the former reactions, hydroperoxide derivatives of the flavoprotein are cleaved to yield superoxide anion (O_2^-), but in the latter a heterolytic cleavage of the hydroperoxide group occurs to yield the peroxide ion (OOH^-). Many flavoproteins contain a metal (e.g., iron, molybdenum, zinc), and the combination of flavin and metal ion is often involved in the adjustments of these enzymes in transfers between single- and double-electron donors. In some flavoproteins, the means for multiple electron transfers is provided by the presence of multiple flavins as well as metals.

Metabolic Roles

The flavoproteome comprises a large group of enzymes that have central roles in the metabolism of carbohydrates, amino acids, lipids, and the activation of pyridoxine and folate to their functional forms (Table 12.3).[46,47] Others (particularly, glutathione reductase) participate in antioxidant protection by maintaining the glutathione redox cycle and providing the reducing equivalents for neutralizing ROS. Intracellular

41. Streptozotocin-induced diabetes.
42. This compound is also called 8-hydroxymethylriboflavin.
43. Rats have been found to oxidize less than 1% of an oral dose of the vitamin.
44. Including six enzymes involved in riboflavin utilization and metabolism.
45. For example, succinate dehydrogenase, monoamine oxidase, monomethylglycine dehydrogenase.
46. See review: Lienhart, W.D., Gudipati, V., Macheroux, P., 2013. Arch. Biochem. Biophys. 535, 150–162.
47. Riboflavin has also been found to play a role in the regulation of gene expression in bacteria by forming mRNA structures called "riboswitches" that repress conformation to cause premature termination of transcription or inhibit initiation of translation. Analogous function in higher animals has not been reported.

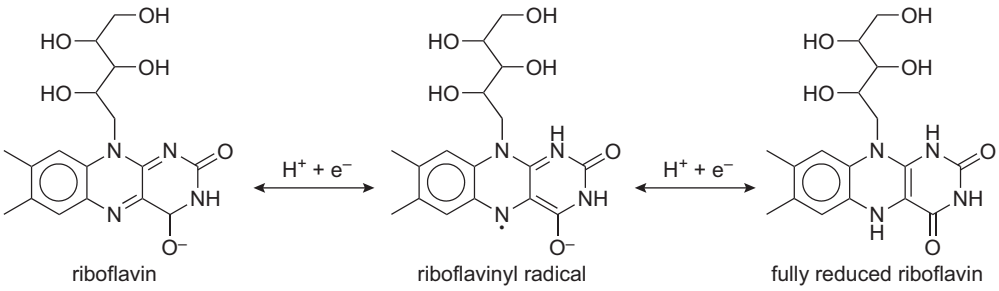


FIGURE 12.2 Two-step, single-electron, redox reactions of riboflavin.

redox balance[48] affects the oxidation state of protein sulfhydryls; complete oxidation (to the sulfenic and sulfinic acid states) is irreversible and can adversely affect protein folding. Such abnormalities are prevented by maintaining GSH:GSSG at adequate levels, which allows protein thiol groups to be protected by S-glutathionylation.

8. BIOMARKERS OF RIBOFLAVIN STATUS

Several clinical biochemical endpoints have been used to assess riboflavin status.[49] Of those, four methods have proven to be the most informative:

- **Degree of FAD/FMN saturation of flavoenzymes** is the most informative biomarker of riboflavin status. The enzyme of choice is typically erythrocyte glutathione reductase (eGR), which responds to riboflavin deprivation. The assay takes advantage of the in vitro binding of FAD by that enzyme in hemolysates. Because riboflavin-adequate subjects typically have 80–90% of eGR bound to FAD, the addition of exogenous FAD should stimulate their eGR activities by no more than XX%; stimulation above that level indicates that less eGR was bound to FAD. Therefore, the eGR response to added FAD measures eGR saturation and, thus, riboflavin status. This is measured as an activity coefficient:

  eGR activity coefficient = baseline eGR activity/eGR activity with added FAD

Subjects with eGR activity coefficients <1.15 are considered to be at low risk of riboflavin deficiency; those with activity coefficients of 1.15–1.25 or >1.25 are considered to be at moderate and high risks, respectively. This biomarker, however, is *not* useful for subjects with the common genetic condition of glucose-6-phosphate dehydrogenase deficiency whose GRs remain saturated with FAD regardless of riboflavin status. **Pyridoxine 5′-phosphate oxidase activity**, which also reflects riboflavin status, has been used for such subjects.

- **Erythrocyte riboflavin content** less than 0.15 mg/L indicates low/deficient riboflavin status.
- **Urinary riboflavin excretion** less than 10% of the amount of the ingested vitamin indicates low/deficient riboflavin status.

9. RIBOFLAVIN DEFICIENCY

Many tissues are affected by riboflavin deficiency (Table 12.4). Therefore, deprivation of the vitamin causes in animals such general signs as loss of appetite, impaired growth, and reduced efficiency of feed utilization, all of which constitute significant costs in animal agriculture. In addition, both animals and humans experiencing riboflavin deficiency show specific epithelial lesions and nervous disorders. These manifestations are accompanied by abnormally low activities of various flavoenzymes. The most rapid and dramatic loss of activity involves eGR. Substantial losses also occur in the activities of flavokinase and FAD synthetase; thus, the biosynthesis of flavoproteins is lost under conditions of riboflavin deprivation. In summary, then, riboflavin deficiency results in impairments in the metabolism of energy, amino acids, and lipids. These metabolic impairments are manifested morphologically as arrays of both general and specific signs/symptoms.

Riboflavin deficiency produces in the small intestine: a hyperproliferative response of the mucosa, characterized by reductions in number of villi, increases in villus length, and increases in the transit rates of enterocytes along the villi. These morphological effects are associated with reduced enteric absorption of dietary iron, resulting in secondary impairments in nutritional iron status in riboflavin-deprived individuals.

Risk Factors for Riboflavin Deficiency

Several factors can contribute to riboflavin deficiency:

- **Inadequate diet** is the most likely cause of riboflavin deficiency. Typically, this involves the low consumption of milk.[50] In industrialized countries, riboflavin deficiency occurs most frequently among alcoholics, whose

48. Intracellular redox potential is maintained by keeping GSH:GSSG 30–100:1 through the use of reducing equivalents from NADPH and cysteine.
49. These have been systematically reviewed (Hoey, L., McNulty, H., Strain, J.J., 2009. Am. J. Clin. Nutr. 89, 1960S–1980S).
50. Children consuming less than a cup of milk per week are likely to be deficient in riboflavin.

TABLE 12.3 Major Components of the Flavoproteome of Humans and Animals

Flavoprotein	Flavin	Metabolic Function
One Electron Transfers		
Mitochondrial electron transfer flavoprotein (ETF)	FAD (flavin adenine dinucleotide)	e^- acceptor for acyl-CoA, branched-chain acyl-CoA, glutaryl-CoA, and sarcosine and dimethylglycine dehydrogenases; links flavoprotein dehydrogenases with respiratory chain *via* ETF–ubiquinone reductase
NADH–ubiquinone reductase	FAD	Transfers reducing equivalent from ETF and ubiquinone in respiratory chain
NADH–cytochrome *P*450 reductase[a]	FMN (flavin mononucleotide)	Transfers reducing equivalent from FMN to cytochrome *P*450
Two Electron Transfers		
Pyridine-Linked Dehydrogenases		
NADP–cytochrome *P*450 reductase[a]	FAD	A key regulatory protein; transfers reducing equivalents from NADP to FAD, then to several acceptor proteins
Adrenodoxin reductase	FAD	Transfers reducing equivalents from NADP to adrenodoxin[b] in Steroid hydroxylation in adrenal cortex
NADP dehydrogenase	FMN	Transfers reducing equivalents from NADP to FMN, then to ubiquinone[c]
NADP-dependent methemoglobin reductase	FAD	Transfers reducing equivalents from NADP to FAD to reduce methemoglobin
NADH–cytochrome b_5 reductase	FAD	Transfers reducing equivalents from NADP to FAD to dehydrogenate stearoyl-CoA to oleoyl-CoA
3-β-Hydroxysterol Δ-24-reductase	FAD	Transfers reducing equivalents from NADPH in the latter steps of cholesterol synthesis
7-Dehydrocholesterol reductase	FAD	Transfers reducing equivalents from NADPH in the latter steps of cholesterol synthesis
Nonpyridine Nucleotide-Dependent Dehydrogenases		
Succinate dehydrogenase	FAD	Transfers reducing equivalents from succinate to ubiquinone yielding fumarate
Acyl-CoA dehydrogenases	FAD	Transfers reducing equivalents from substrate to flavin in the initial step in β-oxidation of fatty acids
Pyridine Nucleotide Oxidoreductases		
Glutathione reductase	FAD	Reduces GSSG to GSH using NADPH
Lipoamide dehydrogenase[d]	FAD	Oxidizes dihydrolipoamide to lipoamide using NAD^+
Reactions of Reduced Flavoproteins With Oxygen		
D-amino acid oxidase	FAD	Dehydrogenates D-amino acid substrates to imino acids, which are hydrolyzed to α-keto acids
L-amino acid oxidase	FMN	Dehydrogenates L-amino acid substrates to imino acids, which are hydrolyzed to α-keto acids
Monoamine oxidase	FAD	Dehydrates biogenic amines[e] to corresponding imines with hydrogen transfer to O_2, forming H_2O_2
Xanthine oxidase	FAD	Oxidizes hypoxanthine and xanthine to uric acid with formation of H_2O_2
L-gulonolactone oxidase	FAD	Oxidizes L-gulonolactone to ascorbic acid
3-Ketosphinganine reductase	FAD	Reduces 3-ketosphinganine to sphinganine in sphingosine biosynthesis
Dihydroceramide desaturase	FAD	Desaturates dihydroceramide to form ceramide in sphingosine biosynthesis
Flavoprotein Monooxygenases		
Microsomal flavoprotein monooxygenase	FAD	Oxidizes N, S, Se, and I centers of various substrates in drug metabolism
Squalene monooxygenase	FAD	Accepts reducing equivalents from ETF to oxidize squalene in the rate-limiting step in cholesterol biosynthesis

[a]*A component of microsomal cytochrome P450, it contains one molecule each of FAD and FMN.*
[b]*An iron–sulfur protein.*
[c]*Also has NADH–ubiquinone reductase activity, reductively releasing iron from ferritin.*
[d]*A component of the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase complexes.*
[e]*For example, serotonin, noradrenaline, benzylamine.*

TABLE 12.4 General Signs of Riboflavin Deficiency

Organ System	Signs
General	
Appetite	Decrease
Growth	Decrease
Dermatologic	Cheilosis, stomatitis
Muscular	Weakness
Gastrointestinal	Inflammation, ulcer
Skeletal	Deformities
Vital organs	Hepatic steatosis
Vascular	
Erythrocytes	Anemia
Nervous	Ataxia, paralysis
Reproductive	
Male	Sterility
Female	Decreased egg production
Fetal	Malformations, death
Ocular	
Retinal	Photophobia
Corneal	Decreased vascularization

TABLE 12.5 Recommended Riboflavin Intakes

The United States		FAO/WHO	
Age–Sex	RDA[a] (mg/day)	Age–Sex	RNI[b] (mg/day)
0–6 months	(0.3)[c]	0–6 months	0.3
7–11 months	(0.4)[c]	7–11 months	0.4
1–3 years	0.5	1–3 years	0.5
4–8 years	0.6	4–6 years	0.6
9–13 years	0.9	7–9 years	0.9
14–18 year females	1.0	10–18 year females	1.0
14–18 year males	1.3	10–18 year males	1.3
>18 year females	1.1	19+ year females	1.1
>18 year males	1.3	19+ year males	1.3
Pregnancy	1.4	Pregnancy	1.4
Lactation	1.6	Lactation	1.6

[a]*Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.*
[b]*Recommended Nutrient Intakes, Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food and Agricultural Org., Rome, 286 pp.*
[c]*RDAs have not been set; AdIs are given instead.*

dietary practices are often faulty, leading to this and other deficiencies.

- **Enhanced catabolism** associated with illness or vigorous physical exercise and involving nitrogen loss can increase riboflavin losses.
- **Alcohol** at high intakes appears to antagonize the utilization of FAD from foods.
- **Phototherapy** of infants with hyperbilirubinemia can lead to riboflavin deficiency by photodestruction of the vitamin[51] if the vitamin is not also administered.[52]
- **Diuretics or hemodialysis** can enhance loss of riboflavin (as well as other water-soluble vitamins).

Clinical signs of riboflavin deficiency are rarely seen in the industrialized world. **Subclinical riboflavin deficiency,** i.e., conditions in which intake is in sufficient to keep flavoproteins saturated with their respective flavin cofactors, however, is not uncommon. In fact, it has been estimated that as much as 27% of urban American teenagers of low-socioeconomic status had subclinical riboflavin deficiency. These changes are prevented by adequate regular intakes of the vitamin (Table 12.5).

Deficiency Signs in Humans

Clinical signs of frank riboflavin deficiency are not common; they are manifest only after 3–4 months of deprivation of the vitamin:

- **Dermal lesions** are the first to present **cheilosis**,[53] **angular stomatitis**,[54] **glossitis** (Fig. 12.3),[55] hyperemia,[56] and

51. Phototherapy can be an effective treatment for infants with mild hyperbilirubinemia; however, the mechanism by which it leads to the degradation of bilirubin (to soluble substances that can be excreted) necessarily leads also to the destruction of riboflavin. It is the photoactivation of riboflavin in the patient's plasma that generates singlet oxygen, which reacts with bilirubin. Thus, plasma riboflavin levels of such patients have been found to drop as the result of phototherapy. Riboflavin supplementation prevents such a drop and has been shown to enhance bilirubin destruction.
52. For example, 0.5 mg of riboflavin sodium phosphate per kg body weight per day.
53. Lesions of the lips.
54. Lesions at the corners of the mouth, beginning as white, thickened foci and then developing a macerated appearance; they may ulcerate and then crusted. Healing may leave linear scars.
55. Inflammation of the tongue. This can involve disappearance of filiform papillae and enlargement of fungiform papillae, with the tongue color changing to a deep red. Subjects with this condition, called **geographical tongue**, have soreness of the tongue and loss of taste sensation.
56. Increased amount of blood present.

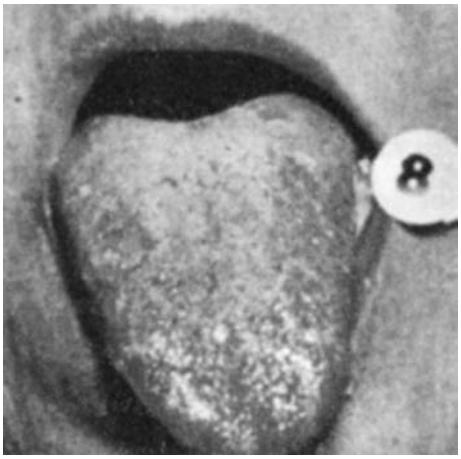


FIGURE 12.3 Geographical tongue in riboflavin deficiency. *Courtesy Cambridge University Press.*

edema[57] of the oral mucosa, seborrheic dermatitis around the nose and mouth and scrotum/vulva. These signs are associated with impaired collagen maturation.

- **Anemia** is later to present **normocytic, hypochromic anemia** with **reticulocytopenia**,[58] **leukopenia,**[59] and **thrombocytopenia**.[60] These signs reflect impaired erythropoiesis, particularly loss of NAD(P)H oxidoreductase, which facilitates cellular uptake of Fe^{2+} by keeping it reduced.
- **Neurological signs** are the last to present demyelinating peripheral neuropathy of the extremities characterized by hyperesthesia,[61] coldness, and pain, as well as decreased sensitivity to touch, temperature, vibration, and position.

Deficiency Signs in Animals

Riboflavin deficiency in animals is potentially fatal. In addition to the general signs already mentioned, animals show other signs that vary with the species. Riboflavin-deficient rodents show dermatologic signs (alopecia, seborrheic inflammation,[62] moderate epidermal hyperkeratosis[63] with atrophy of sebaceous glands) and a generally ragged appearance. Red, swollen lips and abnormal papillae of the tongue are seen. Ocular signs may also be seen (blepharitis,[64] conjunctivitis,[65] and corneal opacity). Feeding a high-fat diet can increase the severity of deficiency signs; high-fat-fed rats showed anestrus, multiple fetal skeletal abnormalities (shortening of the mandible, fusion of ribs, cleft palate, and deformed digits and limbs), paralysis of the hind limbs (degeneration of the myelin sheaths of the sciatic nerves[66]), hydrocephalus,[67] ocular lesions, cardiac malformations, and hydronephrosis.[68]

FIGURE 12.4 Curled toe paralysis in a riboflavin-deficient chick. *Courtesy, G.F. Combs, Sr.*

The riboflavin-deficient chick also experiences myelin degeneration of nerves, affecting the sciatic nerve in particular. This results in an inability to extend the digits, a syndrome called **curled toe paralysis** (Fig. 12.4). In hens, the deficiency involves reductions in both egg production and embryonic survival (decreased hatchability of fertile eggs). Riboflavin-deficient turkeys show severe dermatitis. The deficiency is rapidly fatal in ducks.

Riboflavin-deficient dogs are weak and ataxic. They show dermatitis (chest, abdomen, inner thighs, axillae, and scrotum) and **hypoplastic anemia**[69] with fatty infiltration of the bone marrow. They can have bradycardia and sinus arrhythmia[70] with respiratory failure. Corneal opacity has been reported. The deficiency can be fatal, with collapse and coma. Swine fed a riboflavin-deficient diet grow slowly and develop a scaly dermatitis with alopecia. They can show corneal opacity, cataracts, adrenal hemorrhages, fatty degeneration of the kidney, inflammation of the mucous membranes of the gastrointestinal tract, and nerve

57. Accumulation of excessive fluid in the tissue.
58. Abnormally low number of immature red blood cells in the circulating blood.
59. Abnormally low number of white blood cells in the circulating blood (<5000/ml).
60. Abnormally low number of platelets in the circulating blood.
61. Excessive sensibility to touch, pain, etc.
62. Involving excess oiliness due to excess activity of the sebaceous glands.
63. Hypertrophy of the horny layer of the epidermis.
64. Inflammation of the eyelids.
65. Inflammation of the mucous membrane covering the anterior surface of the eyeball.
66. The nerve situated in the thigh.
67. A condition involving the excessive accumulation of fluid in the cerebral ventricles, dilating these cavities and, in severe cases, thinning the brain and causing a separation of the cranial bones.
68. Dilation of one or both kidneys owing to obstructed urine flow.
69. Progressive nonregenerative anemia resulting from depressed, inadequate functioning of the bone marrow.
70. Irregular heartbeat, with the heart under control of its normal pacemaker, the sinoatrial (S-A) node.

degeneration. In severe cases, deficient individuals can collapse and die.

Riboflavin deficiency in the newborn calf[71] is manifested as redness of the buccal mucosa,[72] angular **stomatitis**,[73] alopecia, diarrhea, excessive tearing and salivation, and inanition. Signs of riboflavin deficiency appear to develop rather slowly in rhesus monkeys. The first signs seen are weight loss (6–8 weeks), followed by dermatologic changes in the mouth, face, legs, and hands and a normocytic hypochromic anemia[74] (2–6 months) and, ultimately, collapse and death with fatty degeneration of the liver. Similar signs have been produced in baboons made riboflavin deficient for experimental purposes.

10. RIBOFLAVIN IN HEALTH AND DISEASE

Vascular Disease

Dietary riboflavin intake has been found to be inversely correlated with serum homocysteine levels.[75] Homocysteinemia has been associated with increased risks to occlusive vascular disease, total and cardiovascular disease-related mortality, stroke, dementia, Alzheimer's disease, fracture, and chronic heart failure.[76] The Framingham Offspring Study found elevated plasma homocysteine levels in subjects with relatively low plasma riboflavin levels (Table 12.6). Such findings are consistent with the role of riboflavin as the essential cofactor (FAD) for methyltetrahydrofolate reductase (MTHFR), which is required for the formation of *N*-5-methyltetrahydrofolate, which, in turn, is required to convert homocysteine to methionine (*see* Chapter 18, Folic Acid). Riboflavin is particularly important in individuals with the 677TT polymorphism of MTHFR, which causes it to lose FAD thus reducing its activity.[77] Individuals with the *TT* genotype[78] have elevated circulating levels of homocysteine, particularly, if they are also low in folate. They are at increased risk for hypertension (Fig. 12.5) and vascular disease in general.[79] Randomized trials have demonstrated that riboflavin supplementation can reduce serum homocysteine levels[80] and blood pressure[81] only in individuals with the 677*TT* genotype.

TABLE 12.6 Relationship of Plasma Riboflavin and Homocysteine Levels Among Subjects in the Framingham Offspring Cohort Study

	Plasma Riboflavin Tertile (nM)		
	<6.89	6.89–10.99	≥11.0
Plasma homocysteine—mean (95% C.I.)	10.3 (9.8–10.8)	9.5 (9.1–10.0)*[a]	9.5 (9.1–10.0)*

[a]p<.05, n=147–152 in each group.
From Jacques, P.F., Boston, A.G., Williams, R.R., et al., 2002. J. Nutr. 132, 283–290.

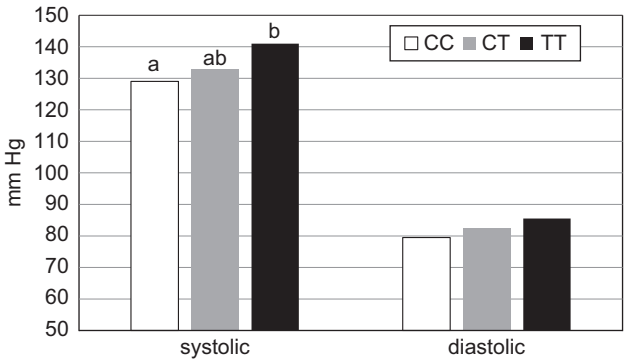


FIGURE 12.5 Relationship of MTHFR (methyltetrahydrofolate reductase) 677T→C genotype and blood pressure. *After Wilson, C.P., Ward, M., McNulty, H. et al., 2012. Am. J. Clin. Nutr. 95, 766–772.*

Anticarcinogenesis

Observational studies have found inverse relationships of riboflavin status and risks of cancers of the esophagus and colon rectum.[82] Riboflavin deprivation has been found to increase aflatoxin B_1-induced DNA damage in the rat, suggesting that suboptimal intakes of the vitamin may enhance carcinogenesis[83]. Plasma levels of RfBP may have utility

71. Ruminants do not normally require a dietary source of riboflavin, as their rumen microbiota synthesize the vitamin in adequate amounts. However, newborn calves and lambs, whose rumen microbiome is not established, require a dietary source of the vitamin, which is supplied by their mothers' milk or by supplements in milk-replacer formula diets.
72. The mucosa of the cheek.
73. Lesions in the corners of the mouth.
74. Anemia involving erythrocytes of normal size but low hemoglobin content.
75. Ganji, G., Kafai, M.R., 2004. Am. J. Clin. Nutr. 80, 1500–1507.
76. Selhub, J., 2006. J. Nutr. 136, 1726S–1730S.
77. McNulty, H., McKinley, M.C., Wilson, B., et al., 2002. Am. J. Clin. Nutr. 76, 436–442.
78. This group has been estimated to comprise 3–32% of various populations.
79. Hustad, S., Ueland, P.M., Volset, S.E., et al., 2000. Clin. Chem. 46, 1065–1072.
80. By as much as 22% (McNulty, H.M., Dowey, L.R.C., et al., 2006. Circulation 113, 74–80).
81. Wilson, C.P., Ward, M., McNulty, H., et al., 2012. Am. J. Clin. Nutr. 95, 766–772.
82. Esophageal squamous cell cancer (He, Y., Shan, B., Song, G., et al., 2009. Asian Pacific J. Cancer Prev. 10, 619–625); colorectal cancer (Figueiredo, J.C., Levine, A.J., Grau, M.V., et al., 2008. Cancer Epidemiol. Biomarkers Prev. 17, 2137–2144; de Vogel, S., Dindore, V., van Engeland, M., et al., 2008. J. Nutr. 138, 2372–2378).
83. Webster, R.P., Gawde, M.D., Bhattacharya, R.K., 1996. Cancer Lett. 98, 129–135.

as a biomarker for some cancers. It has been found to be overexpressed in patients with estrogen-responsive breast cancer, hepatocellular cancer, and prostate cancer.

Malaria

Riboflavin deficiency confers some protection against malaria, decreasing both parasitemia and signs of infection[84] due to the plasmodia autolyzing before they can mature. This protection is thought to involve significant differentials in needs/susceptibilities of the parasite and erythrocytes, the parasite having greater needs for riboflavin,[85] and being more susceptible to oxidative stress.[86] These hypotheses are consistent with genetic defects in certain flavoenzymes being associated with malaria resistance.[87] Flavin analogues[88] that antagonize riboflavin and inhibit glutathione reductase have been shown to have antimalarial activities.[89]

Occular Health

Tryptophan deficiency cataract can be reduced by riboflavin deprivation in the rat. The cataractogenic effect of a low-tryptophan diet appears to the formation of a riboflavinyl tryptophan adduct that accelerates the photooxidation of the amino acid to a prooxidative form.

Mineral Utilization

Riboflavin can form complexes with divalent cations. That correction of riboflavin deficiency can improve the enteric absorption of iron and zinc in the mouse model[90] suggests that riboflavin can be a determinant of the bioavailability of those minerals.

Medical Uses

Riboflavin is photoactive. In its excited state, it can react with amino acids, proteins, or lipids in low-O_2 environments to generate radicals that can, in turn, generate ROS.[91] This property is the basis of riboflavin loss due to photoirradiation treatment of neonatal jaundice.[92] It has also been exploited for medical purposes. These include inducing DNA damage in blood-borne pathogens and catalyzing **corneal collagen cross-linking (CXL)** in the treatment of progressive keratectasia[93] in humans.[94]

High doses of riboflavin in combination with other treatments have been reported to be effective in preventing migraine headaches,[95] although controlled studies have not confirmed that effect.[96]

11. RIBOFLAVIN TOXICITY

The toxicity of riboflavin is *very low* and, thus, problems of hypervitaminosis are not expected. Probably, because it is not well absorbed as high oral doses, riboflavin is essentially nontoxic. Oral riboflavin doses as great as 2–10 g/kg body weight produce no adverse effects in dogs and rats. The vitamin is somewhat more toxic when administered parenterally. The LD_{50} (50% lethal dose) values for the rat given riboflavin by the intraperitoneal, subcutaneous, and oral routes have been estimated to be 0.6, 5, and >10 g/kg, respectively. No tolerable upper limits of exposure have been established.

12. CASE STUDY

Instructions

Review the following summary of a research report, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

Case

An experiment was conducted to determine the basis of protection by riboflavin deficiency against malarial infection. An animal model, which previously showed such protection against *Plasmodium berghei*, was used. It involved depleting 3-week-old male rats of riboflavin by feeding them a sucrose-based purified diet containing <1 mg of riboflavin per kilogram. A control group

84. Da, B.S., et al., 1988. Eur. J. Clin. Nutr. 42, 227–234.
85. Dutta, P., 1991. J. Protozool. 38, 479–486.
86. Dutta, P., 1993. J. Soc. Pharm. Chem. 23, 11–48.
87. Glutathione reductase, pyridoxine phosphate oxidase, glucose-6-phosphate dehydrogenase (López, C., Saravia, C., Gomez, A., et al., 2010. FASEB J. 467, 1–12).
88. e.g., Galactoflavin, 10-(4′-chlorophenyl)-3-methylflavin and some isoalloxazine derivatives.
89. It has also been proposed that high-dose riboflavin may also be useful in treating malaria. This would involve doses sufficiently large to stimulate NADPH–cytochrome b_5/cytochrome b_5 reductase in the parasite. Because plasmodia typically accumulate large amounts of methemoglobin, upregulation of this flavoenzyme, reduces Fe^{3+} in methemoglobin to Fe^{2+} in hemoglobin, could lead to a futile cycle of hemoglobin oxidation and reduction (Akompong, T., Ghori, N., Haldar, K., 2000. Antimicrob. Agents Chemother. 44, 88–96).
90. Agte, V.V., Paknikar. K.M., Chiplonkar, S.A., et al., 1998. Biol. Trace Elem. Res. 65, 109–116.
91. Marin, C.B., Tso, M., Hadan, C.M., 2002. J. Am. Chem. Soc. 124, 7226–7234.
92. Riboflavin and bilirubin have similar absorption maxima; loss of riboflavin causes loss of eGR, which by compromises ROS protection, causes erythrocyte lysis.
93. A group of noninflammatory disorders involving thinning of the cornea; some forms can sometimes occur after corneal surgery.
94. Dahl, B.J., Spotts, E., Truong, J.Q., 2012. Optometry 83, 33–42.
95. Zencirci, B., 2010. J. Pain. Res. 3, 125–130.
96. MacLennan, S.C., Wade, F.M., Forrest, K.M., et al., 2008. J. Child. Neurol. 23, 1300–1304.

was pair-fed[97] the same basal diet supplemented with 8.5 mg of riboflavin per kilogram.[98] At 6 weeks of age, several biochemical characteristics of erythrocytes (RBCs) were measured,i.e., reduced glutathione levels, activities of antioxidant enzymes, stabilities of erythrocytes to hemolysis (measured by incubating 0.5% suspensions of RBCs with prooxidants [500 μM H_2O_2 or 2.5 μM ferriprotoporphyrin IX] or in a hypotonic medium [151 mOsm] for 1 h at 37°C). Oxidative damage was assessed by measuring H_2O_2-induced production of malonyldialdehyde (MDA). Other studies with this and similar animal models have shown that the riboflavin-deficient group, when infected with the parasite *grows better* and shows *reduced parasitemia* than pair-fed controls.

Results of Biochemical Studies of Erythrocytes

Parameter	Riboflavin Defiscient	Control	*p* Value
Reticulocytes (% total RBCs)	1.50 ± 0.29	1.26 ± 0.37	NS[a]
Hemoglobin (g/dL blood)	14.7 ± 0.6	14.9 ± 0.3	NS
GSH (mmol/g Hb)	7.97 ± 2.89	6.19 ± 2.52	<0.001
Glutathione reductase (mU[b]/mg protein)	42 ± 6	124 ± 16	<0.001
Glutathione reductase activity coefficient	2.37 ± 0.19	1.20 ± 0.08	<0.01
Glutathione peroxidase (mU[b]/g Hb)	918 ± 70	944 ± 62	NS
In Vitro Hemolysis (%)			
H_2O_2-induced	32 ± 9	55 ± 9	<0.05
Hypotonicity	69 ± 4	53 ± 7	<0.01
Ferriprotoporphyrin IX	42 ± 3	29 ± 4	<0.001
Malonyldialdehyde (MDA) (nmol/g Hb)			
Before incubation	25.5 ± 3.8	25.9 ± 3.4	NS
Incubated with H_2O_2	34.8 ± 1.2	42.7 ± 1.8	<0.01

[a]*NS, not significant (p > .05).*
[b]*1 mU = 1 nmol NADPH per min.*

97. **Pair-feeding** is a method of controlling for the effects of reduced food intake that may be secondary to the independent experimental variable (e.g., a nutrient deficiency). It involves the matching of one animal from the experimental treatment group with one of similar body weight from the control group, and the feeding of the latter individual a measured amount of feed equivalent to the amount of feed consumed by the former individual on the previous day. In experiments of more than a few days' duration, this approach normalizes the feed intake of both the experimental and control groups.

98. This level is about three times the amount normally required by the rat.

Case Questions

1. What dependent variables did the investigators measure to confirm that riboflavin deficiency had been produced in their experimental animals?
2. Propose a hypothesis to explain the apparently discrepant results regarding the effects of riboflavin deficiency on erythrocyte stability.
3. Propose a hypothesis for the protective effect of riboflavin deficiency against malarial infection. What other nutrients might you expect to influence susceptibility to this erythrocyte-attacking parasite?

13. STUDY QUESTIONS AND EXERCISES

1. Diagram the general roles of FAD- and FMN-dependent enzymes in various areas of metabolism.
2. Construct a decision tree for the diagnosis of riboflavin deficiency in humans or an animal species.
3. What key feature of the chemistry of riboflavin relates to its biochemical functions in flavoproteins?
4. What diet and lifestyle factors would you expect to affect dietary riboflavin needs? Justify your answer.

RECOMMENDED READING

Lienhart, W.D., Gudipati, V., Macheroux, P., 2013. The human flavoproteome. Arch. Biochem. Biophys. 535, 150–162.

McCormick, D.B., 2012. Riboflavin, Chapter 28. In: Erdman, J.W., Macdonald, I.A., Zeisel, S.H. (Eds.), Present Knowledge in Nutrition, tenth ed. Wiley, New York, pp. 280–306.

Pinto, J.T., Rivlin, R.S., 2014. Riboflavin, Chapter 6. In: Zemplini, J., Suttie, J.W., Gregory, J.F., et al. (Eds.), Handbook of Vitamins, fifth ed. CRC Press, New York, pp. 191–265.

Said, H.M., 2004. Recent advances in carrier-mediated intestinal absorption of water-soluble vitamins. Ann. Rev. Physiol. 66, 419–446.

Stuehr, D.J., Tejero, J., Haque, M.M., 2009. Structural and mechanistic aspects of flavoproteins: electron transfer through the nitric oxide synthase flavoprotein domain. FEBS J. 276, 3959–3974.

Walsh, C.T., Wencewicz, 2013. Flavoenzymes: versatile catalysts in biosynthetic pathways. Nat. Prod. Rep. 30, 175–200.

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Chapter 13

Niacin

Chapter Outline

Anchoring Concepts

1. Niacin is the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.
2. The two major forms of niacin, nicotinic acid and nicotinamide, are active metabolically as the pyridine nucleotide coenzymes NAD(H) and NADP(H).
3. Deficiencies of niacin are manifest as dermatologic, gastrointestinal, and neurologic changes and can be fatal.

So far as they have been studied, the foodstuffs that appear to be good sources of the black tongue preventive also appear to be good sources of the pellagra preventive…Considering the available evidence as a whole, it would seem highly probable, if not certain, that experimental black tongue and pellagra are essentially identical conditions and, thus, that the preventive of black tongue is identical with the pellagra preventive, or factor P–P.

Joseph Goldberger[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of niacin
2. To understand the means of enteric absorption and transport of niacin
3. To understand the biochemical function of niacin as a component of coenzymes of different metabolically important redox reactions and the relationship of that function to the physiological activities of the vitamin
4. To understand the factors that can affect low niacin status and the physiological implications of that condition.

VOCABULARY

Acetyl-CoA
ADP-ribosylation
ADP-ribotransferases (ARTs)
α-Amino-β-carboxymuconic-ε-semialdehyde (ACS)
α-Amino-β-carboxymuconic-ε-semialdehyde decarboxylase (ACSD)
Anthranilic acid
Black tongue disease
Casal's collar
Flushing
Formylase
N-Formylkynurenine
"Four Ds" of niacin deficiency
Glucose tolerance factor Hartnup disease
3-Hydroxyanthranilic acid
3-Hydroxyanthranilic acid oxygenase
3-Hydroxykynurenine
Kynurenic acid
Kynurenine
Kynurenine 3-hydroxylase
Leucine
1-Methylnicotinamide
1-Methylnicotinic acid
1-Methyl-2-pyridone 3-carboxamide

1. Joseph Goldberger (1874–1929) was a Hungarian-born American physician who, as member of the U.S. Public Health Service in 1914, demonstrated that pellagra was associated with corn-based diets. Although his work had little immediate public health impact, it ultimately led to the discovery of niacin and the role of the essential amino acid tryptophan.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00013-7

1-Methyl-4-pyridone 3-carboxamide
1-Methyl-6-pyridone 3-carboxamide
Mono-ADP-ribosyltransferases (ARTs)
NAD^+ kinase
NAD^+ synthetase
$NAD(P)^+$ glycohydrolase
Niacin number
Niacin receptor
Nicotinate phosphoribosyl transferase
Nicotinamide
Nicotinamide adenine dinucleotide (NADH)
Nicotinamide adenine dinucleotide phosphate (NADPH)
Nicotinamide methylase
Nicotinamide *N*-methyltransferase
Nicotinamide mononucleotide (NMN)
Nicotinamide riboside (NR)
Nicotinic acid (NA)
Nixtamalization
Nyacitin
Pellagra
Perosis
Phosphodiesterase
Picolinic acid
Poly(ADP-ribose) polymerases (PARPs)
Pyridine nucleotide
Pyridoxal phosphate
Quinolinate phosphoribosyltransferase
Quinolinic acid
Schizophrenia
Sirtuin
Transaminase
Transhydrogenase
Trigonelline
Tryptophan
Tryptophan pyrrolase
Xanthurenic acid

1. THE SIGNIFICANCE OF NIACIN

Niacin is required for the biosynthesis of the **pyridine nucleotides**, **NAD(H)** and **NADP(H)**, through which the vitamin has key roles in virtually all aspects of metabolism. Historically, niacin deficiency was prevalent among people who relied on maize (corn) as their major food staple; before the availability of inexpensive supplements, the deficiency was also a frequent problem of livestock fed maize-based diets.

Great irony characterizes niacin deficiency. Unlike thiamin deficiency, which also involves an unbalanced cereal-based diet, niacin deficiency more frequently results from poor bioavailability rather than scarcity per se. Hence, paradoxical questions have been asked:

- Why does niacin deficiency occur among individuals who can biosynthesize the vitamin?
- Why did pellagra occur among people eating maize (corn), whereas the disease was unknown in the Americas where maize was a historically important part of the diet?
- Why do maize-based diets produce pellagra, although maize contains an appreciable amount of niacin?
- Why does milk, which contains little niacin, prevent pellagra?
- Why does rice, which contains less niacin than maize, not produce pellagra?

Niacin is also of interest for its health value as a pharmacologic, i.e., multigram, supranutritional dose levels. Understanding the bases of both the physiologic and pharmacologic activities of niacin calls for an appreciation of its complexities, which are manifest differently in various species.

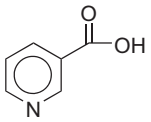
2. PROPERTIES OF NIACIN

The term **niacin** describes pyridine 3-carboxylic acid and its derivatives that exhibit the biological activity of nicotinamide (NAm). That biological activity depends on the following structural features:

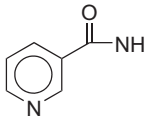
- **a pyridine nucleus** substituted with a β-carboxylic acid or a corresponding amine;
- the pyridine nitrogen being able to undergo reversible oxidation/reduction (i.e., quaternary pyridinium ion to/from tertiary amine); and
- open pyridine carbons adjacent to the nuclear nitrogen atom

The coenzyme forms of niacin are the pyridine nucleotides and their reduced forms: nicotinamide adenine dinucleotide (**NAD[H]**) and nicotinamide dinucleotide phosphate (**NADP[H]**).

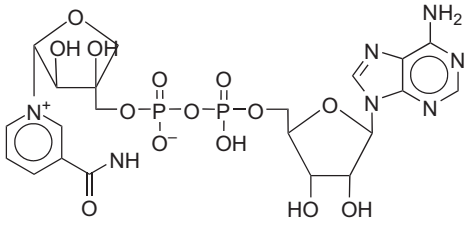
Chemical structures of niacin:



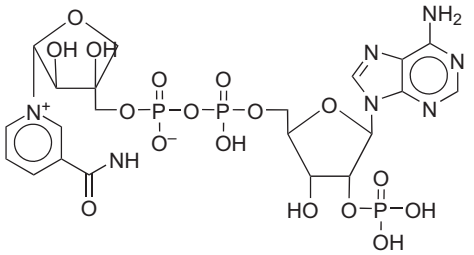
Nicotinic acid



Nicotinamide



Nicotinamide adenine dinucleotide (NADH)



Nicotinamide adenine dinucleotide phosphate (NADPH)

Niacin Chemistry

Nicotinic acid (NA) and **NAm** are colorless and crystalline. Each is insoluble or only sparingly soluble in organic solvents. NA is slightly soluble in water and ethanol; NAm is very soluble in water and moderately soluble in ethanol. The two compounds have similar absorption spectra in water, with an absorption maximum at <262 nm. NA is amphoteric and forms salts with acids as well as bases. Its carboxyl group can form esters and anhydrides, and can be reduced. Both NA and NAm are very stable in dry form, but in solution NAm is hydrolyzed by acids and bases to yield NA.

In each of the coenzyme forms (**NAD[H]** and **NADP[H]**), the electron-withdrawing effect of the *N*-1 atom and the amide group of the oxidized pyridine nucleus enables the pyridine C-4 atom to react with many nucleophilic agents (e.g., sulfite, cyanide, and hydride ions). It is the reaction with hydride ions (H^-) that is the basis of the enzymatic hydrogen transfer by the pyridine nucleotides; the reaction involves the transfer of two electrons in a single step. The hydride transfer of nonenzymatic reactions of the pyridine nucleotides, plus those catalyzed by the pyridine nucleotide-dependent dehydrogenases, is stereospecific with respect to both coenzyme and substrate. At least for reactions of the former type, this stereospecificity results from a specific intramolecular association between the adenine residue and the pyridine nucleus.

Several substituted pyridines are antagonists of niacin in biological systems: pyridine-3-sulfonic acid, 3-acetylpyridine, isonicotinic acid hydrazine, and 6-aminonicotinamide.

3. SOURCES OF NIACIN

Hindgut Microbial Synthesis

Niacin can be produced by the microbiome of the colon. A genomic analysis of 256 representative organisms of the human gut microbiota found most (63%) capable of de novo synthesis of NAD.[2] In addition, some taxa showed the capacity to salvage NAD. Those findings suggested that hindgut microbial synthesis may produce as much as 37% of the daily human need for niacin. However, direct evidence is lacking for the absorption of niacin across the colon. It is likely that a large portion of the microbially produced vitamin is taken up by nonsynthesizing microbes such that noncoprophagous and nonruminant animals derive no benefit from this source of the vitamin.

Distribution in Foods

Niacin occurs in greatest quantities in brewers' yeasts and meats, but significant amounts are also found in many other foods (Table 13.1). The vitamin is distributed unevenly in grains, present mostly in the bran fractions. Niacin occurs predominantly in bound forms, e.g., in plants mostly as protein-bound NA and in animal tissues mostly as NAm in nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). In cow's milk, ~40% of the vitamin is the NAD precursor, nicotinamide riboside (NR). Niacin is added by law to wheat flour and other grain products in the United States.[3]

Stability

Niacin in foods is stable to storage and to most means of food preparation and cooking (e.g., moist heat).

Bioavailability

Niacin is found in many types of foods in forms from which it is not released on digestion, thus rendering it unavailable for absorption. In grains, niacin is present in covalently bound complexes with small peptides and carbohydrates, collectively referred to as **niacytin**.[4] The esterified niacin in these complexes is not normally available; however, its bioavailability can be improved substantially by treatment with base to effect the alkaline hydrolysis of those esters. The tradition in Central American cuisine of soaking and cooking maize in lime[5] water effectively renders available the niacin in that grain.[6] This practice appears to be responsible for effective protection against pellagra in that part of the world. In other foods, niacin is present as a methylated derivative (1-methylnicotinic acid, also called **trigonelline**) that functions as a plant hormone but is also not biologically

2. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.

3. Fortification is mandated for niacin, thiamin, riboflavin, folate, and iron.

4. A polysaccharide extracted from wheat bran has been found to contain more than 1% NA bound via an ester linkage to glucose in a complex also containing arabinose, galactose, and xylose. Although NAD^+ and $NADP^+$, both of which are biologically available to humans and animals, are present in early-stage corn, those levels decline as the grain matures and are replaced by NAm and NA as well as forms of very low bioavailability such as bound niacin and trigonelline.

5. Calcium hydroxide.

6. This process, called **nixtamalization**, renders maize (corn) more easily ground, improves its flavor, and reduces mycotoxin content. It is used in making tortillas, hominy, and corn chips. The term itself derives from the Nahuatl (an Aztec dialect) words *nextli* (ashes) and *tamalii* (corn dough).

TABLE 13.1 Niacin Contents of Foods

Food	Niacin (mg/100 g)
Dairy Products	
Milk	0.09–0.1
Yogurt	0.2
Cheeses	0.02–1
Meats	
Beef	2.4–8.5
Chicken	5–11.1
Pork	4–10
Turkey	6.4–9.6
Fish	
Herring	3.3–4.4
Cod	2.5–7.5
Haddock	4.1
Tuna	10.1–10.5
Cereals	
Barley	2.1
Buckwheat	0.9
Cornmeal	3.6
Rice, brown	2.6
Rice, white	0.4
Rye flour	1.7
Wheat germ	6.8
Wheat bran	13.6
Vegetables	
Asparagus	1.08
Beans	0.6–1.3
Broccoli	0.6
Cabbage	0.2
Carrots	1.0
Cauliflower	0.5
Celery	0.3
Corn	1.7
Kale	0.5
Onions	0.1
Peas	0.6–2
Peppers	0.5–1.2
Potatoes	1.4
Soy beans	1.3
Spinach	0.7
Tomatoes	0.6
Fruits	
Apples	0.9
Bananas	0.7
Grapefruit	0.3
Oranges	0.4
Peaches	0.8
Strawberries	0.4
Nuts	
Most nuts	0.3–4.4
Peanuts	12
Other	
Eggs	0.1
Mushrooms	0.2–14
Baker's yeast	12.3

USDA National Nutrient Database for Standard Reference, Release 28. http://www.ars.usda.gov/ba/bhnrc/ndl.

available to animals. This form, however, is heat labile and can be converted to NA by heating.[7]

Importance of Dietary Tryptophan

A substantial amount of niacin can be synthesized from the indispensable amino acid **tryptophan**. Therefore, the niacin adequacy of diets involves both the level of the preformed vitamin and that of its potential precursor tryptophan. This is expressed as niacin equivalence, which includes available niacin as well as tryptophan (Table 13.2).

4. ABSORPTION OF NIACIN

Digestion of NAD(P)

The predominant forms of niacin in most animal-derived foods are the coenzymes NAD(H) and NADP(H). These are digested to release NAm, in which form the vitamin is absorbed (Fig. 13.1). Both coenzyme forms can be

7. Thus, the roasting of coffee beans effectively removes the methyl group from trigonelline, increasing the NA content from 20 to 500 mg/kg. This practice, too, appears to have contributed to the rarity of pellagra in the maize-eating cultures of South and Central America.

TABLE 13.2 The Niacin-Equivalent Contents of Several Foods

Food	Preformed Niacin (mg/1000 kcal)	Tryptophan (mg/1000 kcal)	Niacin Equivalents[a] (mg/1000 kcal)
Cow's milk	1.21	673	12.4
Human milk	2.46	443	9.84
Beef	2.47	1280	23.80
Eggs (whole)	0.60	1150	19.80
Pork	1.15	61	2.17
Wheat flour	2.48	297	7.43
Corn meal	4.97	106	6.74
Corn grits	1.83	70	3.00
Rice	4.52	290	9.35

[a]*Based on a conversion efficiency of 60:1 for humans.*

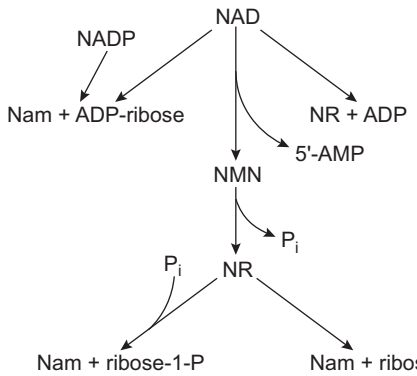


FIGURE 13.1 Metabolic disposition of absorbed niacin.

degraded by the intestinal mucosal enzyme **NAD(P)$^+$ glycohydrolase**, which cleaves the pyridine nucleotides into NAm and ADP-ribose. NAm can also be cleaved at the pyrophosphate bond to yield **nicotinamide mononucleotide (NMN)** and 5′-AMP, or by a **phosphodiesterase** to yield **NR** and ADP. The dephosphorylation of NMN also yields NR, which can be converted to NAm either by hydrolysis (yielding ribose) or phosphorylation (yielding ribose 1-phosphate). The cleavage of NAm to free NA appears to be accomplished by intestinal microorganisms and is believed to be of quantitative importance in niacin absorption.

Facilitated Absorption

Niacin is absorbed in the stomach and small intestine. Studies using everted intestinal sacs prepared from rats have demonstrated that both NA and NAm are absorbed at low concentrations via Na^+-dependent, carrier-mediated facilitated diffusion, by the organic ion transporter-10.[8] At high doses, the vitamers appear to be absorbed via passive diffusion, the rate of diffusion of NA being half that of NAm; although the Na^+-coupled monocarboxylate transporter SLC5A8 may also contribute. The result is that pharmacologic amounts of the vitamin are absorbed nearly completely.[9] The presence or absence of food in the gut appears to have no effect on niacin absorption. Because NR is not found in plasma, it appears not to be absorbed per se, but first converted to NAm.

5. TRANSPORT OF NIACIN

Free in Plasma

Niacin is transported in the portal circulation as both NA and NAm in unbound forms. Because the NA is converted to NAD(H) and subsequently to NAm in the intestine and liver, circulating levels of NAm tend to exceed those of NA.

Cellular Uptake

Both NA and NAm are taken up by erythrocytes, liver, and most peripheral tissues by facilitated diffusion whereupon they are converted to nucleotides. Tissues vary with respect to their uptake systems: erythrocytes use the anion transport system; renal tubules use a Na^+-dependent system; brain uses an energy-dependent system; the choroid plexus appears to have separate systems for accumulating and releasing NA and NAm. A high-affinity, G protein-coupled receptor for NA has been identified in adipose tissue.[10] This "**niacin receptor**" binds

8. Said, H.M., 2011. Biochem. J. **437**, 357–372.
9. In humans at steady state, consuming 3 g of NA per day, 85% of the vitamin is excreted in the urine.
10. Lorensen, A., 2001. Mol. Pharmacol. **59**, 349.

NA only at nonphysiologically high levels;[11] its natural ligand may actually be β-hydroxybutyrate.[12] It is also expressed in spleen and immune cells; in the latter, it is regulated by various cytokines. The receptor appears to play roles in responses (flushing, antihyperlipidemic) to high doses of NA.[13]

Tissue Storage

Niacin is retained in the liver and other tissues, which take it up as NA and/or NAm and convert it to the pyridine nucleotides NAD(H) and NADP(H) (Table 13.3). By far the greater amount is found as NAD(H), most of which, in contrast to NADP(H), is found in the oxidized form (NAD^+).

TABLE 13.3 Pyridine Nucleotide Contents (mg/kg) of Various Organs of Rats

Organ	NAD^+	NADH	$NADP^+$	NADPH
Liver	370	204	6	205
Heart	299	184	4	33
Kidney	223	212	3	54
Brain	133	88	<2	8
Thymus	116	35	<2	12
Lung	108	52	9	18
Pancreas	80	78	<2	12
Testes	80	71	<2	6
Blood	55	36	5	3

Offermanns, K., et al., 1984. Kirk-Othmer Encycl. Chem. Technol. 24, 59–66.

6. METABOLISM OF NIACIN

Niacin Biosynthesis

Tryptophan–niacin conversion. All animal species including humans are capable, to varying degrees, of the de novo synthesis of the metabolically active forms of niacin, NAD(H) and NADP(H). This multistep process (Fig. 13.2) involves as follows:

1. oxidative cleavage of the tryptophan pyrrole ring by **tryptophan pyrrolase** to yield ***N*-formylkynurenine**;
2. removal of the formyl group by **formylase** to yield **kynurenine**;
3. ring hydroxylation of kynurenine by the FAD-dependent **kynurenine 3-hydroxylase** to yield **3-hydroxykynurenine** (**3-OH-Ky**);
4. deamination of 3-OH-Ky by a Zn-activated, pyridoxal phosphate-dependent transaminase, to yield **xanthurenic acid**, which can either be excreted in the urine or further metabolized;
5. removal of an alanine residue from the xanthurenic acid side chain by the pyridoxal phosphate-dependent enzyme **kynureninase** to yield **3-hydroxyanthranilic acid** (**3-OH-AA**);[14]
6. oxidative ring opening of 3-OH-AA by an Fe^{2+}-dependent dioxygenase, **3-hydroxyanthranilic acid oxygenase (3-HAAO)** to yield the semistable **α-amino-β-carboxymuconic-ε-semialdehyde** (**ACS**)—a branch point in this metabolism:
   - **a.** It can be converted by **α-amino-β-carboxymuconic-ε-semialdehyde decarboxylase** (**ACSD**)[15] to **α-aminomuconic-ε-semialdehyde**, which is reduced and further decarboxylated ultimately to yield **acetyl CoA**.
   - **b.** If ACS accumulates, some can spontaneously cyclize in two ways:
     - **i.** with **dehydration** yielding **quinolinic acid**, which is decarboxylated and phosphoribosylated by **quinolinate phosphoribosyl transferase** to yield NMN, which is phosphoadenylated by the ATP-dependent **NAD^+ synthetase** to yield NAD^+;
     - **ii.** with **decarboxylation** yielding **picolinic acid**.

Determinants of tryptophan–niacin conversion. The conversion of tryptophan to NAD is an inefficient process. Balance studies in humans demonstrated that this conversion varies among individuals in the range of 34–86 mg tryptophan per niacin equivalent,[16] on which basis Horwitt recommended the use of a 60:1 ratio for practical purposes.[17] Henceforth, it is normally taken that humans normally require ~60 mg of tryptophan to produce 1 mg of niacin metabolically.[18] This ratio is similarly wide for the chick (45:1) and the rat (50:1), and extremely wide for the duck (175:1). Conversion is reduced under conditions of nutritional iron deficiency and enhanced by niacin deprivation. Niacin-deficient humans are estimated to use nearly 3% of dietary tryptophan for niacin biosynthesis and, thus, are able to satisfy two-thirds of their requirement for the vitamin from the metabolism of

11. Wise, A., Foord, N.J., Fraser, N.J., 2003. J. Biol. Chem. 278, 9869–9876.
12. β-hydroxybutyrate is one of three ketone bodies produced by the liver when glucose cannot be used as an energy substrate. Unlike the others (acetone, acetoacetate), β-hydroxybutyrate has lipolytic activity.
13. It is referred to as HM74A in humans, GPR109A in the rat, and PUMA-G in the mouse.
14. Kynureninase can also convert kynurenine to another urinary metabolite **anthranilic acid**.
15. Previously called picolinic acid carboxylase.
16. Goldsmith, G.A., Rosenthal, H.L., Gibbens, J., et al., 1955. J. Nutr. 56, 371–386; Horwitt, M.K., Harvey, C.C., Rothwell, W.S., et al., 1956. J. Nutr. 60, 1–43S.
17. Horwitt, M.K., 1955. Am. J. Clin. Nutr. 3, 244–245.
18. Hence, food niacin value is defined in terms of niacin equivalents, one unit of which is defined as 1 mg niacin +1/60 mg tryptophan.

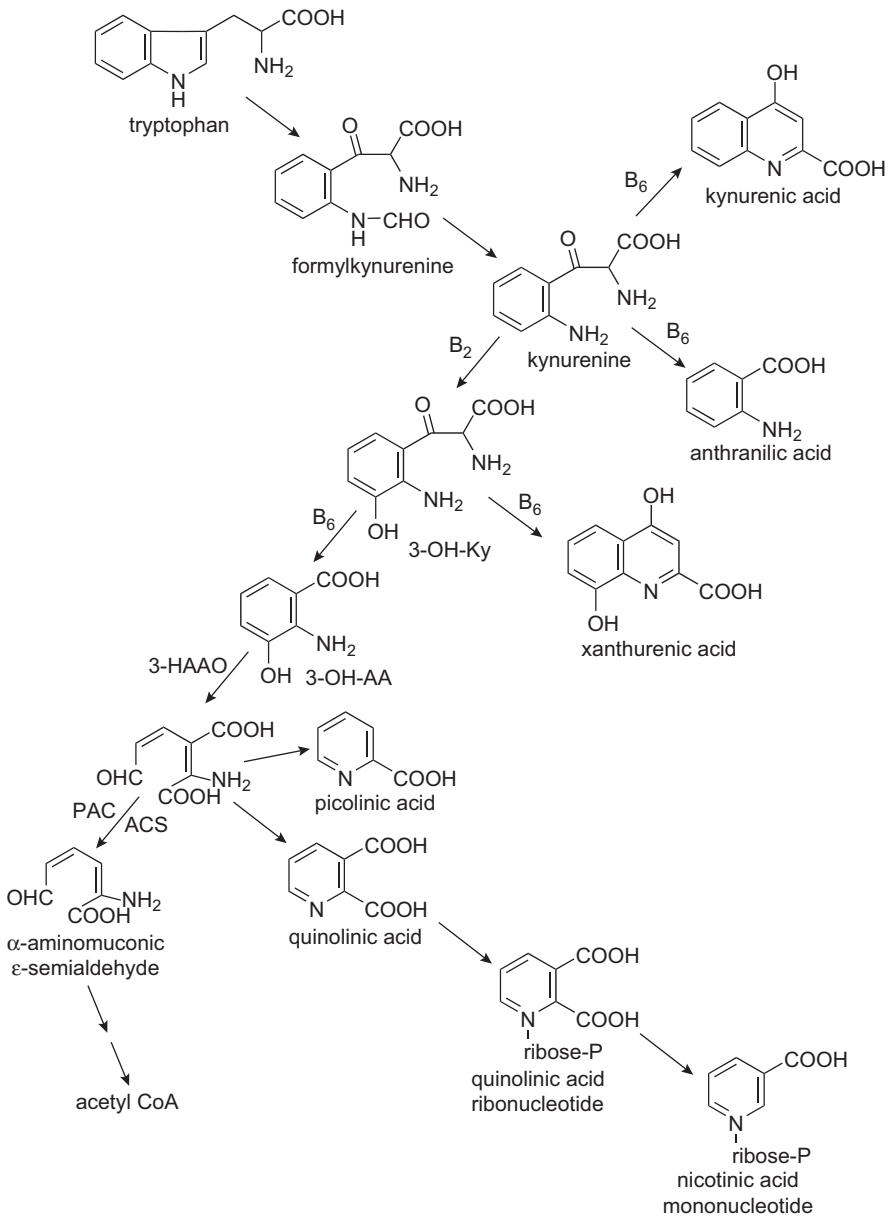


FIGURE 13.2 Metabolic interconversion of tryptophan to niacin. Note: the rate-controlling enzymes, 3-hydroxyanthranilic acid oxidase (3-HAAO) and aminomuconic semialdehyde dehydrogenase (ACSD); the roles of riboflavin (flavin adenine dinucleotide, FAD) and vitamin B_6 (pyridoxyl phosphate, PALP); anthranilic and xanthurenic acids are excreted in the urine.

this indispensable amino acid. Higher niacin-biosynthetic efficiencies are associated with *high* activities of 3-HAAO (enhancing production of ACS, the branch point intermediate in the pathway) and *low* activities of ACSD (which removes the first committed intermediate). Both hepatic activity of ACSD and the ratio of the hepatic activities of 3-HAAO and ACSD vary greatly between species and are inversely correlated with their dietary requirements for preformed niacin (Tables 13.4 and 13.5).

It would appear that protein turnover may preempt niacin synthesis under conditions of limiting tryptophan. In such circumstances the amount of tryptophan available for niacin synthesis would be expected to be low, rendering the calculation of niacin equivalents inaccurate.

Tryptophan–niacin conversion involves pyridoxal phosphate-dependent enzymes at four steps: two transaminases (which catalyze the conversions of kynurenine to kynurenic acid and of 3-hydroxykynurenine to xanthurenic acid) and kynureninase (which catalyzes the conversion of kynurenine to anthranilic acid as well as that of 3-hydroxykynurenine to 3-hydroxyanthranilic acid). While each uses pyridoxal phosphate, only kynureninase is impaired by pyridoxine deprivation. Its affinity for pyridoxal phosphate (K_m,[19] 10^{-3} M) is five orders of magnitude less than those of the transaminases (K_m's about 10^{-8} M); this renders it stripped of its cofactor

19. Michaelis constant; in this case, the concentration of pyridoxal phosphate necessary to support half-maximal enzyme activity.

TABLE 13.4 Relationship Between the 3-HAAO:ACSD (3-Hydroxyanthranilic Acid Oxygenase: α-Amino-β-Carboxymuconic-ε-Semialdehyde Decarboxylase) Ratio and Dietary Niacin Requirement

Animal	3-HAAO:ACSD	Niacin Requirement[a] (mg/kg Diet)
Rat	273	0
Chick:		
Low-niacin requirement strain	48	5
High-niacin requirement strain	27	15
Duck	5.3	40
Cat	5	45
Brook trout, lake trout	2.5	88
Turkey	1.6	70
Rainbow trout, Atlantic salmon	1.3	88
Coho salmon	3.4	175

[a]*Animals fed tryptophan.*
Poston, H.A., Combs, Jr., G.F., 1980. Proc. Soc. Exp. Biol. Med. 163, 452–459.

TABLE 13.5 Variation in Hepatic α-Amino-β-Carboxymuconic-ε-Semialdehyde Decarboxylase (ACSD)[a] Activities in Animals

Animal	ACSD Activity (IU/g)
Cat	50,000
Lizard	29,640
Duck	17,330
Frog	13,730
Turkey	9230
Cow	8300
Pig	7120
Pigeon	6950
Chicken:	
High-niacin requirement strain	5380
Low-niacin requirement strain	3200
Rabbit	4270
Mouse	4200
Guinea pig	3940
Human	3180
Hamster	3140
Rat	1570

[a]*i.e., Picolinic acid carboxylase (PAC).*
DiLorenzo, R. N., 1972. (Ph.D. thesis). Cornell University, Ithaca, New York.

under conditions of pyridoxine deprivation that are not severe enough to reduce cofactor access of the transaminases. Thus, pyridoxine deficiency impairs the overall conversion of tryptophan to niacin by blocking the production of 3-hydroxyanthranilic acid.[20] It does not, however, block the excretion of the urinary metabolites kynurenic acid and xanthurenic acid. This phenomenon has been exploited for the assessment of pyridoxine status by monitoring the urinary excretion of xanthurenic acid after a tryptophan load.

The conversion of tryptophan to niacin is also reduced by high-fat diets or diets containing excess leucine.[21] These effects appear to be due to ketosis, which has been noted as a common feature of diets of individuals with pellagra. NAD synthesis is increased by such factors as caloric restriction and hypoxia and in response to increased **sirtuin**[22] signaling, suggesting that NAD^+ levels may serve as indicators of physiological stress.

Three sources of pyridine nucleotides. The pyridine nucleotides NAD(H) and NADP(H) are produced from three precursors: NA, NAm, and tryptophan (Fig. 13.3). Whereas NA and NAm are formal intermediates in the biosynthesis of NAD^+ from tryptophan, that step (quinolinate phosphoribosyl transferase) actually leads directly to NAD^+ via NMN. Both NA and NAm are converted to NAD^+ by the same pathway after the latter is deamidated to yield NA. As the nicotinamide deamidase activities of animal tissues are low, this step is thought to be carried out by the intestinal microflora. The resulting NA is then phosphoribosylated (by nicotinate phosphoribosyl transferase), adenylated (by deamido-NAD^+ pyrophosphorylase), and amidated (by NAD synthetase) ultimately to yield NAD^+, which can be phosphorylated by an ATP-dependent NAD^+ kinase to yield $NADP^+$.

Although various tissues of the body are capable of pyridine nucleotide synthesis, there is clearly an exchange between the tissues. This involves primarily NAm, which is rapidly transported between tissues. In the rat, NA appears to be the most important precursor of these coenzymes in the liver, kidneys, brain, and erythrocytes; but in the testes and ovaries NAm appears to be a better precursor. Studies

20. It has also been suggested that the deficiency of zinc, an essential cofactor of pyridoxal kinase (*see* Chapter 14), may also impair tryptophan–niacin conversion by reducing the production of pyridoxal phosphate.
21. Shastri, N.V., Nayudu, S.G., Nath, M.C., 1968. J. Vitaminol. 14, 198–205; Bender, D.A., 1983. Br. J. Nutr. 50, 25–32.
22. Named for the yeast gene "*silent mating-type information regulation 2*," sirtuins are protein deacetylases or ribosyltransferases that function in the regulation of transcription and apoptosis.

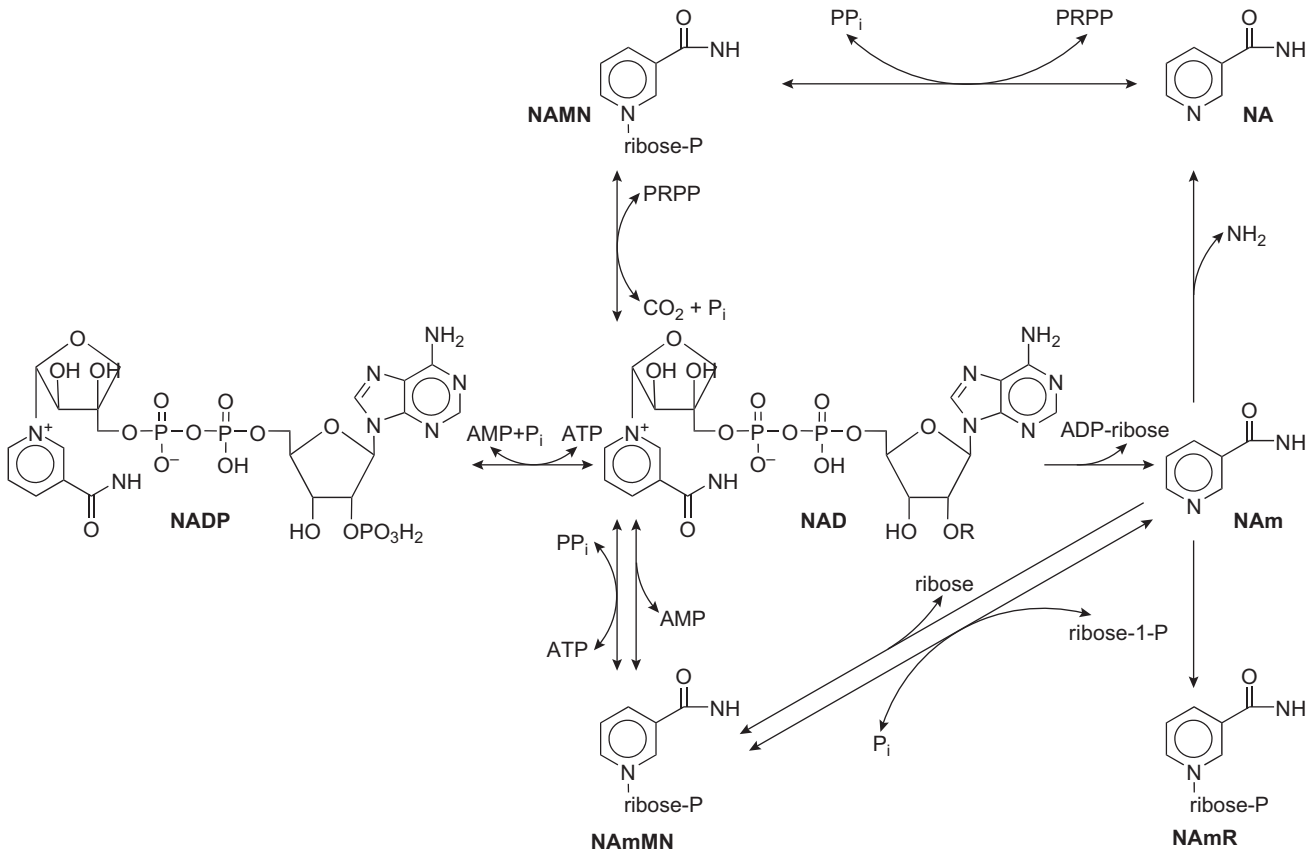


FIGURE 13.3 Niacin metabolism. *NAMN*, nicotinic acid mononucleotide; *NA*, nicotinic acid; *NAm*, nicotinamide; *NAmMN*, nicotinamide mononucleotide; *NAmR*, nicotinamide riboside; *NAD*, nicotinamide dinucleotide; *NADP*, nicotinamide dinucleotide phosphate; *PRPP*, phosphoribosyl pyrophosphate.

with chickens have shown that NAm can be a better dietary source of niacin activity than NA.[23]

Catabolism

The pyridine nucleotides are catabolized by hydrolytic cleavage of their two β-glycosidic bonds, primarily the one at the NAm moiety, by $NAD(P)^+$ glycohydrolase. NAm so released can be deamidated to form NA, in which form it can be reconverted to NAD^+. Alternatively, it can be methylated (mainly in the liver) by NAm ***N*-methyltransferase** to yield **1-methylnicotinamide**,[24,25] which can be oxidized to different products that are excreted in the urine.

Excretion

Niacin is excreted in appreciable amounts under conditions of supranutritional intake, as both vitamers are actively reabsorbed by the renal glomerulus. Excretion involves different water-soluble metabolites in the urine. At typical levels of intake of the vitamin, the major urinary metabolites are 1-methylnicotinamide[26] and its oxidation product **1-methyl-6-pyridone-3-carboxamide**. Under such conditions, intact NA and NAm, as well as other oxidation products, are also excreted, but in much smaller amounts. Most mammals excrete several metabolites: nicotinamide 1-oxide, 1-methyl-4-pyridone-3-carboxamide, 1-methyl-6-pyridone-3-carboxamide, 6-hydroxynicotinamide, and 6-hydroxynicotinic acid; some species also excrete NA/NAm conjugates of ornithine (2,5-dinicotinyl ornithine by birds only) or glycine (nicotinuric acid by rabbits, guinea pigs, sheep, goats, and calves).

The major urinary metabolite in the rat is **1-methyl-4-pyridone-3-carboxamide**. This metabolite is also found in human urine, but at levels substantially less than 1-methylnicotinamide and **1-methyl-2-pyridone-5-carboxamide**. The urinary metabolite profile can be changed by dietary deprivation of protein and/or amino acids, and it has been

23. Ohuho, M., Baker, D., 1993. J. Nutr. 123, 201–208, showed NAm to be utilized some 24% better than NA by broiler chickens.

24. NAm methylase activity is very low in fetal rat liver, increasing only upon maturity stimulation of hepatocyte proliferation (e.g., after partial hepatectomy or treatment with thioacetamide). Such increases in enzyme activity are accompanied by drops in tissue NAD^+ concentrations, as 1-methylnicotinamide reduces NAD^+ synthesis either by inhibiting NAD^+ synthetase and/or stimulating $NAD(P)^+$ glycohydrolase. Thus, it is thought that nicotinamide methylase and its product may be involved in the control of hepatocyte proliferation.

25. NA appears not to be methylated by animals. Trigonelline (1-methylnicotinic acid) does appear, however, in the urine of coffee drinkers, owing to its presence in that beverage.

26. Humans normally excrete up to 30 mg of total niacin metabolites daily, of which 7–10 mg is 1-methylnicotinamide.

suggested that the ratio of the pyridone metabolites to 1-methylnicotinamide may have utility as a biomarker for adequate amino acid intake.

At high rates of niacin intake, the vitamin is excreted predominantly (65–85% of total) in unchanged form. At all rates of intake, however, NAm tends to be excreted as its metabolites more extensively than is NA. Further, the biological turnover of each vitamer is determined primarily by its rate of excretion; thus, at high intakes, the half-life of NAm is shorter than that of NA.

7. METABOLIC FUNCTIONS OF NIACIN

Coenzyme Functions

Niacin functions metabolically as the essential component of the enzyme cosubstrates NAD(H)[27] and NADP(H).[28] The most central electron transport carriers of cells, each acts as an intermediate in most of the hydrogen transfers in metabolism, including some 500 reactions in the metabolism of carbohydrates, fatty acids, and amino acids. Each proceeds according to the following general reaction:

$$\textbf{substrate} + \textbf{NAD(P)}^+ \rightarrow \textbf{product} + \textbf{NAD(P)H} + \textbf{H}^+$$

The hydrogen transport by the pyridine nucleotides is accomplished by two-electron transfers in which the hydride ion (H^-) serves as a carrier for both electrons. The transfer is stereospecific, involving C-4 of the pyridine ring. The two hydrogen atoms at C-4 of NAD(H) and NADP(H) are not equivalent; each is stereospecifically transferred by the enzymes to the corresponding substrates.[29] In general, stereospecificity is independent of the nature of the substrate and the source of the enzyme, and few regularities are apparent except that dehydrogenases with phosphorylated and nonphosphorylated substrates tend to show opposite ste reospecificities.[30] The reactions catalyzed by the pyridine nucleotide-dependent dehydrogenases occur by the abstraction of the proton from the alcoholic hydroxyl group of the donor substrate, and the transfer of hydride ion from the same carbon atom to the C-4 of NAm. In many cases, this reaction is coupled to a further reaction, such as phosphorylation or decarboxylation. Despite similarities in mechanism and structure,[31] NAD(H) and NADP(H) have quite different metabolic roles and most dehydrogenases have specificity for one or the other.[32]

NAD in redox reactions. The oxidized form NAD^+ serves as a hydrogen acceptor at the C-4 position of the pyridine ring, forming NAD(H) which, in turn, functions as a hydrogen donor to the mitochondrial respiratory chain (TCA cycle) for ATP production (Table 13.6). These reactions include the following:

- glycolytic reactions
- oxidative decarboxylations of pyruvate
- oxidation of acetate in the TCA cycle
- oxidation of ethanol
- β-oxidation of fatty acids
- other cellular oxidations.

NADP(H) in reduction reactions. The phosphorylation of NAD^+ facilitates the separation of oxidation and reduction pathways of niacin cofactors by allowing NADP(H) to serve as a codehydrogenase in the oxidation of physiological fuels.[33] Thus, NADP(H) is maintained in the reduced state, NADPH[34] by the pentose phosphate pathway such that reduction reactions are favored. Many of these also involve flavoproteins.[35] These reactions involve reductive biosyntheses, such as those of fatty acids and steroids (Table 13.6). In addition, NADPH also serves as a codehydrogenase for the oxidation of glucose 6-phosphate in the pentose phosphate pathway.

NAD as a Substrate

ADP-ribosylation. NAD^+ functions in the addition of ADP-ribose to various nucleophilic acceptors. This occurs by its first forming a glycosidic linkage with ADP-ribose, which appears to be released by mitochondria under oxidative stress. The energy released upon breaking that bond is then used to drive mono- and poly-(ADP-ribosyl)ation reactions (Fig. 13.4).

- **Mono(ADP-ribosyl)ation**. NAD^+ serves as the donor of an ADP-ribose moiety to an amino acid residue on an acceptor protein. Originally recognized as properties of bacterial toxins,[36] two groups of **mono-ADP-ribotransferases** (**ARTs**) have been identified in mammalian cells. **Ecto-ARTs** are secreted or expressed on the outer surfaces of cells in skeletal and cardiac muscles, lung, testes, and lymphatic tissues. They ADP-ribophoshorylate

27. Previously, coenzyme I or diphosphopyridine nucleotide (DPN).
28. Previously, coenzyme II or triphosphopyridine nucleotide (TPN).
29. Because of this phenomenon, the pyridine nucleotide-dependent enzymes are classified according to the side of the dihydropyridine ring to which each transfers hydrogen, i.e., class A and class B.
30. Dehydrogenases with phosphorylated substrates tend to be B-stereospecific, whereas those with small (i.e., no more than three carbon atoms), nonphosphorylated substrates tend to be A-stereospecific.
31. For example, each contains adenosine, which appears to serve as a hydrophobic anchor.
32. A small number of dehydrogenases can use either NAD(H) or NADP(H).
33. For example, glyceraldehyde-3-phosphate, lactate, alcohol, 3-hydroxybutyrate, and pyruvate and α-ketoglutarate dehydrogenases.
34. The $NADP^+$/NADPH couple is largely reduced in animal cells, owing to the **transhydrogenase** activity that catalyzes the energy-dependent exchange of hydride between the pyridine nucleotides coupled to proton transport across the mitochondrial membrane in which it resides (the so-called *redox-driven proton pump*).
35. The first step in most biological redox reactions is the reduction of a flavoprotein by NADPH.
36. Cholera, diphtheria, pertussis, and *pseudomonas* toxins use NAD^+ to catalyze the ADP-ribosylation of host G-proteins and disrupt host cell function.

TABLE 13.6 Some Important Pyridine Nucleotide-Dependent Enzymes of Animals

Role	Enzyme	
	NAD(H) Dependent	NADP(H) Dependent
Carbohydrate metabolism	3-Phosphoglyceraldehyde dehydrogenase Lactate dehydrogenase Alcohol dehydrogenase	Glucose-6-phosphate dehydrogenase 6-Phosphogluconate dehydrogenase
Lipid metabolism	α-Glycerophosphate dehydrogenase β-Hydroxyacyl-CoA dehydrogenase 3-Hydroxy-3-methylglutaryl-CoA reductase	3-Ketoacyl ACP[a] reductase enoyl-ACP reductase
Amino acid metabolism	Glutamate dehydrogenase	Glutamate dehydrogenase
Other	NADH dehydrogenase/NADH-ubiquinone Dihydrofolate reductase Poly(ADP-ribose) polymerase 4-Hydroxybenzoate hydroxylase NADPH-cytochrome *P*-450 reductase Mono-ADP-ribotransferases	Glutathione reductase Thioredoxin-NADP reductase

[a]*ACP,* acyl carrier protein.

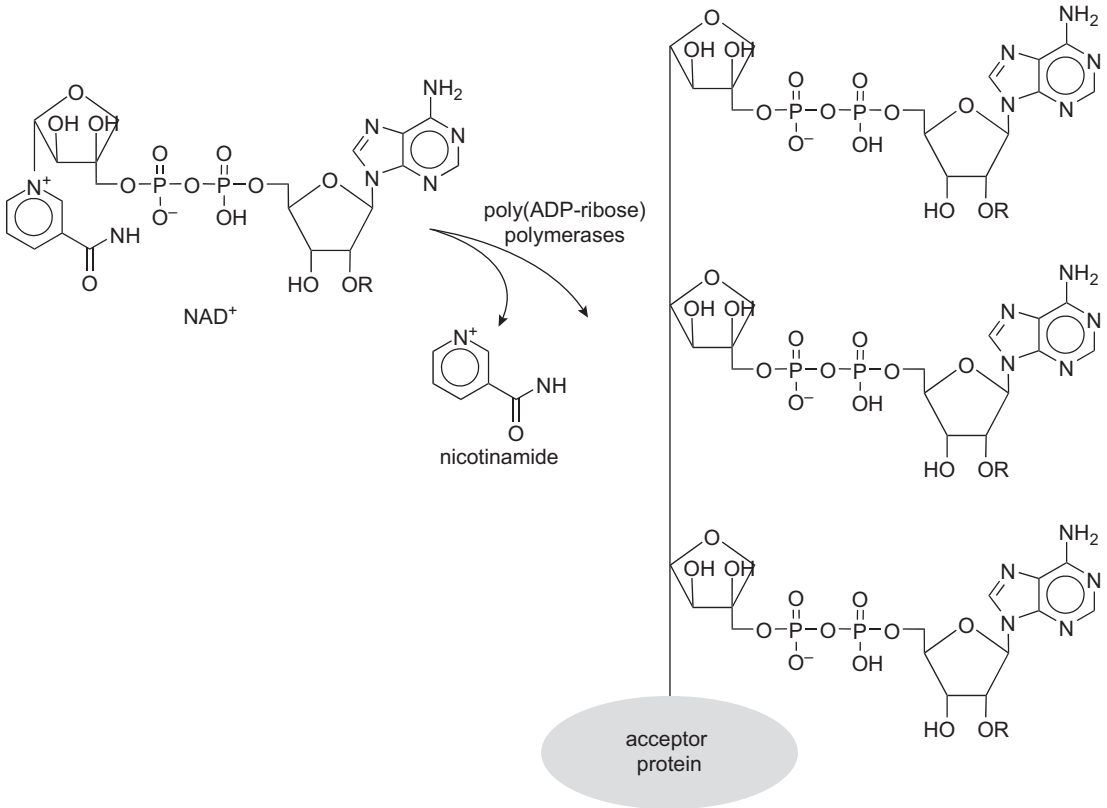


FIGURE 13.4 Poly(ADP-ribosyl)ation of proteins. Branching can occur at ribose links.

several proteins: integrins[37] in the control of myogenesis, defensins[38] in signaling macrophages, and an ATP-gated ion channel[39] to induce apoptosis. Because extracellular NAD concentrations are typically low, it has been suggested that the activities of the ecto-ARTs must depend on NAD being released by damaged cells, which would make NAD a signaling molecule for nearby cell death. **Endo-ARTs** are present in the cytosol

37. Integrins are extracellular receptors that mediate cell–cell attachment and control cell signaling.

38. Defensins are small, cysteine-rich peptides in neutrophils and most epithelial cells that participate in killing phagocytized bacteria by binding to the bacterial cell and forming pore-like structures that facilitate loss of essential ions.

39. P_2X_7.

or inner membranes of cells. They show little homology with the ecto-ARTs. One endo-ART inactivates G-protein β-subunits to serve in cell signaling; others inactivate the protein-folding chaperone, the 78 kDa glucose-regulated protein (GRP78), which reduces protein secretion under conditions of cellular stress. In both cases, the inactivation appears to be reversible through the activity of hydrolases, which recycle the acceptor protein by removing its ADP-ribosyl moiety.

- **Poly(ADP-ribosyl)ation**. NAD(H) functions in the formation of ADP-ribose polymers by **poly(ADP-ribose) polymerases (PARPs)**,[40] which are activated by DNA single-strand breaks to catalyze both auto-ADP-ribosylation (to a glutamatyl or aspartyl residue) and, to a lesser extent, ADP-ribosylation of some 30 other acceptor proteins. In this way, PARPs form chains of ADP-ribose sequences on protein monomers, creating branch points at 40–50 unit intervals with new sites for subsequent elongation and increasingly negative charge. The poly(ADP-ribosyl)ation process involves extensive turnover of NAD^+ with concomitant production of NAm for reutilization.

Genomic Stability

Niacin status can affect genomic stability in several ways. First, poly(ADP-ribose) polymers bind to high-affinity binding sites on histones and other nuclear proteins. This has been shown to draw histones away from the DNA, thus, facilitating interactions of exposed DNA with other DNA-binding proteins (polymerases, ligases, helicases, and topoisomerases) involved in replication and repair. Evidence indicates that this process is involved in preventing nonhomologous recombination between two sites of damage. Niacin deprivation appears to impair the activities of all PARPs, resulting in genomic instability.[41] Second, the NAD-dependent deacylation activities of sirtuins 1–6 function in the maintenance of compact chromatin structure and gene silencing by deacylating histones, and in the regulation of many nonhistone proteins including the tumor suppressor p53 and the transcription factors PGC-1α and FOXO1.[42]

Glucose Tolerance Factor

Niacin has been identified as part of the chromium-containing **glucose tolerance factor** of yeast, which enhances the response to insulin. Its role, if any, in that factor is not clear, as free niacin is without effect. It is possible that this activity involves a metal-chelating capacity of NA such as has been reported for zinc and iron.[43]

Affector of Neurotransmission

NAD has been shown to bind to cell surface receptors of colonic enterocytes (P2Y1) and lymphoid cells (P2Y11). Binding has been proposed to inhibit neurotransmission to inhibit colonic contraction.[44]

Niacin-Responsive Genetic Disorders

Polymorphisms affecting the cofactor binding of NAD(P)-dependent proteins can lead to dysfunction if niacin is not plentiful; they can be treated and prevented with high doses of niacin. Polymorphisms affecting several proteins and enzymes have been identified: aldehyde dehydrogenase, glucose-6-phosphate-1-dehydrogenase, and the neutral amino acid transporter. Mutations in the latter result in **Hartnup disease**,[45] a rare familial disorder involving malabsorption of tryptophan (and other amino acids) and characterized by hyperaminoaciduria,[46] a pellagra-like skin rash (precipitated by stress, sunlight, or fever), ataxia and psychiatric disorders ranging from emotional instability to delirium. Nonreabsorbed tryptophan is degraded by gut microbiota to pyruvate and indole, which is reabsorbed and is neurotoxic.

8. BIOMARKERS OF NIACIN STATUS

Niacin Status Can be Assessed in Two Ways

- **Whole blood NAD** levels are sensitive to niacin deprivation and, therefore, can be used as an indicator of niacin status. Because NADP levels remain fairly stable under deficient conditions, the NAD:NADP ratio is frequently used to assess niacin status. This has been called the "**niacin number**."
- **Urinary niacin metabolites**, N^1-methylnicotinamide and N^1-methyl-2-pyridone-5-carboxamide, can indicate niacin status. While the amount of the former has been associated with dermatitis,[47] the ratio of the two metabolites is affected by protein status.

Dietary assessment based on both the preformed vitamin and tryptophan is subject to the limitations of dietary

40. At least 18 PARP genes have been identified.
41. Oei, S.L., Kel, C., Ziegler, M., 2005. Biochem. Cell Biol. **83**, 263–270.
42. Spronck, J.C., Nickerson, J.L., Kirkland, J.B., 2007. Nutr. Cancer **57**, 88–99.
43. Agte, W., Paknikar, K.M., Chiplonkar, S.A., 1997. Biometals **10**, 271–276.
44. Klein, C., Grahnert, A., Abdelrahman, A., et al., 2009. Cell Calcium 46, 263–272.
45. The disease was named for the first case, described in 1951, involving a boy thought to have pellagra. Since that time some 50 proved cases involving 28 families have been described.
46. The presence of abnormally high concentrations of amino acids in the urine.
47. Dilon, J.C., Malfait, P., Demaux, G., et al., 1992. Am. J. Med. 93, 102–104.

recall methods and the uncertainties of niacin bioavailability in foods.

9. NIACIN DEFICIENCY

Determinants of niacin status. Because a substantial amount of niacin can be synthesized from tryptophan, niacin status depends not only on the level of intake of the preformed vitamin but also that of its potential amino acid precursor. Accordingly, niacin deficiency typically occurs in individuals consuming diets low in both of these essential nutrients and, frequently, pyridoxine. Thus, the occurrence of pellagra, as well as niacin-deficiency diseases in animals, is properly viewed as the result of a multifactorial dietary deficiency rather than that of insufficient intake of niacin per se.

In addition to dietary tryptophan and pyridoxine supplies being important determinants of niacin status, it has been suggested that excess intakes of the branched-chain amino acid leucine may antagonize niacin synthesis and/or utilization and, thus, also may be a precipitating factor in the etiology of pellagra. Excess leucine has been shown to inhibit the production of quinolinic acid from tryptophan by isolated rat hepatocytes; however, the magnitude of this effect is small in comparison with the K_m of quinolinate phosphoribosyl transferase for quinolinate, indicating that excess leucine (and/or its metabolites) is unlikely to affect the rate of NAD^+ biosynthesis by the liver. Some studies with intact animals (rats) have produced results supporting the view that excess leucine can impair the synthesis of NAD^+ from tryptophan (either by inhibiting the enzymatic conversion itself or the cellular uptake of the amino acid); however, others have yielded negative results in this regard. Therefore, the relative contribution of high leucine intake to the etiology of pellagra is not clear at present.

Zinc appears to play a role in the pyridoxine-dependent conversion of tryptophan to niacin.[48] Pellagra patients have been found to have low plasma Zn levels, and Zn supplementation increases their urinary excretion of 1-methylnicotinamide and 1-methyl-2-pyridone-5-carboxamide. Studies with rats have shown that treatment of niacin-deficient animals with the metabolic intermediate picolinic acid increases circulating Zn levels.

General signs. Niacin deficiency leads to tissue depletion of NAD(P) that varies with cell turnover. This results in different species-specific signs usually accompanied by loss of appetite and poor growth (Table 13.7).

TABLE 13.7 Signs of Niacin Deficiency

Organ System	Signs
General	Decreased appetite and growth
Dermatologic	Dermatitis, photosensitization
Gastrointestinal	Inflammation, diarrhea, glossitis
Skeletal	Perosis
Vascular	Anemia
Nervous	Ataxia, dementia

Deficiency Signs in Humans

Niacin deficiency in humans results in changes in the skin, gastrointestinal tract, and nervous system. Individuals with this syndrome, **pellagra**, typically complain of a sore tongue, which involves markedly reduced epithelial thickness and diarrhea. The dermatologic changes are most pronounced in the parts of the skin that are exposed to sunlight (face, neck,[49] backs of the hands and fore) (Figs. 13.5 and 13.6). In some patients, lesions resemble early sunburn; in chronic cases the symmetric lesions feature cracking, desquamation,[50] hyperkeratosis, and hyperpigmentation. Lesions of the gastrointestinal tract include angular stomatitis, cheilosis, and glossitis as well as alterations of the buccal mucosa, tongue, esophagus, stomach (resulting in achlor[51]), and intestine (resulting in diarrhea).[52] Pellagra almost always involves anemia.[53] Early neurological symptoms associated with pellagra include anxiety, depression, and fatigue[54]; later symptoms include depression, apathy, headache, dizziness, irritability, and tremors.

Deficiency Signs in Animals

Most niacin-deficient animals show poor growth and reduced efficiency of feed utilized. Pigs and ducks are particularly sensitive to niacin deficiency. Pigs show diarrhea, anemia, and degenerative changes in the intestinal mucosa and nervous tissue[55]; ducks show severely bowed and weakened legs and diarrhea. Niacin-deficient dogs show necrotic

48. Zinc, which is required by pyridoxal phosphokinase, is also related to the function of pyridoxine in this system. Alcoholics, who are typically of low Zn status, can excrete high levels of the niacin metabolites 1-methyl-6-pyridone-3-carboxamide and 1-methylnicotinamide. This excretion can be increased by Zn supplementation, presumably owing to increased pyridoxal phosphate activity and the consequent activation of pyridoxine to the form (pyridoxal phosphate) that facilitates tryptophan–niacin conversion. Zinc deficiency can also reduce the availability of tryptophan for niacin biosynthesis by enhancing its oxidation.

49. This is referred to as **Casal's collar**.
50. The shedding of the epidermis in scales.
51. The absence of hydrochloric acid from the gastric juice, usually due to gastric parietal cell dysfunction.
52. Many of these gastrointestinal changes also occur in schizophrenia.
53. The anemia associated with pellagra is of the macro- or normocytic, hypochromic types.
54. Many of these symptoms also occur in schizophrenia.
55. The syndrome is called "pig pellagra."

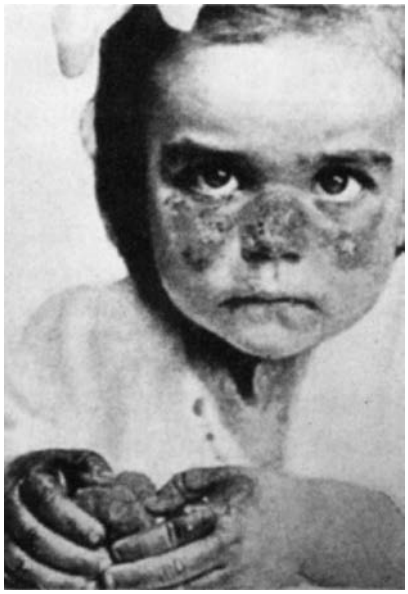


FIGURE 13.5 Pellagra: affected child with facial "*butterfly wing*." *Courtesy Cambridge University Press.*

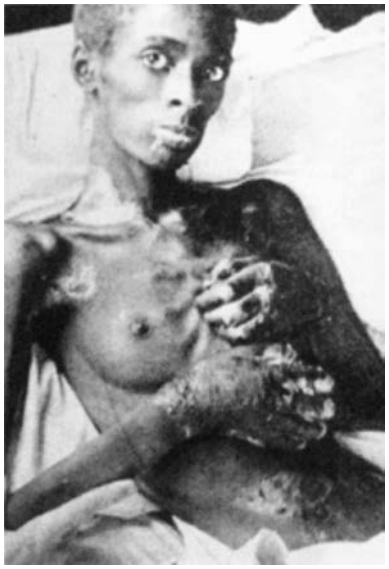


FIGURE 13.6 Pellagra: affected woman with "*pellagra glove*." *Courtesy Cambridge University Press.*

degeneration of the tongue with changes of the buccal mucosa and severe diarrhea.[56] Rodents show alopecia and nerve cell histopathology. Chickens show inflammation of the upper gastrointestinal tract, dermatitis of the legs, reduced feather growth, and **perosis** (Figs. 13.7 and 13.8).[57]

It has been thought that ruminants are not susceptible to niacin deficiency, owing to the synthesis of the vitamin

56. **Black tongue disease**.

57. Inflammation and misalignment of the tibiotarsal joint (*hock*), in severe cases involving slippage of the Achilles tendon from its condyles, which causes crippling due to an inability to extend the lower leg.

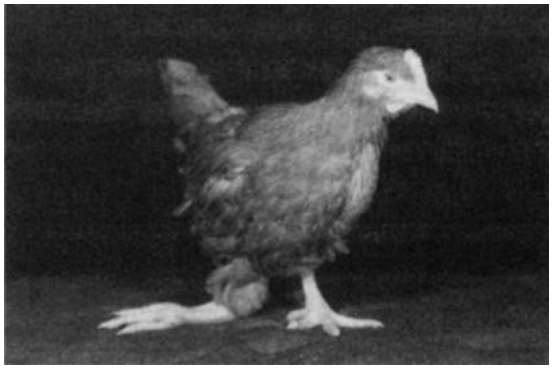


FIGURE 13.7 Perosis (left leg) in niacin-deficient chick. *Courtesy, M.L. Scott, Cornell University.*

by their rumen microflora. Although that appears to be true for most ruminant species, evidence indicates that fattening beef cattle and some high-producing dairy cows can benefit from niacin supplements under some circumstances. Studies have shown niacin treatment of lactating cows to depress circulating levels of ketones, apparently by reducing lipolysis in adipocytes by a process involving increased cyclic 3′,5′-adenosine monophosphate (cAMP) and, consequently, the concentrations of nonesterified fatty acids in the plasma. That ruminal synthesis of the vitamin may not meet the nutritional needs of the host would appear most likely in circumstances wherein rumen fermentation is altered to enhance energy utilization, with associated reductions in rumen microbial growth.

10. NIACIN IN HEALTH AND DISEASE

Niacin has been associated with a number of health effects unrelated to the signs of niacin deficiency.

Cardiovascular Health

High doses of NA have proven to be among the most useful treatments for hyperlipidemias and hypercholesterolemia. A retrospective evaluation of results from the U.S. Coronary Drug Project showed NA doses of 1–2 g/day to have reduced lethal coronary events, resulting in highly significant reduction of mortality from all causes by 11% (*versus* a placebo). A meta-analysis of 11 clinical trials involving nearly 10,000 subjects found niacin use to be associated with significant reductions in cardiovascular end points, major coronary heart disease events, and stroke incidence.[58] However, in 2011 a large trial of combined NA-statin treatment was stopped after 32 months on the basis of its not showing reductions in cardiovascular events, despite showing the expected anti-hyperlipidemic effects.

High-dose NA treatment reduces all major lipids and apoB-containing lipoproteins (VLDL, LDL), while

58. Lavigne, P.M., Karras, R.H., 2013. J. Am. Coll. Cardiol. 61, 440–446.

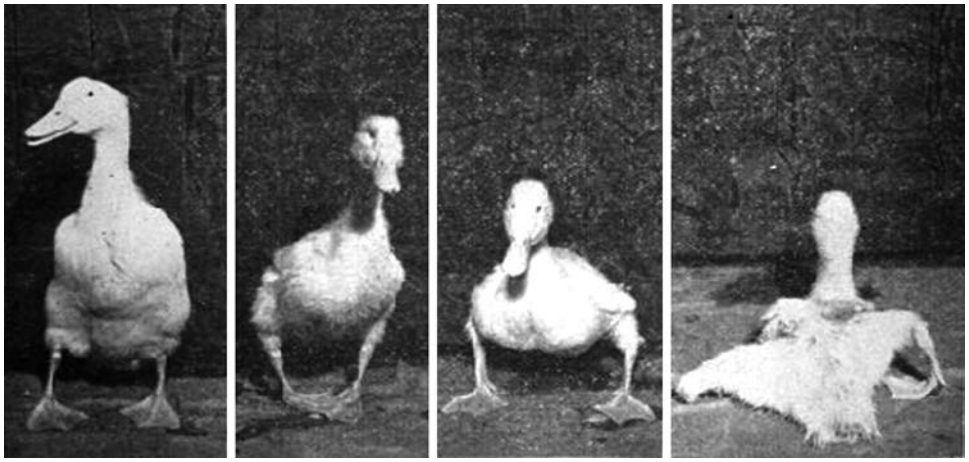


FIGURE 13.8 Leg weakness in ducks fed diets containing adequate (left) or progressively deficient amounts of niacin. *Courtesy M. L. Scott, Cornell University.*

increasing apoA1-containing lipoproteins (HDL) (Tables 13.8 and 13.9). Thus, NA has been considered as an adjunct to statin therapy; limited clinical data indicate that the addition of NA improves lipid profiles in patients with coronary artery disease but does not affect endothelial or microvascular function.[59] The antihyperlipidemic effects of niacin appear to be unrelated to NAD(P) or NAm but instead involve three metabolic phenomena (Fig. 13.9):

- **Reducing hepatic triglyceride synthesis** by NA inhibiting hepatic microsomal diacylglyceride transferase-2 has been demonstrated. That enzyme catalyzes the final reaction in triglyceride synthesis; its inhibition limits the amounts of triglycerides available for the assembly of VLDL.[60] This results in increased degradation of apolipoprotein B and the consequent reduction in both VLDL and its catabolic product, LDL.
- **Reducing the removal of HDL-apoA1** has been demonstrated in cultured cells, with NA inhibiting the catabolism of HDL-apoA1 without affecting apoA-I synthesis. This increases HDL and HDL cholesterol. It has been suggested that the response may involve the putative "HDL catabolism receptor," which may be a β-chain ATP synthase. These increases are thought to reflect reduced exchange of triglycerides and cholesterol esters[61] and the retarded degradation of apoA1.
- **Reducing adipocyte lipolysis** by NA binding to the niacin receptor, GPR109A, which is linked to a G-protein that inhibits adenylate cyclase. That inhibition leads to a decline in cAMP levels, which inhibits the hormone-sensitive lipase and consequently reduces the mobilization of fatty acids from triglycerides in adipose tissue. Reduced release of fatty acids is responsible for at least part of the reduction of hepatic synthesis and secretion of VLDLs and the subsequent decline in circulating LDL levels. Decreased circulating levels of VLDLs are associated with decreased levels of triglycerides and cholesterol. Also contributing to reduced cholesterol levels is a decrease in cholesterol biosynthesis due to NA inhibition of 3-hydroxy-3-methylglutaryl CoA reductase.[62]

TABLE 13.8 Summary of Plasma Lipid Responses to Nicotinic Acid Treatment (>1.5 g/day) of Dyslipidemias

Parameter	Reduction (%)	Increase (%)
Triglycerides	21–44	–
VLDL	25–40	–
LDL cholesterol	2–22	–
HDL cholesterol	–	18–35
Total cholesterol	4–16	–
Lipoprotein Lp(a)	16–36	–

Gille, A., Bodor, E.T., Ahmed, K., et al., 2008. Ann. Rev. Pharmacol. Toxicol. 48, 79–86.

Skin Health

Niacin deficiency increases skin sensitivity to sunlight. This occurs in patients with pellagra and has been produced experimentally in the rainbow trout.[63] This effect appears to be due to inhibition of PARPs and sirtuins, which depend

59. Philpott, A.C., Hubacek, J., Sun, Y.C., et al., 2013. Atherosclerosis 226, 453–458.
60. *See review*: Kamanna, V.S., Kashyap, M.L., 2008. Am. J. Cardiol. 101, 20–26 B.
61. This process is mediated by the cholesterol ester transfer protein.
62. DiPalma, J.R., Thayer, W.S., 2001. Ann. Rev. Nutr. 11, 169–176.
63. Poston, H.A., Wolfe, M.J., 1985. J. Fish Dis. 8, 451–460.

TABLE 13.9 Results of Clinical Trials of Nicotinic Acid (≥1 g/day) in Patients on Statins

Study	Subjects (Duration)	Parameter	Placebo	Nicotinic Acid
1[b]	8341 (5 years)	Myocardial infarction	12.2%	8.9%[a]
		Mortality	20.9%	21.2%
1[c]	8441 (15 years follow-up)	Mortality	58.2%	52.0%[a]
2[d]	555 (5 years)	Mortality	29.7%	21.8%[a]
3[e]	146 (2.5 years)	Cardiovascular events	19.2%	4.2%[a]
6[f]	167 (1 year)	Carotid intima-media thickness	0.044 mm	0.014 mm[a]

[a]$p < .05$.
[b]*Coronary Drug Project Research Group, 1975. JAMA 231, 360–366.*
[c]*Canner, P.L., Berge, K.G., Wenger, N.K., 1986. J. Am. Coll. Cardiol. 8, 1245–1252.*
[d]*Carlson, L.A., Rosenhamer, G., 1988. Acta Med. Scan. 223, 405–41.*
[e]*Brown, G., Albers, J.J., Fisher, L.D., 1990. N. Engl. J. Med. 323, 1289–1296.*
[f]*Taylor, A.J., Sullenberger, L.E., Lee, H.J., et al., 2004. Circulation 110, 3512–3519.*

on NAD^+ to protect against DNA damage. At high doses, NA has vasodilatory activity; this is due to its binding to hydroxycarboxylic acid receptors, which increase prostaglandin production, increasing microvascular blood flow. Niacin has been used for topical treatment of acne vulgaris and rosacea.[64] Most forms of the vitamin are water soluble and, therefore, not absorbed across the skin; fatty esters of niacin can be absorbed dermally.

Lung Health

Studies in animal models have demonstrated that niacin status can be affected by pulmonary oxidant injury in different ways. Acute oxidative stress, caused by treatment with DNA-damaging agents (lipopolysaccharide, cyclophosphamide, and bleomycin) produces NAD(P) depletion, which can be prevented by supplemental niacin.[65] Nonacute oxidative stress, caused by hyperoxia, increases NAD levels in the lungs and induces poly(ADP-ribose) synthesis even of niacin-deprived animals. These effects increase inflammatory and apoptotic responses.[66]

Anticarcinogenesis

Metabolic considerations would predict that niacin deprivation might reduce tumorigenesis. Tumor cells are known to be far more dependent on glycolysis than oxidative phosphorylation for generating ATP,[67] and their demands for and turnover of NAD are substantially greater than nontumor cells. However, experimental evidence does not indicate that tumor risk can be reduced by niacin deprivation. In fact, epidemiological evidence has associated marginal niacin intakes and/or the reliance on maize-based diets with increase risks of cancers of the esophagus.[68] Further, studies in animal models have found NA supplementation to reduce yields of esophageal tumors in the *N*-nitrosomethylbenzylamine-treated rat model.[69] Supranutritional doses of niacin have been shown to reduce dramatically the yield of skin tumors in UV-treated mice in a dose-dependent manner that correlated with skin NAD levels.[70]

Neurocognitive Health

NAm has been shown to enhance the effect of tryptophan in supporting brain serotonin levels. It does so by reducing the urinary excretion of tryptophan metabolites and reducing the conversion of tryptophan to niacin. This increases the availability of tryptophan for the synthesis of serotonin, the general effect of which is antidepressive. At the same time, neurons and glial cells have metabolic needs for NAD(P), as they depend on glycolysis for generating ATP. Niacin status has been associated with several neurological disorders:

- **Schizophrenia** is associated with NAD deficiency in critical areas of the brain. Affected individuals oxidize NAm more readily than unaffected people: they excrete greater amounts of 1-methyl-6-pyridone-3-carboxamide. As the excretion of this methylated product is increased by treatment with methylated hallucinogens (e.g., methylated indoles) and is decreased by treatment with tranquilizers, it has been suggested that schizophrenics suffer a depletion of NAm (via its methylation and excretion), which limits NAD^+ synthesis. Patients with first episodes show diminished flushing responses to niacin; that response is mediated by vasodilators

64. Kirkland, J.B., Millman, C.G., Jacobson, J., 2011. J. Evid. Based Complement. Med. 16, 91–101.
65. Giri, S.N., Blaisdell, R., Rucker, R., et al., 1994. Environ. Health Perspect. 102 S10, 137–147.
66. Kirkland, J.B., 2010. Exp. Biol. Med. 235, 561–568.
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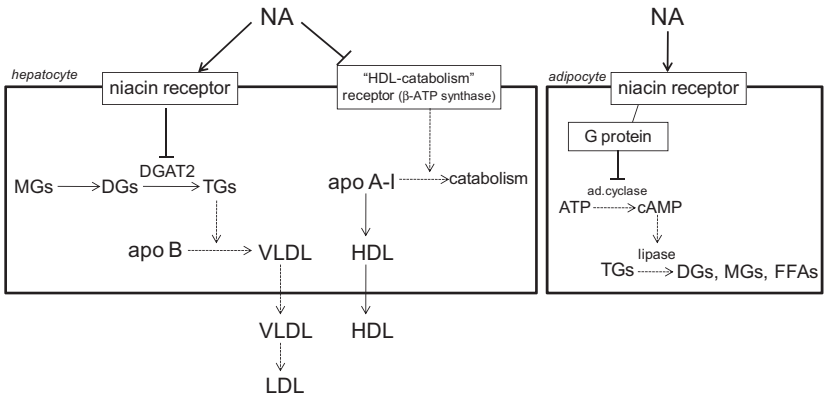

FIGURE 13.9 Schematic representation of apparent metabolic bases for the antihyperlipidemic effects of nicotinic acid. *Dashed arrows* indicate steps reduced by NA. *NA*, nicotinic acid; *MGs*, monoglycerides; *DG*, diglycerides; *TGs*, triacylglycerides; *apo B*, apolipoprotein B; *apo A-I*, apolipoprotein A-I; *DGAT2*, diacylglycerol acyltransferase 2; *ATP*, adenosine triphosphate; *cAMP*, cyclic adenosine monophosphate; *ad. cyclase*, adenylate cyclase; *lipase*, hormone-sensitive lipase.

derived from arachidonic acid, which is typically low in such patients. High doses of NA (e.g., 1 g/day) given with ascorbic acid have been found effective in eliminating psychotic symptoms and preventing relapses of acute cases.

- **Alzheimer's disease** incidence has been associated inversely with niacin intake. A cohort study found that subjects with the greatest intakes of dietary niacin (22 mg/day) experienced cognitive declines over 6 years that averaged 44% less than those of subjects with lower niacin intakes.[71] It is possible that this protection may be due to niacin increasing expression of apoA-1, which is associated with decreased Alzheimer's disease risk, or to sirtuins, which have been shown to reduce amyloid precursor protein in animal models.
- **Parkinson's disease** is characterized by high urinary excretion of methylnicotinamide, suggesting aberrant niacin metabolism.[72]
- **Depression** can be associated with diminished flushing responses to niacin. Studies have shown that 5% of depressed subjects do not show the niacin-induced flushing. Nonresponders are likely to be severely ill, with depressed mood, anxiety, and physical symptoms.[73]
- **Progeny effects of maternal alcohol** (anxiety, neural damage) have been found in animal models to be reduced by maternal NAm treatment.[74]

Type 2 Diabetes (T2D)

T2D is associated with a reduced state of the pyridine nucleotides in the cytosol and mitochondria due to the increased levels of glucose, free fatty acids, lactate, and branched-chain amino acids. This affects gene expression via the NADH-activated transcriptional corepressor, C-terminal binding protein (CtBP), and the NADH enzyme—glyceraldehyde-3-phosphate dehydrogenase. NAm has been found to delay or prevent the development of diabetic signs in the nonobese diabetic mouse model,[75] to decrease the severity of diabetic signs associated with β-cell proliferation induced by partial pancreatectomy,[76] and to protect against diabetes induced by agents[77] that cause DNA strand breakage in β-cells. This appears to be due to preventing extreme NAD(P) depletion, which would be expected to enhance poly(ADP-ribose)-induced signaling leading to inflammation and apoptosis. In clinical trials, NAm has been found to protect high-risk children from developing clinically apparent insulin-dependent diabetes,[78] to improve small artery vasodilatory function in statin-treated T2D patients,[79] and to increase blood glucose levels but not T2D.[80] However, a large randomized trial found NAm ineffective in reducing diabetes risk.[81]

Other Effects

High doses of NA and NAm have been shown to

- reduce the hyperphosphatemia in chronic renal disease and renal dialysis, by inhibiting the Na^+-dependent cotransport of phosphate in both the renal tubule and intestine;[82]

71. Morris, M.C. Evans, D.A., Bienas, J.L., et al., 2004. J. Neurol. Neurosurg. Psychiatr. 75, 1093–1099.
72. Ying, W., 2007. Front. Biosci. 12, 1863–1888.
73. Smensy, S., Baur, K., Rudolph, N., et al., 2010. J. Affect. Disord. 124, 335.
74. Feng, Y., Paul, I.A., LeBlanc, M.H., 2006. Brain Res. Bull. 69, 117–123; Ieraci, A., Herrara, D.G., 2006. PLoS Med. 3, e101.
75. Reddy, S. Bibby, N.J., Elliott, R.B., 1990. Diabetes Res. 15, 95.
76. Yonemura, Y., Takashima, T., Miwa, K., et al., 1984. Diabetes 33, 401.
77. Alloxan, streptozotocin.
78. Elliott, R.B., Chase, H.P., 1991. Diabetologia 34, 362; Manna, R., Milgore, A., Martin, L.S., et al., 1992. Br. J. Clin. Pract. 46, 177–184.
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80. Phan, B.A.P., Muñoz, L., Shadzi, P., et al., 2013. Am. J. Cardiol. 111, 352–355.
81. Gale, E.A., Binley, P.J., Emmett, C.L., et al., 2004. The European-Canadian Nicotinamide Diabetes Intervention Trial. Lancet 363, 925–931.
82. Lenglet, A., Liabeuf, S., Guffroy, P., et al., 2013. Drugs R.D. 13, 165–173.

- reduce hypertensions, probably due to vasodilation; however, results of clinical trials have been inconsistent;[83]
- increase circulating levels of adiponectin; these effects were not accompanied by changes in insulin sensitivity or endothelial function;[84]
- suppress postprandial trigyceridemia, a predictor of cardiovascular events.[85]

11. NIACIN TOXICITY

In general, the toxicity of niacin is low. Nonruminant animals can tolerate oral exposures of at least 10- to 20-fold their normal requirements for the vitamin. The toxic potential of NAm appears to be greater than that of NA, probably by a factor of four. Side effects of high doses appear to result from metabolic disturbances due to the depletion of methyl groups as the result of the metabolism of the vitamin.

Nicotinic Acid

The most common side effect of high-dose NA is **skin flushing** caused by cutaneous vasodilation. This response is transient (30–90 min) and accompanied by erythema, tingling, itching, and elevated skin temperature. It affects some 70% of subjects in which it is seen at the beginning of NA therapy and subsides over time with the development of tolerance. Still, for some it can be disagreeable to the point of discontinuing treatment.[86] The flushing response can be evoked by either oral or topical exposure to NA, which triggers COX-1[87]-derived release of prostaglandin D_2 from platelets and dendritic cells. These effects involve the niacin receptor, which is expressed by macrophages and bone marrow-derived cells of the skin. The response can be minimized by using a slow-release formulation of NA or by using a cyclooxygenase inhibitor (e.g., aspirin, indomethacin) prior to taking NA.[88] High doses of NA have also been reported to cause itching urticaria (hives) and gastrointestinal discomfort (heartburn, nausea, vomiting, and rarely diarrhea) in humans. Animal studies have shown that high levels of NAm can raise circulating homocysteine levels, particularly on a high-methionine diet.

The longer-term effects of high NA doses include insulin resistance, which may involve a rebound in lipolysis that results in increased free fatty acid levels. A few cases of transient elevations in the plasma activities of liver enzymes without associated hepatic dysfunction have been reported and chronic doses of NA have been reported to cause hepatic damage.[89]

Nicotinamide

While acute adverse effects of NAm have not been reported for doses used to treat insulin-dependent diabetes (c. 3 g/day), larger doses (10 g/day) have been found to cause hepatic damage. It is possible that chronic, high intakes of NAm may deplete methyl groups due to the increased demand for methylation to excrete the vitamin. Such effects would be exacerbated by low intakes of methyl donors, methionine and choline, and suboptimal status with respect to folate and/or vitamin B_{12}. NAm can inhibit uricase, depressing intestinal microbial uricolysis, which could lead to uricemia.

12. CASE STUDY

Instructions

Review the following report, paying special attention to the responses to the experimental treatments. Then, answer the questions that follow.

Case

Fourteen patients with alcoholic pellagra and 7 healthy controls, all ranging in age from 21 to 45 years, were studied in the metabolic unit of a hospital. None had severe hepatic dysfunction on the basis of medical history, clinical examination, and routine laboratory tests. The nutritional status of each subject was evaluated at the beginning of the study by clinical examination, anthropometric measurements [body mass index (BMI, weight divided by the square of the height), triceps skinfold thickness, arm and muscle circumference], biochemical tests [24-hr urinary creatinine, serum albumin, total iron-binding capacity (TIBC)], and 24-hr recalls of food consumption. Results indicated that, before admission, the patients with alcoholic pellagra consumed a daily average of 270 g of ethanol. Each showed signs of protein–calorie malnutrition (reduced BMI, skinfold thickness, arm and muscle circumference, serum albumin, and TIBC). In addition, their plasma zinc concentrations were significantly lower than those of controls, although their urinary zinc concentrations were not different from the control group.

The pellagra patients were assigned to one of two experimental treatment groups and the healthy controls to another (three treatments, each with n = 7). During the 7-day study, each group received enteral diets prepared from 10% crystalline amino acids (adequate amounts of each, except

83. Bays, H.E., Rader, D.J., 2009. Int. J. Clin. Pract. 63, 1–11.
84. Westphal, S., Borucki, K., Taneva, E., et al., 2007. Atherosclerosis 193, 361–368.
85. Usman, M.H., Qamar, A., Gadi, R., et al., 2012. Am. J. Med. 125, 1026–1035.
86. Dropout for this reason has been seen in 5–20% of patients.
87. Cyclooxygenase-1.
88. 81 mg.
89. Rader, J.I., Calvert, R.J., Hathcock, J.N., 1992. Am. J. Med. 92, 77–83.

for tryptophan) and 85% sucrose, which supplied daily amounts of 0.8 g of protein per kilogram of body weight and 200 kcal/g N. In addition, each patient was given weekly by vein 500 mL of an essential fatty acid emulsion as well as a vitamin–mineral supplement. The diets were administered by intubation directly to the midportion of the duodenum. The control diet was supplemented with tryptophan and the vitamin–mineral supplement contained both niacin and zinc. The diets provided to each group of pellagra patients contained no tryptophan; neither did their vitamin supplement contain niacin. One group of pellagra patients received supplemental zinc (220 mg of $ZnSO_4$) whereas the other did not. Several biochemical measurements were made at the beginning of the experiment and, again, after 4 days. Each of the biochemical measurements was repeated after 7 days of treatment. In most cases, the results showed the same effects but of greater magnitudes.

Results

Subject Group	Parameter	Initial Value	Day 4 Value
Healthy controls	Plasma Zn (mmol/L)	14.2 ± 1.5	16.0 ± 2.2
	Plasma tryptophan (mmol/L)	50.8 ± 12.5	74.3 ± 18.5
	Urine Zn (mmol/day)	7.34 ± 1.38	9.18 ± 2.91
	Urine 6-pyridone[a] (mmol/day)	70 ± 22	640 ± 235
	Urine CH_3-NAm[b] (mmol/day)	78 ± 32	143 ± 48
Pellagra patients	Plasma Zn (mmol/L)	9.9 ± 1.1	9.6 ± 2.0
	Plasma tryptophan (mmol/L)	33.3 ± 15.3	29.5 ± 6.1
	Urine Zn (mmol/day)	9.79 ± 3.06	11.93 ± 10.55
	Urine 6-pyridone[a] (mmol/day)	16 ± 10	19 ± 12
	Urine CH_3-NAm[b] (mmol/day)	6 ± 3	9 ± 6
Pellagra patients fed Zn	Plasma Zn (mmol/L)	9.8 ± 1.0	15.8 ± 3.2
	Plasma tryptophan (mmol/L)	37.3 ± 17.8	23.7 ± 7.6
	Urine Zn (mmol/day)	9.80 ± 3.10	24.02 ± 8.11
	Urine 6-pyridone[a] (mmol/day)	16 ± 11	55 ± 18
	Urine CH_3-NAm[b] (mmol/day)	6 ± 3	33 ± 20

[a]1-Methyl-6-pyridone-3-carboxamide.
[b]1-Methylnicotinamide.

Case Questions

1. What signs support the diagnosis of protein–calorie malnutrition in these alcoholic patients with pellagra?
2. Propose a hypothesis for the mechanism of action of zinc in producing the responses that were observed in these patients with alcoholic pellagra. Outline an experiment (using either pellagra patients or a suitable animal model) to test that hypothesis.
3. List the probable contributing factors to the pellagra observed in these patients.

13. STUDY QUESTIONS AND EXERCISES

1. Diagram the several general areas of metabolism in which NAD(H)- and NADP(H)-dependent enzymes are involved.
2. In general, how do the pyridine nucleotides interact with the flavoproteins in metabolism? What is the fundamental metabolic significance of this interrelationship?
3. Construct a decision tree for the diagnosis of niacin deficiency in humans or an animal species.
4. What key feature of the chemistry of NAm relates to its biochemical functions as an enzyme cosubstrate?
5. What parameters might you use to assess niacin status of a human or animal?
6. Does niacin meet the definition of a vitamin? Why or why not?

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Chapter 14

Vitamin B_6

Chapter Outline

Anchoring Concepts

1. Vitamin B_6 is the generic descriptor for all 3-hydroxy-2-methylpyridine derivatives exhibiting the biological activity of Pn [3-hydroxy-4,5-*bis*(hydroxymethyl)-2-methylpyridine].
2. The metabolically active form of vitamin B_6 is pyridoxal phosphate, which functions as a coenzyme for reactions involving amino acids.
3. Deficiencies of vitamin B_6 are manifested as dermatologic, circulatory, and neurologic changes.

Had we been able to afford Monel metal or stainless steel cages, we would have missed xanthurenic acid.

Samuel Lepkovsky[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of vitamin B_6.
2. To understand the means of absorption and transport of vitamin B_6.
3. To understand the biochemical function of vitamin B_6 as a coenzyme of different reactions in the metabolism of amino acids, and the relationship of that function to the physiological activities of the vitamin.
4. To understand the physiological implications of low-vitamin B_6 status.

VOCABULARY

Acrodynia
Aldehyde dehydrogenase (NAD+)
Aldehyde oxidase
Alkaline phosphatase
γ-Aminobutyric acid (GABA)
Anthranilic acid
Cheilosis
C-reactive protein
Cystathionine β-synthase
Cystathionine γ-lyase
Cystathioninuria
Decarboxylases
Deoxypyridoxine
Epinephrine
Erythrocyte aspartate aminotransferase (EAAT)
Glossitis
Glycine decarboxylase
Glycogen phosphorylase
Hemoglobin
Homocystinuria
Histamine
Hydrogen sulfide
Hyperoxaluria
Isonicotinic acid hydrazide (INH)
Kynureninase
Methionine load
Norepinephrine

1. Samuel Lepkovsky (1899–1984) was a Polish-born American nutritionist who spent his professional career on the faculty of the University of California at Berkeley. He conducted pioneering work in nutrition, biochemistry, and physiology and is best known for winning the "race for the filtrate factor" by isolating and crystallizing pyridoxine. His observation of a green pigment below the cages of his deficient test animals led to his discovering xanthurenic acid (which reacted with ferric iron on the rusted cage bottoms) in the urine of deficient animals.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00014-9

Phosphorylases
Premenstrual syndrome
Pyridoxal (Pal)
Pyridoxal dehydrogenase
Pyridoxal kinase
Pyridoxal oxidase
Pyridoxal phosphate (PalP)
Pyridoxal phosphate synthase
Pyridoxamine (Pm)
Pyridoxamine phosphate (PmP)
Pyridoxamine phosphate oxidase
4-Pyridoxic acid
Pyridoxine (Pn)
Pyridoxine glycosides
Pyridoxine hydrochloride
Pyridoxol
Racemases
Schiff base
Schizophrenia
Selenocysteine β-lyase
Selenocysteine γ-lyase
Serine hydroxymethyl transferase
Serine palmitoyltransferase
Serotonin
Sickle cell anemia
Sideroblastic anemia
Steroid hormone–receptor complex
Stomatitis
Transaminases
Tryptophan load test
Vitamin B_6-responsive seizures Xanthurenic acid

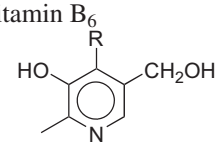
1. THE SIGNIFICANCE OF VITAMIN B_6

The biological functions of the three naturally occurring forms of vitamin B_6, **pyridoxine** (**Pn**), **pyridoxal** (**Pal**), and **pyridoxamine** (**Pm**), depend on the metabolism of each to a common coenzyme form, **pyridoxal phosphate** (**PalP**). That coenzyme plays critical roles in several aspects of metabolism, giving the vitamin importance in such diverse areas as growth, cognitive development, depression, immune function, fatigue, and steroid hormone activity. Vitamin B_6 is fairly widespread in foods of both plant and animal origin; therefore, problems of primary deficiency are not prevalent. Still, vitamin B_6 status can be antagonized by alcohol and other factors that displace the coenzyme from its various enzymes to increase the rate of its metabolic degradation.

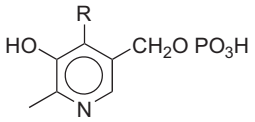
2. PROPERTIES OF VITAMIN B_6

The term **vitamin B_6** describes all 3-hydroxy-2-methylpyridine derivatives exhibiting the biological activity of pyridoxine in the rat. The term **pyridoxine** (**Pn**) is used to designate one of the vitamin B_6-active compounds, 3-hydroxy-4,5-bis(hydroxymethyl)-2-methylpyridine. Derivatives of 3-hydroxy-2-methyl-5-hydroxypyridine can be biologically active if they have a phosphorylatable 5-hydroxymethyl group, and a substituent on the ring-carbon *para* to the pyridine nitrogen that can be converted to an aldehyde. Two such analogs are biologically active: the aldehyde **pyridoxal** (**Pal**) and the amine **pyridoxamine** (**Pm**).

Structures of vitamin B_6



Pyridoxine (Pn): R=CH_2OH
Pyridoxal (Pal): R=CHO
Pyridoxoic acid: R=COOH
Pyridoxamine (Pm): R=CH_2NH_2



Pyridoxal phosphate (PalP): R=CHO
Pyridoxamine phosphate (PmP): R=CH_2NH_2

Vitamin B_6 Chemistry

Vitamers B_6 are colorless crystals at room temperature. Each is very soluble in water, weakly soluble in ethanol, and either insoluble or sparingly soluble in chloroform. Each is fairly stable in dry form and in solution. Pyridoxine is oxidized in vivo and under mild oxidizing conditions in vitro to yield pyridoxal. The prominent feature of the chemical reactivity of pyridoxal is the ability of its aldehyde group to react with primary amino groups (e.g., of amino acids) to form Schiff bases. The electron-withdrawing effect of the resulting Schiff base labilizes the other bonds on the bound carbon, thus serving as the basis of the catalytic roles of pyridoxal and pyridoxamine.

3. SOURCES OF VITAMIN B_6

Hindgut Microbial Synthesis

Vitamin B_6 can be produced by the microbiome of the colon. A genomic analysis of 256 representative organisms of the human gut microbiota found half capable of de novo synthesis of the vitamin.[2] In addition, the majority of genomes (all phyla except Fusobacterial) also showed the capacity to salvage PalP and its precursors. Those findings suggested that hindgut microbial synthesis may produce as much as

2. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.

86% of the daily human need for vitamin B_6, a finding consistent with the fact that primary deficiency of the vitamin is rarely observed. Colonocyte uptake of the vitamin has been demonstrated;[3] however, it is likely that a large portion of the microbially produced vitamin is taken up by nonsynthesizing microbes such that noncoprophagous animals derive little benefit from this source of the vitamin. In contrast, ruminants benefit from their rumen microflora, which produces vitamin B_6 in adequate amounts to meet their needs proximal to where it is absorbed.

Distribution in Foods

Vitamin B_6 is widely distributed in foods, occurring in greatest concentrations in meats, whole grain products (especially wheat), vegetables, and nuts (Table 14.1). In the cereal grains, vitamin B_6 is concentrated primarily in the germ and aleuronic layer. Thus, the refining of grains in the production of flours, which removes much of these fractions, results in substantial reductions in vitamin B_6 content. White bread, therefore, is a poor source of vitamin B_6 unless it is fortified.

The chemical forms of vitamin B_6 tend to vary among foods of plant and animal origin; plant tissues contain mostly Pn (the free alcohol form), whereas animal tissues contain mostly Pal and Pm. A large portion of the vitamin B_6 in many foods is phosphorylated or bound to proteins via the ε-amino groups of lysyl residues or the sulfhydryl groups of cysteinyl residues. The vitamin is also found in glycosylated forms such as 5′-*O*-(β-D-glucopyranosyl) Pn. Vitamin B_6 glycosides are found in varying amounts in different foods but little, if at all, in animal products.

Stability

Vitamin B_6 in foods is stable under acidic conditions but unstable under neutral and alkaline conditions, particularly when exposed to heat or light. Of the several vitamers, Pn is far more stable than either Pal or Pm. Therefore, the cooking and thermal processing losses of vitamin B_6 tend to be highly variable (0–70%), with plant-derived foods (which contain mostly Pn) losing little, if any, of the vitamin and animal products (which contain mostly Pal and Pm) losing substantial amounts. Milk, for example, can lose 30–70% of its inherent vitamin B_6 on drying. The storage losses of naturally occurring vitamin B_6 from many foods and feedstuffs, although they occur at slower rates, can also be substantial (25–50% within a year). Because it is particularly stable, **pyridoxine hydrochloride** is used for food fortification and in multivitamin supplements.

3. Said, Z.M., Subramanian, V.S., Vaziri, N.D., et al., 2008. Am. J. Physiol. Cell Physiol. 294, C1192–C1197.

Bioavailability

The bioavailability of vitamin B_6 in most commonly consumed foods appears to be in the range of 70–80%. However, appreciable amounts of the vitamin in some foods are not biologically available. The determinants of the bioavailability of vitamin B_6 in a food include as follows:

- **Pn glycoside content**—The pyridoxal-5′-β-D-glycosides are poorly digested, being taken up intact, and converted to Pn by a hydrolase in the cytosol. Compared to free Pn, the bioavailabilities of the glycosides have been estimated to be 20–30% in the rat and about 60% in humans. In addition, the presence of Pn glycosides has been found to reduce the utilization of coingested free Pn.
- **Peptide adducts**—Vitamin B_6 can condense with peptide lysyl and/or cysteinyl residues during food processing, cooking, or digestion; such products are less well utilized than the free vitamin. The reductive binding of Pal and Pal 5′-phosphate to e-amino groups of lysyl residues in proteins or peptides produces adducts that are not only biologically unavailable but that also have vitamin B_6-antagonist activity.[4] For example, linatine, the dipeptide of glutamic acid and 1-amino-D-proline in flaxseed, impairs homocysteine metabolism in moderately vitamin B_6-deficient rats.[5] Wheat bran also contains vitamin B_6 in largely unavailable form(s), the presence of which reduces the bioavailability of the vitamin from other foods consumed at the same time.[6] Because plants generally contain complexed forms of Pn, bioavailability of the vitamin of plant foods tends to be greater than that of foods derived from animals.

4. ABSORPTION OF VITAMIN B_6

Digestion of Food Forms

The enteric absorption of vitamin B_6 depends on ingested forms being converted largely to Pm, Pn, and Pal. This involves digestion of the binding proteins in foods followed by metabolism by brush border enzymes:

- **dephosphorylation** of protein-bound PalP and PmP (the major species in animal products) by **alkaline phosphatase** and other phosphatases;
- **deglycosylation** by **lactase–phlorizin hydrolase**.

4. Gregory, J.F., 1980. J. Nutr. 110, 995–1005.
5. Mayengbam, S., Raposo, S., Aliani, M., et al., 2015. J. Nutr. 146, 14–20.
6. Vitamin B_6 is poorly available from the bran fraction of the grain; therefore, the bioavailability of the vitamin from whole wheat bread is less than that of Pn-fortified white bread.

TABLE 14.1 Vitamin B_6 Contents of Foods

Food	Total Vitamin B_6, mg/100g[a]	Vitamin B_6 Vitamers[b]			
		% Glycosylated	% Pn	% Pal	% Pm
Dairy Products					
Milk	0.046–0.05		3	76	21
Yogurt	0.06				
Cheeses	0.01–0.42	4	8	88	
Meats					
Beef	0.13–0.81		16	53	31
Chicken	0.25–0.52	7	74	19	
Lamb	0.13				
Pork	0.28–0.74		8	8	84
Fish					
Haddock	0.33				
Herring	0.17–0.41				
Oysters	0.05–0.10				
Salmon	0.28–0.83		2	9	89
Shrimp	0.10–0.30				
Tuna	0.53		19	69	12
Cereals					
Barley, pearled	0.12		52	42	6
Corn meal	0.30		11	51	38
Oats	0.12		12	49	39
Rice, white	0.09	20	64	19	17
Rice, brown	0.12	23	78	12	10
Wheat, flour, whole	1.30	28	71	16	13
Wheat, white flour	0.04		55	24	21
Vegetables					
Asparagus	0.08				
Beans	0.06–0.20	15–57	62	20	18
Broccoli	0.18	66			
Cabbage	0.12	46	61	31	8
Carrots	0.14	51–86	75	19	6
Cauliflower	0.18	66	16	79	5
Celery	0.07				
Corn	0.09		6	68	26
Onions	0.12				
Peas	0.17	15	47	47	6
Potatoes	0.30	32	68	18	14
Spinach	0.20	50	36	49	15

Continued

TABLE 14.1 Vitamin B_6 Contents of Foods—cont'd

Food	Total Vitamin B_6, mg/100g[a]	Vitamin B_6 Vitamers[b]			
		% Glycosylated	% Pn	% Pal	% Pm
Fruits					
Apples	0.04		61	31	8
Grapefruit	0.04				
Oranges	0.08	47	59	26	15
Peaches	0.03	22	61	30	9
Strawberries	0.05				
Tomatoes	0.08	46	38	29	15
Nuts					
Almonds	0.14				
Pecans	0.19		71	12	17
Walnuts	0.58	7	31	65	4
Other					
Eggs	0.17		0	85	15
Human milk	0.01				

[a]*USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).*
[b]*Leklem, J.E., 1996. In: Ziegler, E.E., Filer, L.J. (Eds.), Present Understanding in Nutrition, seventh ed. ILSI Press, Washington, DC, p. 75; Orr, M.L., 1969. In: Foods: Home Economics Res. Rep. 36. USDA, Washington, DC, 52 pp.*

Diffusion Linked to Phosphorylation

The vitamers Pn, Pal, Pm as well as some Pn glycosides can be absorbed by passive diffusion throughout the gut. For Pn and Pal, the process is driven by the intracellular trapping of the vitamin via the formation of 5′-phosphates through that action of a cytosolic ATP-dependent **Pal kinase**. Intact Pn glycosides taken up by diffusion are later converted to Pn by cytosolic β-glucosidase and then oxidized to PalP.

Uptake by Facilitated Transport

There is also evidence for carrier-mediated absorption of the vitamin.[7]

5. TRANSPORT OF VITAMIN B_6

Plasma Vitamin B_6

Vitamin B_6 is transported primarily in the plasma. Most (>90%) of the circulating vitamin is PalP derived from the hepatic turnover of flavoenzymes. Plasma PalP, typically <1 mmol, comprises a small portion (<0.1%) of total body vitamin B_6. The circulating vitamin is tightly bound to albumin and other plasma proteins via Schiff base linkages.[8]

The vitamin is present in erythrocytes at more than six times the levels in plasma. In erythrocytes, it forms a Schiff base with hemoglobin by binding to the amino group of the N-terminal valine residue of the hemoglobin α-chain. This binding, twice as strong as that to albumin, drives uptake of the vitamin by erythrocytes. Erythrocyte vitamin B_6 levels are particularly high in infants but decline to adult levels by about 5 years of age. PalP content of erythrocytes is often used as a parameter of vitamin B_6 status. In humans and other animals, plasma PalP concentrations decline during pregnancy, as a result of a shift in the distribution of the vitamer in favor of erythrocytes over plasma, as neither the absorption, excretion, or hepatic uptake of the vitamin is affected. Renal failure has been found to reduce the plasma PalP level;[9] whereas, submaximal exercise has been shown to increase it.

7. Said, Z.M., Subramanian, V.S., Vaziri, N.D., et al., 2008. Am. J. Physiol. Cell Physiol. 294, C1192–C1197.

8. Schiff bases are condensation products of aldehydes and ketones with primary amines; they are stable if there is at least one aryl group on either the N or the C that is linked. Vitamin B_6 forms Schiff base linkages with proteins by the bonding of the keto-C of PalP to a peptidyl amino ($-NH_2$) group. The vitamin also forms a Schiff base with the amino acid substrates of the enzymes for which it functions as a coenzyme; this occurs by the bonding of the amino nitrogen of PalP and the α-C of the substrate.

9. One study showed this depression to be >40% in rats.

TABLE 14.2 Concentrations (nM) of Vitamers B_6 in the Plasma of Several Species

Species	Pal	PalP	Pol	Pm	PmP	Pyridoxic Acid
Pig	29	139	167	—	—	139
Human	62	13	33	6	<3	40
Calf	308	96	50	—	9	91
Sheep	626	57	43	—	466	318
Dog	417	268	66	—	65	109
Cat	2443	139	93	44	271	17

Cellular Uptake

Pal crosses cell membranes more readily than PalP. Its preferential uptake by tissues suggests roles of phosphatases in the cellular retention of the vitamin. After being taken into the cell, the vitamin is phosphorylated by Pal kinase to yield the predominant tissue form, PalP. Small quantities of vitamin B_6 are stored, mainly as PalP, but also as **Pm phosphate**.

Tissue Distribution

The total body pool of vitamin B_6 in the human adult is estimated to be 40–150 mg, constituting a supply sufficient to satisfy normal needs for 20–75 days. This amount is composed of two pools: one with a rapid turnover rate (0.5 day) and a second with a longer turnover rate (25–33 days).[10] Muscle contains most (70–80%) of body's vitamin B_6 in the form of Pal 5′-phosphate bound to **glycogen phosphorylase**. Other tissues (liver, brain, kidney, and spleen) (Table 14.2) contain the vitamin bound to other proteins (e.g., glycogen phosphorylase binding accounts for only 10% of the vitamin in liver) with which it has coenzyme functions. Protein binding is thought to protect PalP from hydrolysis while providing storage of the vitamin.

Moderate exercise has been found to increase plasma PalP concentrations substantially, e.g., by >20% within 20 min. This appears to be related to the increased need for gluconeogenesis, which results in the release of PalP from glycogen phosphorylase. The rapidity of this response suggests either that the vitamer rapidly undergoes hydrolysis, discharge from the muscle, and then rephosphorylation in the liver, or that it is released intact through interstitial fluid.[11]

10. Shane, B., 1978. Human Vitamin B_6 Requirements. National Academies Press, Washington, DC, pp. 111–128.
11. Crozier, P.G., Coredain, L., Sampson, D.A., 1994. Am. J. Clin. Nutr. 40, 552–558.

6. METABOLISM OF VITAMIN B_6

Interconversion of Vitamers

The vitamers B_6 are readily interconverted metabolically by phosphorylation/dephosphorylation, oxidation/reduction, and amination/deamination (Fig. 14.1). Because the nonphosphorylated vitamers cross membranes more readily than their phosphorylated analogues, phosphorylation appears to be an important means of retaining the vitamin intracellularly. Several enzymes are involved in this metabolism:

- **Pyridoxal kinase**—This hepatic enzyme catalyzes the phosphorylation of Pn, Pal, and Pm, yielding the corresponding phosphates. It requires a Zn–ATP complex, the formation of which is facilitated by Zn–metallothionein (MT), and is stimulated by K^+. The role of MT in Pal kinase activity suggests that Zn status may be important in the regulation of vitamin B_6 metabolism. Erythrocyte Pal kinase activity in African-Americans has been reported to be about half that of white Americans, although lymphocytes, granulocytes, and fibroblasts show no such differences. This may indicate reduced erythrocyte retention of vitamin B_6, which depends on the phosphorylation. Pyridoxal kinase also binds the antianxiety drug benzodiazepine,[12] suggesting that the mode of drug action may involve enhancement of neuronal γ-aminobutyrate levels.
- **Alkaline phosphatases**—Phosphorylated forms of the vitamin can be dephosphorylated by membrane-bound alkaline phosphatases in many tissues (e.g., liver, brain, and intestine).
- **Pyridoxamine phosphate oxidase**—This enzyme catalyzes the limiting step in vitamin B_6 metabolism. It requires flavin mononucleotide (FMN); therefore,

12. Hanna, M.C., Turner, A.J., Kirkness, E.F., 1997. J. Biol. Chem. 272, 10756–10760.

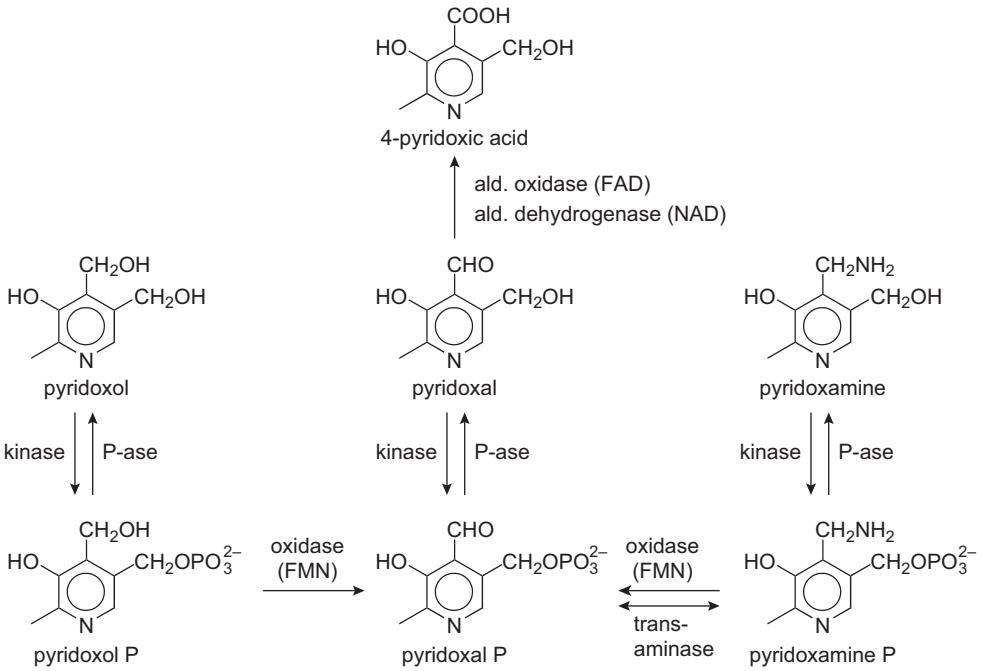


FIGURE 14.1 Metabolism of vitamin B_6.

deprivation of riboflavin may reduce the conversion of Pn and Pm to the active coenzyme PalP.

- **Pyridoxal-5′-phosphate synthase**[13]—This enzyme catalyzes the oxidation of PnP and PalP to PalP.

The liver is the central organ for vitamin B_6 metabolism, containing all of the enzymes involved in its interconversions. The major forms of the vitamin in that organ are PalP and PmP, which are maintained at fairly constant intracellular concentrations in endogenous pools that are not readily accessible to newly formed molecules of those species. The latter, instead, comprise a second pool that is readily mobilized for metabolic conversion (mostly to PalP, Pal, and pyridoxic acid) and release to the blood.

Catabolism

Pyridoxal phosphate is dephosphorylated and oxidized primarily in the liver by the FAD-dependent **aldehyde oxidase** as well as the NAD-dependent **aldehyde dehydrogenase** to yield **4-pyridoxic acid**, the major excretory metabolite. At high intakes, 5-pyridoxic acid is also produced and excreted. Catabolism of the vitamin increases under conditions of systemic inflammation.[14] This is manifest as reductions in PalP levels in several tissues, apparently a consequence of movement of the coenzyme to sites of inflammation where it can function in PalP-dependent enzymes.[15]

Excretion

It has been estimated that humans oxidize 40–60% of ingested vitamin B_6 to 4-pyridoxic acid. In the rat, urinary excretion of 4-pyridoxic acid increases with age in parallel with increases in the hepatic activities of Pal oxidase and Pal dehydrogenase. Small amounts of Pal, Pm, and Pn and their phosphates, as well as the lactone of pyridoxic acid and a ureido–pyridoxyl complex,[16] are also excreted when high doses of the vitamin have been given.[17] Urinary levels of 4-pyridoxic acid are inversely related to protein intake (Table 14.3). This effect appears to be greater for women than for men. However, 4-pyridoxic acid is not detectable in the urine of vitamin B_6-deficient subjects, making it useful in the clinical assessment of vitamin B_6 status.[18,19]

13. i.e., Pyridoxal/pyridoxamine phosphate oxidase.
14. Ulvik, A., Midttun, Ø., Pedersen, E.R., et al., 2014. Am. J. Clin. Nutr. 100, 250–255.
15. Paul, L., Ueland, P.B., Selhub, J., 2014. Nutr. Rev. 71, 239–244.
16. This is formed by the reaction of an amino group of urea with a hydroxyl group of the hemiacetal form of the aldehyde at position 4 of Pal.
17. For example, humans given 100 mg of Pal excrete about 60 mg 4-pyridoxic acid and 2 mg Pal over the next 24 h.
18. In humans, excretion of less than 0.5 mg/day (men) or 0.4 mg/day (women) is considered indicative of inadequate intake of the vitamin. Typical excretion of total vitamin B_6 by adequately nourished humans is 1.2–2.4 mg/day. Of that amount, 0.5–1.2 mg (men) or 0.4–1.1 mg (women) is in the form of 4-pyridoxic acid.
19. Although no explanation has been offered for the correlation, it is of interest that excretion of relatively low amounts (<0.81 mg/24 h) of 4-pyridoxic acid is associated with increased risk of relapse after mastectomy.

TABLE 14.3 Effect of Protein Intake on Vitamin B_6 Status

Dietary Treatment	Intakes		
Protein intake (g/kg)	0.5	1.0	2.0
Vitamin B_6 intake (mg/g protein)	0.04	0.02	0.01
Parameter (adequate value)	% Subjects with low values		
Urinary 4-pyridoxic acid (>3 mmol/day)	11	22	78
Urinary total vitamin B_6 (>0.5 mmol/day)	56	56	67
Plasma PalP (>30 nmol/L)	33	67	78
Urinary xanthurenic acid (<65 mmol/day)	11	11	44

Adapted from Hansen, C.M., Leklem, J.E., Miller, L.T., 1996. J. Nutr. 126, 1891–1901.

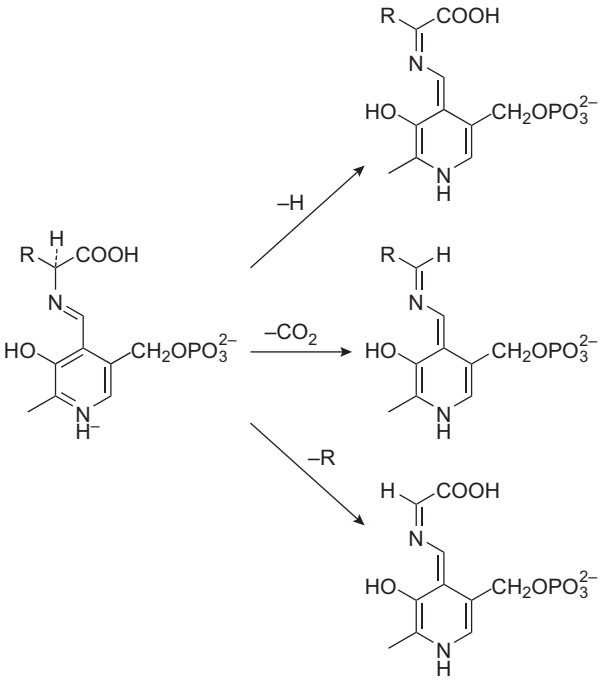


FIGURE 14.2 General reactions of PalP-dependent enzymes in amino acid metabolism.

Effects of Alcohol and Other Drugs

Several drugs can antagonize vitamin B_6. Among these is alcohol; its degradation product, acetaldehyde, displaces PalP from proteins, resulting in enhanced catabolism of the coenzyme. Acetaldehyde also stimulates the activity of alkaline phosphatase, enhancing the dephosphorylation of PalP. The antituberculosis drug **isonicotinic acid hydrazide**[20] (**INH**) also antagonizes vitamin B_6; it does so by binding the vitamin directly. For this reason, vitamin B_6 must be given to patients treated with INH. Pal kinase binds the antianxiety drug benzodiazepine and can be inhibited by the antiasthmatic drug theophylline. Short-term theophylline therapy induces biochemical signs of vitamin B_6 deficiency due to this effect.

7. METABOLIC FUNCTIONS OF VITAMIN B_6

The metabolically active form of vitamin B_6 is PalP. That form serves as a coenzyme of more than 140 enzymes, most of which are involved in the metabolism of amino acids (Fig. 14.2), functioning in the decarboxylations; transamination, racemization, elimination, and replacement reactions; and β-group interconversions. This group of enzymes shows considerable variation in affinity for PalP, some being more sensitive to deprivation of vitamin B_6 than others.

20. Isoniazid.

Mechanisms of Action

Vitamin B_6-dependent enzymes have structural similarities in their coenzyme-binding regions at which PalP or Pm phosphate is bound through the formation of a Schiff base. Accordingly, the mechanisms of the reactions catalyzed by the vitamin B_6-dependent enzymes are also similar. Each involves the binding of an α-carbon of an α-amino acid substrate to the pyridine nitrogen of PalP. The delocalization of the electrons from the α-carbon by the action of the protonated pyridine nitrogen as an electron sink results in the conversion of the former to a carbanion (C^-) at the α-carbon and the labilization of its bonds. This results in the heterolytic cleavage of one of the three bonds to the α-carbon (Table 14.4, Fig. 14.2). The particular bond to be cleaved is determined by the particular PalP-dependent enzyme; each involves the loss of the cationic ligand of an amino acid.

Amino Acid Metabolism

PalP is involved in practically all reactions involved in amino acid metabolism being involved in both their biosynthesis as well as their catabolism:

- **Transaminations**—PalP-dependent transaminases catabolize most amino acids[21]. The response of

21. The only amino acids that are not substrates for PalP-dependent transaminases are threonine, lysine, proline, and hydroxyproline.

TABLE 14.4 Important PalP-Dependent Enzymes of Animals

Type of Reaction	Enzyme
Decarboxylation	Aspartate 1-decarboxylase
	Glutamate decarboxylase
	Ornithine decarboxylase
	Aromatic amino acid decarboxylase
	Histidine decarboxylase
R-group interconversion	Serine hydroxymethyltransferase
	δ-Aminolevulinic acid synthase
Transamination	Aspartate aminotransferase
	Alanine aminotransferase
	γ-Aminobutyrate aminotransferase
	Cysteine aminotransferase
	Tyrosine aminotransferase
	Leucine aminotransferase
	Ornithine aminotransferase
	Glutamine aminotransferase
	Branched-chain amino acid aminotransferase
	Serine–pyruvate aminotransferase
	Aromatic amino acid transferase
	Histidine aminotransferase
Racemization	Cystathionine β-synthase
α,β-Elimination	Serine dehydratase
γ-Elimination	Cystathionine γ-lyase
	Kynureninase

erythrocyte aspartate aminotransferase (**EAAT**) to in vitro additions of PalP has been used as a biochemical maker of vitamin B_6 status.[22]

- **Transsulfuration**—PalP-dependent enzymes **cystathionine β-synthase** and **cystathionine γ-lyase** catalyze the transsulfuration of methionine to cysteine. Vitamin B_6 deprivation, therefore, reduces the activities of these enzymes, affected individuals show **homocystinuria** (due to impaired conversion to cystathionine) and **cystathioninuria** (due to impaired cleavage of cystathionine to cysteine and α-ketobutyrate). These conditions can be exacerbated for diagnostic purposes by the use of an oral methionine load. Plasma homocysteine concentrations, however, usually do not change in vitamin B_6 deficiency and are therefore not suitable for assessment of vitamin B_6 status.
- **Selenoaminoacid metabolism**—Vitamin B_6 is essential for the utilization of selenium (Se) from the major dietary form, selenomethionine, after that Se is transferred to selenohomocysteine. PalP is a cofactor for two enzymes, **selenocysteine ß-lyase** and **selenocysteine γ-lyase**, which catalyze the elimination of the Se from selenohomocysteine to yield hydrogen selenide (H_2Se). Selenide is the obligate precursor for the incorporation of Se into selenoproteins in the form of selenocysteinyl residues produced during translation.[23]

Single-Carbon Metabolism

PalP-dependent enzymes function in single-carbon metabolism (Fig. 14.3):

- **Serine hydroxymethyl transferase (SHMT)**, in both the cytoplasm and mitochondria, catalyzes the interconversion of glycine and serine, providing single-carbon units to the folate single-carbon pool;
- **Glycine decarboxylase** catalyzes the transfer of single-carbon units to/from tetrahydrofolate, working with SHMT feed the folate single-carbon pool in the form of 5,10-methylenetetrahydrofolate;
- **Cystathionine β-synthetase** catalyzes this first step in the transsulfuration pathway, the addition of serine and homocysteine to yield cystathionine; it is stimulated by S-adenosylmethionine and is minimally affected by vitamin B_6 deprivation;
- **Cystathionine γ-lyase** catalyzes the second step in the transsulfuration pathway, the cleavage of cystathionine to yield cysteine.

Niacin Synthesis

Vitamin B_6 is an essential cofactor for two key enzymes in the synthesis of the vitamin niacin from the indispensable amino acid tryptophan (Fig. 14.4):

- **Kynureninase**—This enzyme catalyzes the removal of an alanyl residue from 3-hydroxykynurenine in the metabolism of tryptophan to the branch point intermediate α-amino-β-carboxymuconic-ε-semialdehyde in the

22. However, EAAT activity coefficients can be affected by factors unrelated to vitamin B_6 status (e.g., intake of protein and alcohol, differences in body protein turnover, certain drugs, genetic polymorphism of the enzyme), which can compromise its use without careful controls.

23. These include the Se-dependent glutathione peroxidases and thioredoxin reductases, which have antioxidant functions; the iodothyronine 5′-deiodinases, which are involved in thyroid hormone metabolism; selenophosphate synthase, which is involved in selenoprotein synthesis; selenoproteins P and W, which are major selenoproteins in plasma and muscle, respectively; and at least a dozen other proteins.

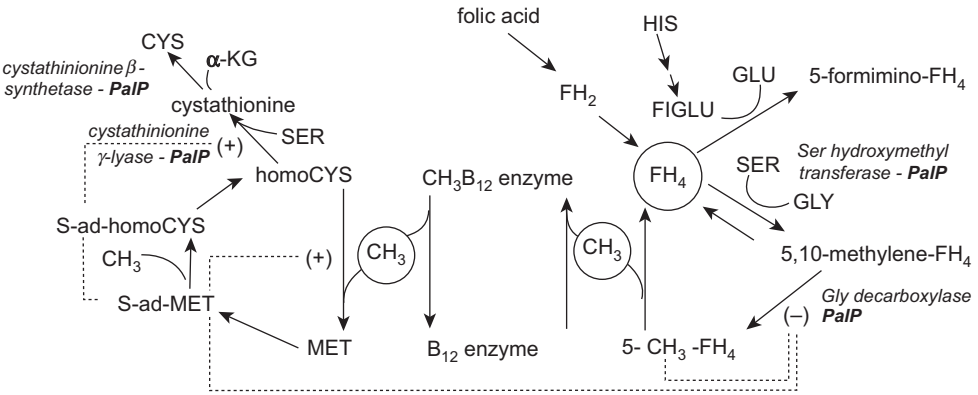


FIGURE 14.3 PalP-dependent enzymes (labeled) in single-carbon metabolism.

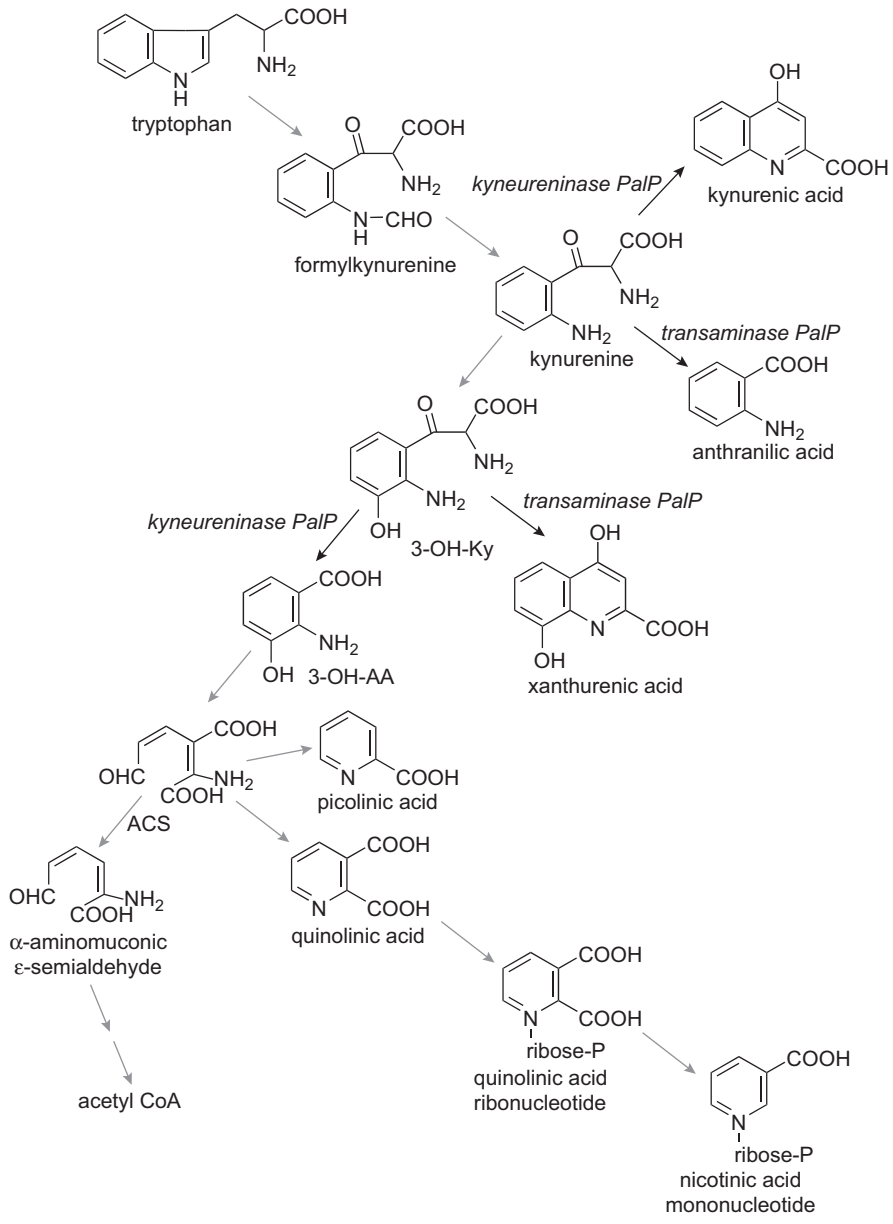


FIGURE 14.4 PalP-dependent enzymes (labeled) in the conversion of tryptophan to NAD.

tryptophan–niacin conversion pathway. Kynureninase also catalyzes the analogous reaction (removal of alanine) using nonhydroxylated kynurenine as substrate and yielding the nonhydroxylated analog of 3-hydroxykynurenine, anthranilic acid.

- **Transaminases**—Vitamin B_6-dependent transaminases metabolize kynurenine and 3-hydroxykynurenine, yielding kynurenic and xanthurenic acids, respectively. The transaminases have much greater binding affinities for PalP than kynureninase[24] and are, therefore, affected preferentially by vitamin B_6 deprivation. This results in blockage in the tryptophan–niacin pathway, with an accumulation of 3-hydroxykynurenine that gets diverted by transamination to yield xanthurenic acid, which appears in the urine.[25] This phenomenon is exploited in the assessment of vitamin B_6 status: deficiency is indicated by urinary excretion of xanthurenic acid after a tryptophan load.

Gluconeogenesis

Vitamin B_6 has two roles in gluconeogenesis:

- **Transaminations**—Amino acid catabolism depends on PalP is a cofactor for transaminases (see "amino acid metabolism" above).
- **Glycogen utilization**—PalP is required as a coenzyme of **glycogen phosphorylase**. Unlike its role in other PalP enzymes, it is the phosphate group of the coenzyme that is catalytically important. It participates in the transfer of inorganic phosphate to the glucose units of glycogen to produce glucose-1-phosphate, which is released. The shift of the enzyme from its inactive to active forms involves an increase in the binding (2–4 moles per mole of enzyme) of PalP. This accounts for more than half of the vitamin B_6 in the body, owing to the abundance of both muscle and glycogen phosphorylase (5% of soluble muscle protein).

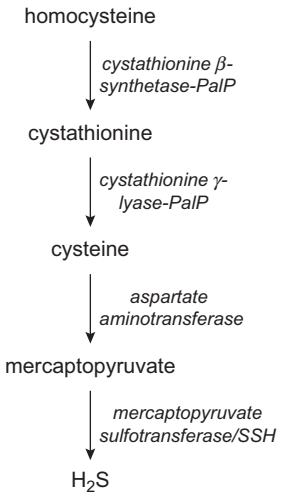


FIGURE 14.5 Role of PalP in the biogenesis of H_2S.

Hydrogen Sulfide Biogenesis

The endogenous mediator, hydrogen sulfide (H_2S), is produced in the heart, kidney, lungs, and the reproductive and central nervous systems. This is accomplished primarily by the transsulfuration pathway, i.e., the PalP enzymes, cystathionine β-synthetase and cystathionine γ-lyase,[26] plus a non-PalP enzyme, 3-mercaptopyruvate sulfotransferase, which can catalyze the desulfuration of cysteine or homocysteine to generate sulfane-S, subsequently reduced to H_2S (Fig. 14.5).[27] Through a process still poorly understood, H_2S is maintained at low, steady-state levels. It functions in paracrine intracellular signaling in the regulation of vascular tone, apoptosis, inflammation, and cellular stress responses. Patients with coronary heart disease have been found to produce significantly lower levels of H_2S than healthy controls, suggesting a role in vascular disease. It appears to exert these effects by regulating Ca^{2+} channels.[28]

Neurologic Function

Vitamin B_6 has a key role in the synthesis of the neurotransmitters: dopamine, norepinephrine, serotonin, and **γ-aminobutyric acid (GABA)**, as well as sphingolipids and polyamines. PalP-dependent enzymes function in the biosynthesis of neurotransmitters: tryptophan decarboxylase and aromatic L-amino acid decarboxylase in the synthesis of serotonin; tyrosine carboxylase in the synthesis of **epinephrine** and **norepinephrine**; glutamate decarboxylase serves in regulating turnover of a major source

24. The Michaelis constants (K_m's) for the transaminases are on the order of 10^{-8} M; whereas the K_m for kynureninase is on the order of 10^{-3} M.

25. Xanthurenic acid was discovered unexpectedly by Lepkovsky (University of California), who during the Great Depression sought to elucidate the nature of rat **adermin**. He wrote of his surprise in finding that the urine voided by his adermin-deficient rats was green, whereas that of his controls were the normal yellow color. In pursuing this observation, he found that urine from deficient animals was normally colored when voided but turned green only on exposure to the rusty dropping pans their limited budget had forced them to use. Thus, he recognized that adermin-deficient rats excreted a metabolite that reacted with Fe^{3+} to form a green derivative. This small event, which might have been missed by someone "too busy" to observe the experimental animals, resulted in Lepkovsky's identifying the metabolite as xanthurenic acid and discovering the role of vitamin B_6 in the tryptophan–niacin conversion pathway. His message: "The investigator has to do more than sit at his desk, outline experiments and examine data."

26. The PalP-dependent aspartate aminotransferase, which can have cysteine aminotransferase activity, may also be involved.

27. Kabil, O., Banerjee, R., 2014. Antioxid. Redox. Signal. 20, 770–782.

28. Tan, H.H., Wong, P.T.H., Bian, J.S., 2010. Neurochem. Int. 56, 3–10.

of energy for the brain, GABA. The apoenzymes involved in these various steps have widely different affinities for PalP. Therefore, vitamin B_6 deprivation affects preferentially those decarboxylases with low affinities for the coenzyme. Accordingly, moderate deficiency of vitamin B_6 reduces brain serotonin levels without affecting other neurotransmitters.

Animal studies of long-term potentiation, a synaptic model of learning and memory, have revealed that maternal deprivation of the vitamin during gestation and lactation specifically reduces the development of the *N*-methyl-D-aspartate receptor subtype in the young. Although the metabolic basis is not understood, these effects appear to be related to the loss of dendritic arborization in vitamin B_6 deficiency. These lesions are thought to underlie reported effects of impaired learning on the part of the progeny of vitamin B_6-deficient animals and humans.

Histamine Synthesis

PalP functions in the metabolism of the vasodilator and gastric secretagogue histamine as a cofactor for histidine decarboxylase.

Hemoglobin Synthesis and Function

PalP functions in the synthesis of heme from porphyrin precursors as a cofactor for δ-aminolevulinic acid synthase. The vitamin also binds to hemoglobin at two sites on the β chains (the N-terminal valine and Lys-82 residues) and the N-terminal valine residues of the α chains. Binding of Pal or PalP at these sites enhances the O_2-binding capacity of the protein and inhibits the physical deformation of sickle cell hemoglobin.

Lipid Metabolism

Vitamin B_6 is required for the biosynthesis of sphingolipids via the PalP-dependent **serine palmitoyltransferase** and other enzymes in phospholipid synthesis. Diminution in the activities of these enzymes is thought to account for the changes observed in phospholipid contents of linoleic and arachidonic acids in vitamin B_6-deficient animals.

Antioxidant Function

Studies with cell systems have demonstrated antioxidant properties of B_6 vitamers. Pn, Pm, and PalP have been shown to reduce the production of superoxide (O_2^-) and lipid peroxides in response to prooxidative conditions.[29] These effects are thought to be due to the high reactivity of the vitamin with hydroxyl radicals, which preferentially abstract hydrogen atom from either of the vitamin's methanolic carbons (C8 or C9). These reactions can include additions and cyclizations such that the vitamin can be capable of high antioxidant activity by scavenging up to eight hydroxyl radicals.[30]

Cardiovascular Function

Vascular function. That vitamin B_6 plays a role in normal vascular function is evidenced by the fact that low-vitamin B_6 status has been associated with increased risk to coronary artery disease.[31] This relationship has been linked to altered platelet aggregation due to reduced Ca^{+2} influx caused by impaired adenosine-5′-diphosphate receptors and to increased chronic inflammation marked by elevated plasma levels of C-reactive protein.[32] Studies in a rat model found Pm to prevent age-related aortic stiffening and vascular resistance by reducing the formation of collagen cross-linking induced by advanced glycation end products.[33] Such effects would not appear to involve vitamin B_6-dependent enzymes; but, instead, the antioxidant capacity of Pm is to scavenge carbonyls and prevent glycation reactions.[34]

Hypertension. Deprivation of vitamin B_6 has been shown to produce moderate hypertension in the rat. These effects were associated with elevations in plasma levels of epinephrine and norepinephrine, and reduced levels of serotonin in the brain and 5-hydroxytryptophan in nerves.[35] Those observations, and the rapid reversibility of hypertension by vitamin B_6 supplementation, suggest that the condition results from impaired neurotransmitter regulation.

Homocysteinemia. Low-vitamin B_6 status can also cause homocysteinemia as a result of diminished conversion to cystathionine due to impaired activities of the PalP-dependent enzyme cystathionine β-synthase. Homocysteinemia has been associated with increased risks to occlusive vascular disease, total and cardiovascular disease-related mortality, stroke, and chronic heart failure.[36] Low-plasma PalP levels have also been associated with increased risk to vascular disease independent of plasma

29. Jain, S.K., Lim, G., 2001. Free Radic. Biol. Med. 30, 232–237; Mahfouz M.M., Zhou, S.Q., Kummerow, F.A., 2009. Int. J. Vitam. Nutr. Res. 79, 218–229.

30. Matxain, J.M., Padro, D., Ristilä, M., et al., 2009. J. Phys. Chem. B. 113, 9629–9632.

31. Robinson, K., Arheart, D., Refsum, H., 1998. Circulation 97, 437–443.

32. Morris, M.S., Sakakeeny, L., Jacques, P.F., et al., 2010. J. Nutr. 140, 103–110.

33. Wu, E.T., Liang, J.T., Wu, M.S., et al., 2011. Exp. Gerontol. 46, 482–488.

34. Voziyan, P.A., Hodson, B.G., 2005. Cell. Mol. Life Sci. 62, 1671–1681.

35. Viswanathan, M., Paulose, C.S., Lal, K.J., et al., 1990. Neurosci. Lett. 111, 201–205.

36. Selhub, J., 2006. J. Nutr. 136, 1726S–1730S.

TABLE 14.5 Effects of Vitamin B_6 Status on Mitogenic Responses and Interleukin 2 Production by Peripheral Blood Mononucleocytes of Elderly Humans

Parameter	Baseline	B_6-Deprived	B_6-Supplemented
Mitogenic Response to			
Concanavalin A	120	70	190
Phytohemagglutinin	100	70	100
Staphylococcus aureus	115	60	200
IL-2 production (kU/liter)	105	40	145

Adapted from Meydani, S.N., Ribaya-Mercado, J.D., Russell, R.M., et al., 1991. Am. J. Clin. Nutr. 53, 1275–1280.

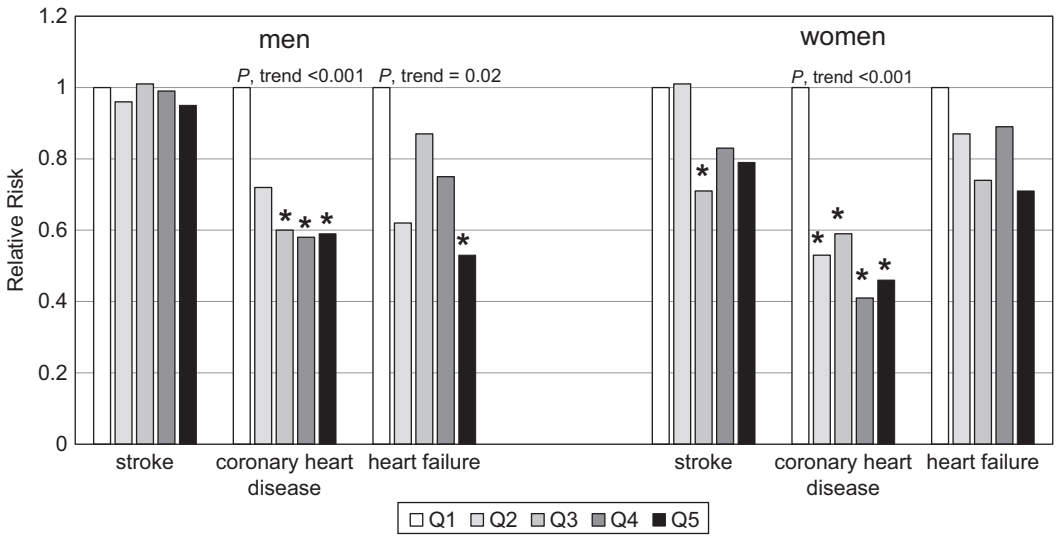


FIGURE 14.6 Cardiovascular Disease Risk by quintile of plasma PalP concentration. *Adapted from Cui, R., Iso, H., Date, C., et al., 2010. Stroke 41, 1285–1289.*

homocysteine level.[37] High doses of vitamin B_6 and folic acid have been found effective in reducing both plasma homocysteine and the incidence of abnormal exercise electrocardiography tests, suggesting reductions in risk of atherosclerotic disease.[38] However, a meta-analysis of 12 randomized trials of vitamin (folate, vitamin B_6, vitamin B_{12}) supplements to lower homocysteine levels in free-living people found no evidence for vitamin B_6 supplements being effective.[39]

Cardiovascular disease. Dietary intakes of vitamin B_6 and plasma levels of PalP have been found to be inversely associated with risk of stroke, coronary heart disease, and heart failure (Table 14.5). A review of six clinical trials with a total of some 8500 chronic kidney disease patients found interventions with vitamin B_6 combined with folate and vitamin B_{12} not to affect risk of cardiovascular mortality (Fig. 14.6).[40]

Immune Function

Vitamin B_6 has a role in the support of immune competence that has not been elucidated. Animal and human studies have demonstrated effects of vitamin B_6 deprivation on both humoral (diminished antibody production) and cell-mediated immune responses (increased lymphocyte proliferation, reduced delayed-type hypersensitivity responses, reduced

37. Robinson, K., Arheart, K., Refsum, H., et al., 1998. Circulation 97, 437–443.

38. Vermuelen, E.G.J., Stehouwer, C.D., Twisk, J.W., et al., 2000 Lancet 355, 517–522.

39. Folic acid produced an average 25% reduction, and vitamin B_{12} produced an average 7% reduction (Clarke, R., Armitage, J., 2000. Semin. Thromb. Hemost. 26, 341–348.).

40. Nursalim, A., Siregar, P., Widyahening, I.S., 2013. Acta Med. Indones. 45, 150–156.

TABLE 14.6 Congenital Disorders of Vitamin B_6-Dependent Metabolism

Disorder	Enzyme Deficiency	Clinical Manifestations
Homocysteinuria[a]	Cystathionine β-synthase	Dislocation of lenses, thromboses, malformation of skeletal and connective tissue, mental retardation
Cystathioninuria	Cystathionine γ-lyase	Mental retardation
GABA deficiency	Glutamate decarboxylase	Seizures
Sideroblastic anemia	δ-Aminolevulinic acid synthase	Anemia, cystathioninuria, xanthurenic aciduria

[a]*Another form is caused by impaired vitamin B_{12}-dependent methionine synthesis.*

T cell-mediated cytotoxicity, reduced cytokine production). Suboptimal status of the vitamin has been linked to declining immunologic changes among the elderly (Table 14.6), persons with human immunodeficiency virus (HIV), and patients with uremia or rheumatoid arthritis. These effects appear to be due to reduced activities of such PalP enzymes as serine transhydroxymethylase and thymidylate synthase, resulting in impaired single-carbon metabolism and reduced DNA synthesis. The vitamin has also been shown to bind to the α-glycoprotein surface receptor (CD4) on a T-helper cells by which it affects photoimmunosuppression.[41] It has also been shown to noncompetitively inhibit HIV-1 reverse transcriptase.[42]

Gene Expression

PalP has been shown to modulate gene expression. Elevated intracellular levels of the vitamin are associated with decreased transcription in responses to glucocorticoid hormones (progesterone, androgens, estrogens). Such diminished responses include hydrocortisone induction of rat liver cytosolic aspartate aminotransferase. Inhibition is caused by the formation of Schiff base linkages of the vitamin to the DNA-binding site of the receptor–steroid complex. This inhibits the ligand binding to the glucocorticoid-responsive element in the regulatory region of the gene. Vitamin B_6 deficiency increases the expression of albumin mRNA sevenfold. The effect appears due to the action of PalP inactivating tissue-specific transcription factors by directly interacting with DNA ligand-binding sites.[43] PalP appears to modulate glycoprotein IIb gene expression by interacting directly to with tissue-specific transcription factors.[44] This results in inhibition of platelet aggregation due to impaired binding of fibrinogen or other adhesion proteins to glycoprotein complexes. PalP has also been shown to suppress mRNA levels for glycogen phosphorylase, apolipoprotein A-1, phenylalanine hydroxylase, glyceraldehyde-3-phosphate dehydrogenase, and β-actin but to decrease mRNA levels for RNA polymerases I and II in the rat model.[45] The effect on glycogen phosphorylase appears to be tissue-specific, as deprivation of the vitamin was found to reduce phosphorylase mRNA levels in muscle but to increase them in liver.[46]

Congenital Disorders of Vitamin B_6 Metabolism

Several rare familial disorders have been identified, each thought to be caused by the expression of deficient amounts or dysfunctional forms of PalP-dependent enzyme (Table 14.7).

- **Homocystinuria** occurs due to a rare (three cases per million), hereditary deficiency of cystathionine β-synthase. The impaired homocysteine catabolism is manifest as elevations in plasma levels of homocysteine, methionine, and cysteine with dislocation of the optic lens,[47] osteoporosis and abnormalities of long bone growth, mental retardation, and thromboembolism. The condition is treated with a low-methionine diet. Half of cases respond to high doses (250–500 mg/day) of Pn.[48] Of more than a hundred alleles that have been studied, mutations of the cystathionine β-synthase associated with disease phenotypes have been found in almost one-third.[49] Some mutations, including some of the most frequent ones in the

41. Salhany, J.M., Schopfer, L.M., 1993. J. Biol. Chem. 268, 7643–7645.
42. Mitchell, L.L.W., Cooperman, B.S., 1992. Biochemistry 31, 7707–7713.
43. *See review*: Oka, T., 2001. Nutr. Res. Rev. 14, 257–266.
44. Chang, S.J., Chuang, H.J., Chen, H.H., 1999. J. Nutr. Sci. Vitaminol. 45, 471–479.
45. Oka, T., Komori, N., Kuwahata, M., et al., 1993. FEBS Lett. 331, 162–164.
46. Oka, T., Komori, N., Kuwahata, M., et al., 1994. Experientia 50, 127–129.
47. *Ectopia lentis*
48. Berber, G., Spaeth, G., 1969. J. Pediatr. 75, 463–478.
49. Kraus, J.P., Janosik, M., Kozich, V., et al., 1999. Hum. Mutat. 13, 362–375.

TABLE 14.7 Effects of Nonsteroidal Antiinflammatory Drugs (NSAIDs) on Biomarkers of Vitamin B_6 Status in Rheumatoid Arthritis Patients

Biomarker	Control	NSAIDs, ≤ 6 Months	NSAIDS, > 6 Months
Plasma PalP, nM	42.3 (39.6, 73.8)[a]	35.1 (34.5, 64.9)[a]	29.1 (33.9, 44.9)[a,b]
Plasma Pal, nM	15.3 (12.3, 19.8)	17.1 (16.3, 33.7)	15.9 (15.9, 26.1)
Plasma homocysteine, μM	6.67 (6.50, 8.74)	6.45 (6.156, 8.19)	6.48 (6.47, 7.62)

[a]*Mean (95% C.L.).*
[b]*Significantly different from control* ($p < .05$).
Adapted from Chang, H.Y., Tang, F.Y., Chen, D.Y., et al., 2013. Am. J. Clin. Nutr. 98, 1440–1449.

human populations studied to date,[50] have been shown to correlate with Pn responsiveness; these would appear to involve the expression of a mutant enzyme with low affinity for PalP.

- **Vitamin B_6-responsive seizures**[51] have been reported.[52] These are manifest as intractable seizures appearing within hours after birth; they are resistant to antiepileptic drugs but stop immediately upon intravenous administration of high levels (100–500 mg) of PalP and are controlled with daily oral doses of PalP (10–30 mg/kg body weight).[53] If untreated, progressive cerebral atrophy ensues. Two types of recessive traits appear to underlie vitamin B_6-responsive seizures. One group involves mutations in the *ALDH7A1* gene, which encodes for antiquitin, an enzyme found in both the cytosol and mitochondria that functions as an aldehyde dehydrogenase in the catabolism of lysine. Deficient expression of antiquitin is characterized by marked elevations in intracellular concentrations of α-amino adipic semialdehyde, piperidine-6-carboxylate, and pipecolic acid, which inhibit the uptake of GABA.[54] The other group involves mutations in Pn/Pm oxidase;[55] these respond only to PalP.
- **Hyperoxaluaria** (Type I) is due to a variant hepatic alanine glyoxylate transferase with abnormally low PalP-binding capacity. High oral doses of vitamin B_6 (e.g., 400 mg/day) reduce hyperoxaluria in some 30% of patients, reducing the risk of formation of oxalate stones and renal injury.[56]

TABLE 14.8 Signs of Vitamin B_6 Deficiency

Organ System	Signs
General	Reduced appetite, growth
Dermatologic	Acrodynia, cheilosis, stomatitis, glossitis, seborrheic and scaling dermatitis (around nose)
Muscular	Weakness
Skeletal	Dental caries
Vital organs	Hepatic steatosis
Vascular	Arteriosclerosis, anemia
Nervous	Paralysis, convulsions, peripheral neuropathy
Reproductive	Decreased egg production; fetal malformations, death

8. BIOMARKERS OF VITAMIN B_6 STATUS

Vitamin B_6 status can be assessed in several ways:[57]

- **Blood metabolites**—The most common means of assessing long-term vitamin B_6 status is the measurement of PalP in plasma. This can be accomplished by direct analysis using HPLC or was formerly done using a tyrosine decarboxylase assay. Plasma PalP concentrations ≥30 nM are considered adequate; those of 20–30 nM are generally considered as marginal, i.e., carrying risk of metabolic dysfunction. These measurements are sensitive to the effects of pregnancy, sex, exercise, age, NSAID (nonsteroidal antiinflammatory drug)[58] use (Table 14.8), smoking, and alcohol consumption. Plasma total vitamin B_6 and erythrocyte PalP concentrations have also been used.

50. Such studies have been conducted only in Europe; no information is available for other populations.
51. Also referred to as pyridoxine-dependent epilepsy.
52. Vitamin B_6 has also been recommended (0.1–1 g/day alone or in combination with tryptophan or magnesium) for reducing seizures in alcoholics and for the treatment of schizophrenia.
53. Gupta, V., Mishra, D., Mathur, I., et al., 2001. J. Pediatr. Child Health 37, 592–596; Gospe, Jr., S.M., 2002. Pediatr. Neurol. 26, 181–185.
54. Jagadeesh, S., Surech, B., Murugan, V., et al., 2013. Paediatr. Int. Child Health 33, 113–115.
55. Gospe, Jr., S.M., 2006. Curr. Opin. Neurol. 19, 148–153.
56. Bhasin, B., Ürekli, H.M., Atta, M.G., 2015. World Rev. Nephrol. 4, 235–244.
57. A microbiological assay employing *Lactobacillus plantarum* is commonly used for the analysis of pantothenic acid. This requires enzymatic pretreatment of specimens to liberate free pantothenic acid.
58. i.e., Nonsteroidal antiinflammatory drugs, such as celecoxib and naproxen.

- **Urinary metabolites**—The urinary excretion of total vitamin B_6, or 4-pyridoxic acid, which comprises half of total vitamin B_6 intake, has been used. The latter is affected by short-term intake as well as tissue stores of the vitamin.
- **Load tests**—Vitamin B_6 function can be assessed by measuring concentrations of downstream metabolites in pathways safely perturbed using large oral doses of key upstream metabolites:
  - **Urinary xanthurenic acid after a tryptophan load**—Because the affinity of kynureninase for PalP is much lower than those of the PalP-dependent transaminases, vitamin B_6 deficiency results in increased production and urinary excretion of xanthurenic acid, which effect is amplified after an oral bolus (2 g) of tryptophan. These results can be affected by gender, estrogens, glucocorticoids, and pregnancy and protein intake, which upregulate tryptophan-2,3-dioxygenase, the rate-limiting enzyme in tryptophan metabolism.
  - **Plasma homocysteine after a methionine load**—Because cystathionine γ-lyase is very sensitive to inadequate PalP supply, plasma homocysteine concentration after an oral bolus (3 g) of methionine, which suppresses homocysteine remethylation, can indicate vitamin B_6 status.
- **Degree of PalP saturation of PalP-dependent enzymes**—Vitamin B_6 status can be assessed by taking advantage of the in vitro binding of PalP by alanine aminotransferase or aspartic aminotransferase from hemolysates. Stimulation of either of these activities upon the addition of exogenous PalP indicates inadequate vitamin B_6 status.

TABLE 14.9 Recommended Vitamin B_6 Intakes

The United States		FAO/WHO	
Age/Sex	RDA[a], µg/day	Age/Sex	RNI[b], µg/day
0–6 months	[0.1][c]	0–6 months	0.1
7–11 months	[0.3][c]	7–11 months	0.3
1–3 year	0.5	1–3 year	0.5
4–8 year	0.6	4–6 year	0.6
9–13 year	1.0	7–9 year	1.0
14–18 year		10–18 year	
females	1.2	females	1.2
Males	1.3	Males	1.3
19–59 year		19–50 year	1.3
females	1.1		
Males	1.3		
>50 year		>50 year	
females	1.5	females	1.5
Males	1.7	Males	1.7
Pregnancy	1.9	Pregnancy	1.9
Lactation	2.0	Lactation	2.0

[a]Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.
[b]Recommended Nutrient Intakes, Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements, WHO, Rome, 286 pp.
[c]RDA has not been set; Adequate Intake (AI) is given.

9. VITAMIN B_6 DEFICIENCY

Severe deficiency of vitamin B_6 results in dermatologic and neurologic changes in most species (Table 14.9). Less obvious are the metabolic lesions associated with insufficient activities of the coenzyme PalP. The latter include impaired tryptophan–niacin conversion and impaired transsulfuration of methionine to cysteine. Deprivation of vitamin B_6 also impairs glucose tolerance, due to reduced activities of PalP-dependent transaminases and glycogen phosphorylase, although it may not affect fasting glucose levels. Recommended intakes of vitamin B_6 have been established for humans (Table 14.10).

Deficiency Signs in Humans

Clinical deficiency. Clinical vitamin B_6 deficiency is not common. Vitamin B_6-deficient humans exhibit symptoms that can be quickly corrected by administration of the vitamin: weakness, sleeplessness, nervous disorders (peripheral neuropathies), **cheilosis**,[59] **glossitis**,[60] **stomatitis**, and impaired cell-mediated immunity.[61] Behavioral differences have been associated with low-vitamin B_6 status: a study in Egypt found that mothers of marginal (subclinical) vitamin B_6 status were less responsive to their infants' vocalizations, showed less effective response to infant distress, and were more likely to use older siblings as caregivers than were mothers of better vitamin B_6 status. In addition, studies with volunteers fed a vitamin B_6-free diet or a vitamin B_6 antagonist[62] have shown elevated urinary xanthurenic acid concentrations[63] and increased susceptibility to infection. Because plasma concentrations of PalP decrease with

59. The lesion is morphologically indistinguishable from that produced by riboflavin deficiency.
60. The lesion is morphologically indistinguishable from that produced by niacin deficiency.
61. These can be produced by the antagonist deoxypyridoxine.
62. For example, 4´-deoxyPn.
63. After tryptophan loading, vitamin B_6-deficient subjects also had elevated urinary concentrations of kynurenine, 3-hydroxykynurenine, kynurenic acid, acetylkynurenine, and quinolinic acid.

TABLE 14.10 Relationship of Vitamin B_6 Status and Inflammatory Status in Humans

Characteristic	Tertile of Plasma PalP, nM			*p*, Trend
	35 (34, 36)[a]	69 (67, 71)	177 (173, 181)	
Vitamin B_6 intake, g/day	2.7 (0.9, 4.5)[a]	5.2 (3.4, 6.9)	18.6 (16.8, 20.3)	<.001
C-reactive protein, mg/L	3.1 (2.9, 3.4)[a]	2.1 (1.9, 2.3)	1.8 (1.6, 1.9)	<.001

[a]*Mean (95% C.L.).*
Adapted from Sakakeeny, L., Roubenoff, R., Oben, M., et al., 2012. J. Nutr. 142, 1280–1285.

age, it is expected that elderly people may be at greater risk of vitamin B_6 deficiency than younger people.

Subclinical deficiency. Low-vitamin B_6 status is associated with inflammation as indicated by increased circulating levels of C-reactive protein (Table 14.10)[64] and is observed in chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease. Low-vitamin B_6 status has also been associated with an increase in the ratio of n-6:n-3 polyunsaturated fatty acids in plasma, a feature associated with increased risk of inflammation.[65] It has been suggested that the reduction in circulating PalP reflects the mobilization of that vitamer to the sites of inflammation where is functions in PalP-dependent enzymes.[66] High-vitamin B_6 intakes are associated with protection against inflammation.[67] Low circulating PalP levels have been reported in patients with asthma, and one small study found vitamin B_6 treatment (100 mg/day) to reduce the severity and frequency of attacks.[68] These effects may be secondary to those of theophylline, which inhibits Pal kinase.

Deficiency Signs in Animals

Vitamin B_6 deficiency in animals is generally manifest as symmetrical scaling dermatitis. In rodents, the condition is called **acrodynia** and is characterized by hyperkeratotic[69] and acanthotic[70] lesions on the tail, paws, face, and upper thorax, as well as by muscular weakness, hyperirritability, anemia, hepatic steatosis,[71] increased urinary oxalate excretion, insulin insufficiency,[72] hypertension, and poor growth. Neurological signs include convulsive seizures (epileptic form) that can be fatal.[73] Reproductive disorders include infertility, fetal malformations,[74] and reduced fetal survival. Some reports indicate effects on blood cholesterol levels and immunity. That tissue carnitine levels are depressed in vitamin B_6-deficient animals has been cited as evidence of a role of the vitamin in carnitine synthesis.

Similar changes are observed in vitamin B_6-deficient individuals of other species. Chickens and turkeys show reduced appetite and poor growth, dermatitis, marked anemia, convulsions, reduced egg production, and low fertility. Pigs show paralysis of the hind limbs, dermatitis, reduced feed intake, and poor growth. Monkeys show an increased incidence of dental caries and altered cholesterol metabolism with arteriosclerotic lesions. Vitamin B_6 deficiency has been reported to cause hyperirritability, hyperactivity, abnormal behavior, and performance deficits in several species. These signs accompany an underlying neuropathology that reduces axonal diameter and dendritic arborization and, thus, impairs nerve conduction velocity.

Ruminants are rarely affected by vitamin B_6 deficiency, as their rumen microflora appears to satisfy their needs for the vitamin. Exceptions are lambs and calves, which, before their rumen microflorae are established, are susceptible to dietary deprivation of vitamin B_6, showing many dermatologic and neurologic changes observed in nonruminant species.

10. VITAMIN B_6 IN HEALTH AND DISEASE

Anticarcinogenesis

Epidemiological studies have yielded inconsistent findings regarding the association of vitamin B_6 intake and cancer risk; however, they have consistently found individuals with higher plasma PalP levels to be 30–50% less likely to develop colorectal cancer risk than individuals with lower plasma PalP levels.[75] Studies in animal models

64. Morris, M.A., Picciano, M.F., Jacques, P.F., et al., 2008. Am. J. Clin. Nutr. 87, 1446–1454; Sakakeeny, L., Roubenoff, R., Oben, M., et al., 2012. J. Nutr. 142, 1280–1285.
65. Zhao, M., Lamers, Y., Ralat, M.A., et al., 2012. J. Nutr. 142, 1791–1797.
66. Lotto, V., Choi, S.W., Friso, S., 2011. Br. J. Nutr. 106, 183–195.
67. Morris, M.S., Sakakeeny, L., Jacques, P.F., et al., 2010. J. Nutr. 140, 103–110.
68. Simon, R.A., Reynolds, R.D., 1988. In: Leklem, J.E., Reynods, R.D. (Eds.), Clinical and Physiological Applications of Vitamin B_6. Alan R. Liss, New York, pp. 307–315.
69. Involving hypertrophy of the horny layer of the epidermis.
70. Involving an increase in the prickle cell layer of the epidermis.
71. This can be precipitated by feeding a vitamin B_6-deficient diet rich in protein.
72. This is believed to be due to reduced pancreatic synthesis of the hormone.
73. Nervous dysfunction is believed to be due to nerve tissue deficiencies of GABA due to decreased activities of the PalP-dependent **glutamate decarboxylase**. The seizures can be controlled by administering either the vitamin or GABA.
74. For example, omphalocele (protrusion of the omentum or intestine through the umbilicus), exencephaly (defective skull formation with the brain partially outside of the cranial cavity), cleft palate, micrognathia (impaired growth of the jaw), splenic hypoplasia.
75. Zhang, X.H., Ma, J., Smith-Warner, S.A., et al., 2007. World Rv. Gastroenterol. 19, 1005–1010.

have found that supranutritional doses of the vitamin can reduce tumorigenesis through effects on cell proliferation and production of reactive oxygen and nitrogen species and angiogenesis.[76] The two, small, randomized intervention trials that have been conducted with human subjects have not found vitamin B_6 supplementation to reduce colorectal cancer risk.[77]

Effects of High-Vitamin B_6 Doses

Vitamin B_6 at relatively high doses has been reported to produce positive effects in a number of conditions affecting individuals who were not apparently deficient in the vitamin:

- **Sideroblastic anemia**. Dosage as great as 200 mg/day (usually as Pn HCl) has been found to stimulate δ-aminolevulinic acid synthase activity and, thus, enhance hematopoiesis in patients.
- **Sickle cell anemia**. A small study found patients to have lower plasma PalP levels than controls, which responded to oral supplementation (100 mg/day) of Pn within 2 months. Both Pn and PalP have been found to protect sickle cells in vitro,[78] but it is not clear whether supplementation with the vitamin may benefit sickle cell anemia patients.
- **Iron storage disease**. Complexes of Pal, which chelate iron (e.g., the isonicotinyl and benzoyl hydrazones), have been found effective in stimulating the excretion of iron in patients with iron-storage disease.
- **Suppression of lactation**. A few studies have reported vitamin B_6 as effective in suppressing lactation probably through the stimulation of dopaminergic activity in the hypothalamus.
- **Adverse drug effects**. Vitamin B_6 is used at doses of 3–5 mg/kg body weight to counteract adverse effects of several types of drugs. The antituberculin drug INH produces a peripheral neuropathy similar to that of vitamin B_6 deficiency by inhibiting the activities of PalP-dependent glutamate decarboxylase and γ-aminobutyrate aminotransferase, which produce and degrade GABA, respectively, in nerve tissue. Two antibiotics antagonize vitamin B_6 by reacting with PalP to form inactive products. Cycloserine reacts with the coenzyme to produce an oxime. Penicillamine produces thiazolidine. L-3,4-dihydroxyphenylalanine antagonizes vitamin B_6 by reacting with PalP to form tetrahydroquinolines. Ethanol increases PalP catabolism. Synthetic estrogens can alter tryptophan–niacin conversion by increasing the synthesis of PalP-dependent enzymes in that pathway, thus, increasing the need for vitamin B_6.
- **Carpal tunnel syndrome**. This disorder, involving pain and paresthesia of the hand, is caused by irritation and compression of the medial nerve by the transverse ligaments of the wrist in ways that are exacerbated by redundant motions. The condition has been associated with low circulating levels of PalP and low-erythrocyte glutamic–oxaloacetic transaminase activities. It has been suggested that such deficiencies lead to edematous changes to and proliferation of the synovia, causing compression of the nerve in the carpal tunnel. Some investigators have reported high doses (50–300 mg/day for 12 weeks) of Pn to be effective as treatment;[79] however, there is no evidence from randomized clinical trials supporting such use of the vitamin.
- **Diabetes**. Several studies have found vitamin B_6 supplementation to improve glucose tolerance. It has been suggested that this may involve the reactivation of kynureninase, which leads to inactivation of insulin through complexation with xanthurenic acid. Studies have also shown that vitamin B_6 supplements (100 mg/day) useful in preventing complications of diabetes mellitus caused by the nonenzymatic glycation of critical proteins.[80] Pyridoxamine has been shown to be a potent inhibitor of the formation of advance glycation products from glycated proteins by scavenging reactive carbonyls; this would appear to be the basis of its protection against the development of renal disease in the diabetic rat model.[81]
- "Chinese restaurant" **syndrome**. The syndrome, which involves headache, sensation of heat, altered heartbeat, nausea, and tightness of the neck induced by oral intake of monosodium glutamate has been reported to respond to Pn (50 mg/day).
- **Premenstrual syndrome**. This syndrome affects some 40% of women 2–3 days before their menstrual flow. It involves tension of the breasts, pain in the lumbar region, thirst, headache, nervous irritability, pelvic congestion, peripheral edema, and usually, nausea and vomiting. Premenstrual syndrome has been reported to respond to vitamin B_6, presumably by affecting levels of the neurotransmitters, serotonin, and GABA that control depression, pain perception, and anxiety. Women experiencing premenstrual symptoms appear to have circulating PalP levels comparable to unaffected women;

76. Komatsu, S., Yanaka, N., Matsubara, K., et al., 2003. Biochem. Biophys. Acta 1647, 127–130.
77. Bønaa, K.H., Njølstad, I., Ueland, P.M., et al., 2006. N. Engl. J. Med. 354, 1578–1588; Ebbing, M., Bleie, Ø., Ueland, P.M., et al., 2008. JAMA 300, 795–804.
78. Kark, J.A., Tarassoff, P.G., Bongiovanni, R., 1983. J. Clin. Invest. 71, 1224–1229.
79. Aufiero, E., Stitik, T.P., Foye, P.M., et al., 2004. Nutr. Rev. 62, 96–104; Goodyear-Smith, F., Arroll, B., 2004. Ann. Fam. Med. 2, 267–273; Ellis, J.M., Pamplin, J., 1999. Vitamin B6 Therapy. Avery Publishing Group, pp. 47–56.
80. Solomon, L.R., Cohen, K., 1989 Diabetes 38, 881–886.
81. Metz, T.O., Alderson, N.L., Thorpe, S. R., et al., 2003. Arch. Biochem. Biophys. 419, 41–49.

nevertheless, high doses of the vitamin have been found to alleviate at least some symptoms in many cases. A review of randomized, clinical trials concluded that Pn doses of up to 100 mg/day are likely to be of benefit in treating these symptoms.[82]

- **Nausea and vomiting of pregnancy**. Plasma concentrations of PalP normally decline in the third trimester of pregnancy at which time fetal stores increase, indicating fetal sequestration of the vitamin.[83] While there is no evidence that this contributes to the nausea and vomiting that affects 85% of all pregnancies, randomized clinical trials have shown that the use of Pn used alone (25 mg every 8 h for 3 days) or in combination with doxylamine[84] significantly reduced nausea and vomiting of pregnancy.[85]

11. VITAMIN B_6 TOXICITY

The toxicity of vitamin B_6 appears to be relatively low, although high doses of the vitamin (several grams per day) have been shown to induce sensory neuropathy marked by changes in gait and peripheral sensation. The primary target, thus, appears to be the peripheral nervous system; although massive doses of the vitamin have produced convulsions in rats, central nervous abnormalities have not been reported frequently in humans. The potential for toxicity resulting from the therapeutic or pharmacologic uses of the vitamin for human disorders (which rarely exceed 50 mg/day) is small. Reports of individuals taking massive doses of the vitamin (>2 g/day) indicate that the earliest detectable signs were ataxia and loss of small motor control. Many of the signs of vitamin B_6 toxicity resemble those of vitamin B_6 deficiency; it has been proposed that the metabolic basis of each condition involves the tissue-level depletion of PalP. Doses up to at least 500 mg/day for extended periods of time (several years) have been found safe.[86] In doses of 10–25 mg, vitamin B_6 increases the conversion of L-dopa to dopamine[87] which, unlike its precursor, cannot cross the blood–brain barrier. The vitamin can, thus, interfere with L-dopa in the management of Parkinson's disease; it should not be administered to individuals taking L-dopa without the concomitant administration of a decarboxylase inhibitor. Upper tolerable intakes have not been established for vitamin B_6.

12. CASE STUDIES

Instructions

Review the following case reports, paying special attention to the diagnostic indicators on which the treatments were based. Then, answer the questions that follow.

Case 1

A 16-year-old boy was admitted with *dislocated lenses* and *mental retardation*. 4 years earlier, an ophthalmologist had found dislocation of the lenses. On the present occasion, he was thin and blond-headed, with ectopia lentis,[88] an anterior thoracic deformity (*pectus excavatum*[89]), and normal vital signs. His palate was narrow with crowding of his teeth. He had mild scoliosis[90] and genu valgum, which caused him to walk with a toe-in, *Chaplin-like* gait. His neurological examination was within normal limits. On radiography, his spine appeared osteoporotic. His performance on the Stanford–Binet Intelligence Scale gave him a development quotient of 60. His hematology, blood glucose, and blood urea nitrogen values were all within normal limits. His plasma homocysteine level (undetectable in normal patients) was 4.5 mg/dL, and his blood *methionine* level was 10-fold normal; the levels of all other amino acids in his blood were within normal limits. Both homocysteine and methionine were increased in his urine, which also contained traces of *S*-adenosylhomocysteine.

The patient was given oral Pn HCl in an ascending dose regimen. Doses up to 150 mg/day were without effect but, after the dose had been increased to 325 mg/day for 200 days, his plasma and urinary homocysteine and methionine levels decreased to normal. These changes were accompanied by a striking change in his hair pigmentation: dark hair grew out from the scalp (the cystine content of the dark hair was nearly double that of the blond hair, 1.5 versus 0.8 mEq/mg). On maintenance doses of Pn, he attained relatively normal function, although the connective tissue changes were irreversible.

Case 2

A 27-year-old woman had experienced increasing difficulty in walking. Some 2 years earlier, she had been told that

82. Wyatt, K.M., Dimmock, P.W., Jones, P.W., et al., 1999. Br. Med. J. 318, 1375–1381.
83. Contractor, S.F., Shane, B., 1970. Am. J. Obstet. Gynecol. 107, 635–640.
84. A first-generation antihistamine.
85. Sahakian, V., Rouse, D., Sipes, S., et al., 1991. Obstet. Gynecol. 78, 33–36; Madjunkova, S., Maltepe, C., Koren G., 2014. Paediatr. Drugs 16, 199–211.
86. Bendich, A., Cohen, M., 1990. Ann. N.Y. Acad. Sci. 585, 320–330; Mpofu, C., Alani, S.M., Whitehouse, C., et al., 1991. Arch. Dis. Child. 66, 1081–1082.
87. It has been claimed that, via its effect on dopamine, vitamin B_6 can inhibit the release of prolactin, thus inhibiting lactation in nursing mothers. Although this proposal is still highly disputed, there is no evidence that daily doses of less than about 10 mg of the vitamin (in multivitamin preparations) has any such effect on lactation.
88. Dislocated lenses.
89. Funnel chest.
90. Lateral curvature of the spine.

vitamin B_6 prevented premenstrual edema and she began taking 500 mg/day of *Pn HCl*. After a year, she had increased her intake of the vitamin to 5 g/day. During the period of this increased vitamin B_6 intake, she noticed that flexing her neck produced a tingling sensation down her neck and to her legs and soles of her feet.[91] During the 4 months immediately before this examination, she had become progressively unsteady when walking, particularly in the dark. Finally, she had become unable to walk without the assistance of a cane. She had also noticed difficulty in handling small objects and changes in the feeling of her lips and tongue; although she reported no other positive sensory symptoms and was not aware of any weaknesses. Her gait was broad-based and stamping, and she was not able to walk at all with her eyes closed. Her muscle strength was normal, but all of her limb reflexes were absent. Her sensations of touch, temperature, pinprick, vibration, and joint position were severely impaired in both the upper and lower limbs. She showed a mild subjective alteration of touch-pressure and pinprick sensation over her cheeks and lips, but not over her forehead. Laboratory findings showed the spinal fluid and other clinical tests to be normal. Electrophysiologic studies revealed that no sensory nerve action potentials could be elicited in her arms and legs, but that motor nerve conduction was normal.

The patient was suspected of having vitamin B_6 intoxication and was asked to stop taking that vitamin. 2 months after withdrawal, she reported some improvement and a gain in sensation. By 7 months, she could walk steadily without a cane and could stand with her eyes closed. Neurologic examination at that time revealed that, although her strength was normal, her tendon reflexes were absent. Her feet still had severe loss of vibration sensation, despite definite improvements in the senses of joint position, touch, temperature, and pinprick. Electrophysiologic examination revealed that her sensory nerve responses were still absent.

Case Questions

1. Propose a hypothesis consistent with the findings in Case 1 for the congenital metabolic lesion experienced by that patient.
2. Would you expect supplements of methionine and/or cystine to have been effective in treating the patient in Case 1? Defend your answer.
3. If the toxicity of Pn involves its competition, at high levels, with PalP for enzyme-binding sites, which enzymes would you propose as potentially being affected in the condition described in Case 2? Provide a rationale for each of the candidate enzymes on your list.

91. Lhermitte's sign.

13. STUDY QUESTIONS AND EXERCISES

1. Diagram schematically the several steps in amino acid metabolism in which PalP-dependent enzymes are involved.
2. Construct a decision tree for the diagnosis of vitamin B_6 deficiency in humans or an animal species.
3. What key feature of the chemistry of vitamin B_6 relates to its biochemical functions as a coenzyme?
4. What parameters might you measure to assess vitamin B_6 status of a human or animal?
5. What factors might be expected to affect the dietary need for vitamin B_6?

RECOMMENDED READING

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Gregory, J.F., 1997. Bioavailability of vitamin B-6. Eur. J. Clin. Nutr. 51, S43–S48.

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Chapter 15

Biotin

Chapter Outline

Anchoring Concepts

1. Biotin is the trivial designation of the compound hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid.
2. Biotin functions metabolically as a coenzyme for carboxylases, to which it is bound by the carbon at position 2 (C-2) of its thiophene ring via an amide bond to the ε-amino group of a peptidyl lysine residue.
3. Deficiencies of biotin are manifested predominantly as dermatologic lesions.

We started with a bushel of corn, and at the end of the purification process, when the solution was evaporated in a small beaker, nothing could be seen, yet this solution of nothing greatly stimulated growth (of propionic acid bacteria). We now know that the factor was biotin, which is one of the most effective of all vitamins.

H.G. Wood[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of biotin.
2. To understand the means of absorption and transport of biotin.
3. To understand the biochemical function of biotin as a component of coenzymes of metabolically important carboxylation reactions.
4. To understand the metabolic bases of biotin-responsive disorders, including those related to dietary deprivation of the vitamin and those involving inherited metabolic lesions.

VOCABULARY

Acetyl-CoA carboxylase 1
Acetyl-CoA carboxylase 2
Achromotrichia
Alopecia
Apocarboxylase
Avidin
Biocytin
Biotin-binding proteins
Biotin sulfoxide
Biotinidase
Biotinylation
Biotinyl 5′-adenylate
Bisnorbiotin
Egg white injury
Fatty liver and kidney syndrome (FLKS)
Footpad dermatitis glucokinase
Histone
Holocarboxylase synthetase (HCS)
3-Hydroxyisovalerate
3-Hydroxyisovaleryl carnitine
Kangaroo gait
β-Methylcrotonoyl-CoA carboxylase
Monocarboxylate transporter (MCT1)

1. Harland G. Wood (1907–91) was an American biochemist who spent most of his career at Case Western Reserve University. He is best known for discovering heterotrophic CO_2 fixation and for establishing the mechanism of transcarboxylase—both facilitated by his pioneering use of radiotracers in biochemistry. In 1989, he received the U.S. National Medal of Science.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00015-0

Multiple carboxylase deficiencies
Ornithine transcarbamylase
Phosphoenolpyruvate carboxykinase (PEPCK)
Propionyl-CoA carboxylase
Pyruvate carboxylase
Sodium-dependent vitamin transporter (SMVT).
Spectacle eye
Streptavidin
Sudden infant death syndrome (SIDS)
Thiophene ring
Transcarboxylase
Ureido nucleus

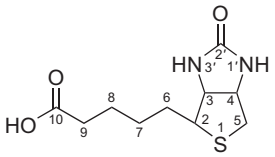
1. THE SIGNIFICANCE OF BIOTIN

Biotin was discovered in the search for the nutritional factor that prevents **egg white injury** in experimental animals. The biotin antagonist in egg white, **avidin**, produces experimental biotin deficiency in animal models. Practical cases of biotin deficiency in humans were encountered with the advent of total parenteral nutrition (TPN) before the vitamin was routinely added to tube-feeding solutions, and biotin-responsive cases of footpad dermatitis remain a problem in commercial poultry production. Biotin deficiency manifests itself differently in different species, most often with dermatologic lesions. A key feature of biotin metabolism is that it is recycled by proteolytic cleavage from the biotin-dependent carboxylases by the enzyme **biotinidase**. This recycling and the prevalent hindgut microbial synthesis of the vitamin allow quantitative dietary requirements for biotin to be relatively small. Inborn errors of biotin absorption and metabolism have been identified; some respond to large doses of the vitamin.

2. PROPERTIES OF BIOTIN

Biotin is the trivial designation of the compound *cis*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-4-pentanoic acid.[2] It consists of conjoined ureido and tetrahydrothiophene nuclei in which the ureido 3′-nitrogen is sterically hindered, preventing substitution and the ureido 1′-nitrogen is weakly nucleophilic. Biotin functions in metabolism bound to certain enzymes by covalent linkages of the carboxyl group of the biotin side chain with ε-amino groups of peptidyl lysyl residues. This bound form is called **biocytin**.

Chemical Structure of Biotin



Biotin

2. Formerly, **vitamin H** or **coenzyme R**.

Biotin Chemistry

Biotin is a white crystalline substance that, in dry form, is fairly stable to air, heat, and light. In solution, however, it is sensitive to degradation under strongly acidic or basic conditions. Its structure consists of a planar **ureido nucleus** and a folded **tetrahydrothiophene** (**thiophane**) **nucleus**, which results in a boat configuration with a plane of symmetry passing through the S-1, C-2′, and O positions in such a way as to elevate the sulfur atom above the plane of the four carbons. The molecule has three asymmetric centers; however, of the eight possible stereoisomers, only the (+)-isomer (called *d*-biotin) has biological activity. Biotin is covalently bound to its enzymes by an amide bond to the ε-amino group of a lysine residue and C-2 of the thiophane nucleus. This bond is flexible, allowing the coenzyme to move between the active centers of some enzymes. The biotin molecule is activated by polarization of the O and N-1′ atoms of the ureido nucleus. This leads to increased nucleophilicity at N-1′, which promotes the formation of a covalent bond between the electrophilic carbonyl phosphate formed from bicarbonate and ATP and allows biotin to serve as a transport agent for CO_2.

3. SOURCES OF BIOTIN

Hindgut Microbial Synthesis

The microbiota of the monogastric hindgut synthesize significant amounts of biotin. In both rats and humans, fecal excretion of biotin typically exceeds the amount consumed. A genomic analysis of 256 representative organisms of the human gut microbiota found 40% capable of de novo synthesis of the vitamin.[3] In addition, the majority of genomes (all phyla except Fusobacterial) also showed the capacity to salvage PalP and its precursors. Those findings suggested that hindgut microbial synthesis may produce nearly 5% of the daily human need for biotin. Evidence indicates that biotin can be absorbed from the hindgut. Studies with the rat have shown the biotin transport capacity of the colon to be as great as 25% that of the jejunum[4]; and a Na^+-dependent biotin carrier has been identified in human-derived colonic epithelial cells. Accordingly, the dietary biotin requirement of the rat has been determined only under gnotobiotic[5] conditions or with avidin feeding. Still, questions remain as to the nutritional significance of biotin of microbial origin for humans and other noncoprophagous animals, as intracecal treatment with antibiotics to inhibit microbial growth or with lactulose to

3. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.
4. Bowman, B.B., Rosenberg, I.H., 1987. J. Nutr. 117, 2121–2128.
5. Germ-free.

stimulate microbial growth failed to affect plasma biotin levels in the pig.[6]

Distribution in Foods

Biotin is widely distributed in foods and feedstuffs, mostly in very low concentrations (Table 15.1). Only a couple of foods (royal jelly,[7] brewers' yeast) contain biotin in large amounts. Milk, liver, egg (egg yolk), and a few vegetables are the most important natural sources of the vitamin in human nutrition; the oilseed meals, alfalfa meal, and dried yeasts are the most important natural sources of the vitamin for the feeding of nonruminant animals. The biotin contents of foods and feedstuffs can be highly variable[8]; for the cereal grains at least, it is influenced by such factors as plant variety, season, and yield (endosperm-to-pericarp ratio). Most foods contain the vitamin as free biotin and as biocytin bound to food proteins.

However, in milk, biotin occurs almost exclusively as the free vitamin in the skim fraction.[9]

Stability

Biotin is unstable to oxidizing conditions and, therefore, is destroyed by heat, especially under conditions that support simultaneous lipid peroxidation.[10] Therefore, such processing techniques as canning, heat curing, and solvent extraction can result in substantial losses of biotin. These losses can be reduced by the use of a food grade antioxidant (e.g., vitamin C, vitamin E, butylated hydroxytoluene, butylated hydroxyanisole).

Bioavailability

Studies of the bioavailability of food biotin to humans have not been conducted. However, biotin bioavailability has been determined experimentally using two types of bioassay: healing of skin lesions in avidin-fed rats, support of growth and maintenance of pyruvate carboxylase activity in chicks. Such assays have shown that the nutritional availability of biotin can be low and highly variable among different foods and feedstuffs (Table 15.2).

6. Kopinski, J.S., Leibholz, J., Love, R.J., 1989. Br. J. Nutr. 62, 781–788.
7. Royal jelly is secreted by the labial glands of worker honeybees and is rich in biotin (>400 µg/100 g). The few female larvae that are fed royal jelly develop reproductively as queens; whereas, most larvae are fed a mixture of honey and pollen and fail to develop reproductively ability and become workers. The active factor in royal jelly may be a lipid, 10-hydroxy-Δ2-decenoic acid; the role of biotin in royal jelly is unclear.
8. In one study, the biotin contents of multiple samples of corn and meat meal were 56–115 µg/kg ($n=59$) and 17–323 µg/kg ($n=62$), respectively.
9. These levels are 20- to 50-fold greater than those found in maternal plasma. Human milk also contains biotinidase, which is presumed to be important in facilitating infant biotin utilization.
10. About 96% of the pure vitamin added to a feed was destroyed within 24 h after the addition of partially peroxidized linolenic acid.

TABLE 15.1 Biotin Contents of Foods

Food	Biotin, µg/100 g
Dairy Products	
Milk	2
Cheeses	3–5
Meats	
Beef	3
Chicken	11
Pork	5
Calf kidney	100
Cereals	
Barley	14
Cornmeal	7.9
Oats	24.6
Rye	8.5
Sorghum	28.8
Wheat	10.1
Wheat bran	36
Oilseed Meals	
Rapeseed meal	98.4
Soybean meal	27
Vegetables	
Asparagus	2
Brussels sprouts	0.4
Cabbage	2
Carrots	3
Cauliflower	17
Corn	6
Kale	0.5
Lentils	13
Onions	4
Peas	9
Potatoes	0.1
Soybeans	60
Spinach	7
Tomatoes	4
Fruits	
Apples	1
Bananas	4
Grapefruit	3

Continued

TABLE 15.1 Biotin Contents of Foods—cont'd

Food	Biotin, μg/100 g
Grapes	2
Oranges	1
Peaches	2
Pears	0.1
Strawberries	1.1
Watermelons	4
Nuts	
Peanuts	34
Walnuts	37
Other	
Eggs	20
Brewers' yeast	80
Alfalfa meal	54
Molasses	108

USDA National Nutrient Database for Standard Reference, Release 18.

In general, less than one-half of the biotin present in feedstuffs is biologically available. Although all of the biotin in corn is available, only 20–30% of that in most other grains and none in wheat is available. The bioavailability of biotin in meat products also tends to be very low.

Differences in biotin bioavailability appear to be due to differential susceptibilities to digestion of the various biotin–protein linkages in which the vitamin occurs in foods and feedstuffs. Those linkages involve the formation of covalent bonds between the carboxyl group of the biotin side chain with ε-amino groups of peptidyl lysyl residues, constituting the means by which biotin binds to the enzymes for which it serves as an essential prosthetic group. The utilization of such biocytin biotin thus depends on the hydrolytic digestion of the proteins and/or the hydrolysis of those amide bonds. Biotins in purified preparations, such as those used in dietary supplements, are highly bioavailable.

4. ABSORPTION OF BIOTIN

Digestion of Protein-Bound Biotin

In the digestion of food proteins, protein-bound biotin is released by the hydrolytic action of the intestinal proteases to yield the e-N[1] biotinyl lysine adduct, biocytin, from which free biotin is liberated by the action of an intestinal biotin amide aminohydrolase, **biotinidase**.

Facilitated Transport

At low concentrations, free biotin is absorbed across the enterocyte by two carrier-mediated processes. Its uptake is facilitated by a **Na^+-dependent multivitamin transporter** (**SMVT**) bound to the apical membrane (brush border).[11] It can also be inhibited by certain anticonvulsant drugs[12] and ethanol or its major metabolite acetaldehyde (Table 15.3). The intracellular trafficking of SMVT involves distinct trafficking vesicles, the microtubular network, and the microtubule motor protein dynein.[13] The process is not specific for the vitamin, as SMVT also functions in the cellular uptake of pantothenic acid and lipoic acid, which it binds with similar affinities and can inhibit biotin uptake. At the basolateral membrane, another Na^+-dependent transporter is involved in translocating biotin to the plasma. The SMVT is regulated by protein kinase C, which can phosphorylate the transporter. Suboptimal SMVT expression is thought to underlie the low biotin absorption observed in alcoholics, pregnant women and patients with inflammatory bowel disease, seborrheic dermatitis or on anticonvulsants, or long-term parenteral nutrition. Four SMVT splicing variants have been identified in the rat.[14]

Passive Diffusion

Both free biotin and nonhydrolyzed biocytin can be absorbed by diffusion, mainly in the jejunum. This becomes physiologically significant only at luminal concentrations >5 μM; even then, biocytin is less well absorbed than the free vitamin.

5. TRANSPORT OF BIOTIN

Transport in Plasma

Biotin is present in low amounts in plasma, most of which is soluble. Less than half is free biotin, the balance being composed of **bisnorbiotin**, **biotin sulfoxide**, and other unidentified metabolites. Some 7% is weakly bound nonspecifically to albumin, α- and β-globulins, and other proteins. An estimated 12% is bound covalently to proteins, predominantly biotinidase, which has both a high-affinity and a low-affinity biotin-binding sites and is thought to function as a biotin transporter. Biotinidase also occurs in human milk and at particularly high levels in colostrum, where it is thought to function in the transport of biotin by the mammary gland to the nursing infant. Biotin is also covalently bound to a plasma glycoprotein present in the serum of the pregnant rat.

11. Encoded by the *SLC5A6* gene.
12. Carbamazepine, primidone.
13. Subramanian, V.S., Marchant, J.S., Said, H.M., 2007. Gastroenterology 132, A583.
14. Said, H.M., 2004. Ann. Rev. Physiol. 66, 419–446.

TABLE 15.2 Biotin Availability in Several Feedstuffs

Feedstuff	Total Biotin[a] (µg/100 g)	Available Biotin[b] (µg/100 g)	Bioavailability[c] (%)
Barley	10.9	1.2	11
Corn	5.0	6.5	133
Wheat	8.4	0.4	5
Rapeseed meal	93.0	57.4	62
Sunflower seed meal	119.0	41.5	35
Soybean meal	25.8	27.8	108

[a]*Determined by microbiological assay.*
[b]*Determined by chick growth assay.*
[c]*Compared to* d*-biotin.*
Whitehead, C.C., Armstrong, J.A., Waddington, D., 1982. Br. J. Nutr. 48, 81–88.

TABLE 15.3 Inhibition of Enteric Biotin Transport by Ethanol

Ethanol, %, v/v	Biotin Transport, pmol/g tissue/15 min
0	16.89 ± 0.80[a]
0.5	15.16 ± 1.02
1	12.56 ± 1.03[b]
2	11.59 ± 1.16[b]
5	6.61 ± 0.42[a]

[a]*Mean ± SD.*
[b]*p > .05.*
Said, H.M., Sharifian, A., Bergherzadeh, A., et al., 1990. Am. J. Clin. Nutr. 52, 1083–1086.

Cellular Uptake

The uptake of biotin into cells is facilitated an SMVT, which also functions in the cellular uptake of pantothenic acid and lipoic acid. Biotin uptake by peripheral blood mononuclear cells, and perhaps other lymphoid cells, appears to be facilitated by and occurs by a **monocarboxylate transporter (MCT1)**, which shows a K_m three orders of magnitude less than SMVT-mediated transport and is not competitively inhibited by either pantothenic or lipoic acids. The intracellular distribution of biotin closely parallels that of its carboxylases. It is found mostly in the cytoplasm (the primary location of acetyl-CoA carboxylase) and mitochondria (in which MCT1 has been detected). A small amount (<1%) is found in the nucleus,[15] which is thought to reflect binding to histones, as it increases (to c.1% of total cellular biotin) with cell proliferation.

Biotin appears to sense and regulate its own intracellular levels. This involves a novel function of **holocarboxylase synthetase (HCS)**, which moves into the nucleus when biotin is available and silences the biotin transporter SMVT by biotinylating histone 4 (H4) at the promoter. Gene silencing results from changes in chromatin structure affecting specific loci.[16] HCS translocation is thought to be regulated by tyrosine kinases and Zn finger proteins that direct the protein to specific chromatin regions.

Tissue Distribution

Appreciable storage of the vitamin appears to occur in the liver, where concentrations of 800–3000 ng/g have been found in various species.[17] Most of this appears to be in mitochondrial acetyl-CoA carboxylase. Hepatic stores, however, appear to be poorly mobilized during biotin deprivation and, thus, do not show the reductions measurable in plasma under such conditions. Biotin is transported to the fetus by specific carriers including SMVT. Concentrations of the vitamin in fetal plasma are 3- to 17-fold greater than maternal levels. That milk biotin levels exceed those of maternal plasma by 10- to 100-fold evidences a mammary transport system. **Biotin-binding proteins** have been identified in the egg yolks of many species of birds where they are believed to function in transporting the vitamin into the oocyte, as their binding is weak enough to be reversible.[18] The yolk biotin-binding protein also occurs in the plasma of the laying hen.

15. Stanley, J.S., Griffen, J.B., Zempleni, J., 2001. Eur. J. Biochem. 268, 5424.

16. Gralla, M., Camporeale, G., Zempleni, J., 2008. J. Nutr. Biochem. 19, 400; Bao, B., Pestinger, V., Hassan, Y.I., et al., 2011. J. Nutr. Biochem. 22, 470.

17. These levels contrast with those of plasma/serum, typically c.300 ng/L (humans, rats).

18. The yolk biotin-binding protein is a 74.3 kDa glycoprotein with an homologous tetrameric structure, each subunit of which binds a biotin molecule. This protein is not to be confused with avidin, the biotin-binding protein of egg white, which irreversibly binds biotin with an affinity three orders of magnitude greater.

6. METABOLISM OF BIOTIN

Linkage to Apoenzymes

Free biotin is attached to its apoenzymes via the formation of an amide linkage to the ε-amino group of a specific lysyl residue. In each of the biotin-dependent enzymes, this binding occurs in a region containing the same amino acid sequence, -Ala-Met-biotinyl-Lys-Met-. The linkage is catalyzed by HCS.[19]

Biotin HCS. HCS is a small, monomeric enzyme that catalyzes the formation of the covalent linkage of the biotin prosthetic group to an ε-amino group of a lysyl residue on the apoenzyme (Fig. 15.1). The process is driven thermodynamically by the hydrolysis of ATP, which occurs in two steps:

1. activation of the vitamin as **biotinyl 5′-adenylate**; and
2. attachment to the **apocarboxylase** by an amide bond with the lysyl residue, with release of AMP.

HCS expression is dependent on adequate biotin status and appears to involve several variants in the range of 62–86 kDa. A high percentage of cellular HCS is present in the nucleus where it is thought to function also in the biotinylation of histones.

Recycling the Vitamin

The normal turnover of the biotin-containing holocarboxylases involves their degradation to yield biocytin, as the biotinyl lysine bond is not hydrolyzed by cellular proteases. Instead, biocytin is cleaved by biotinidase to yield free biotin. Biotinidase is the major biotin-binding protein in plasma; it is also present in breast milk, in which its activity is particularly high in colostrum. The proteolytic liberation of biotin from its bound forms is essential for the reutilization of the vitamin, which is accomplished by its reincorporation into another holoenzyme. Biotinidase may also catalyze the debiotinylation of histones. Mitochondrial biotinyl acetyl-CoA carboxylase may serve as a reservoir to maintain hepatic acetyl-CoA at appropriate levels in the cytosol. This would also provide biotin indirectly to support other biotinyl mitochondrial enzymes under biotin-limiting conditions.

Catabolism

Relatively, little catabolism of biotin is apparent in mammals. A small fraction is oxidized to biotin D- and L-sulfoxides; however, the ureido ring system is not otherwise degraded. The side chain of a larger portion is metabolized via mitochondrial β-oxidation to yield bisnorbiotin and its degradation products. Evidence suggests that biotin catabolism is greater in smokers than nonsmokers.[20]

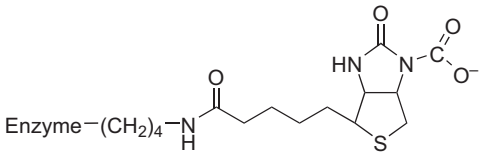


FIGURE 15.1 Biotin is bound covalently to its carboxylases.

Excretion

Biotin is rapidly excreted in the urine (Fig. 15.2). Studies have shown the rat to excrete about 95% of a single oral dose (5 mg/kg) of the vitamin within 24 h. Half of urinary biotin occurs as free biotin, the balance being composed of bisnorbiotin, bisnorbiotin methyl ketone, biotin sulfone, tetranorbiotin-L-sulfoxide, and various side chain products. Although unabsorbed biotin appears in the feces, much fecal biotin is of gut microbial origin and benefits the host by way of hindgut absorption. Thus, at low dietary levels of the vitamin, urinary excretion of biotin can exceed intake. Only a small amount (<2% of an intravenous dose) of biotin is excreted in the bile. The urinary excretion of patients with **achlorhydria** is very low; this may reflect impaired release of bound biotin for subsequent absorption.

7. METABOLIC FUNCTIONS OF BIOTIN

Biotin functions as essential cofactors for five carboxylases. It also functions as a regulator of gene expression, as a substrate for the posttranslational modification of proteins by biotinylation. Evidence also suggests additional biotin-containing proteins.

Carboxylations

Biotin functions in fatty acid metabolism, gluconeogenesis, and amino acid metabolism in key steps involving the transfer of covalently bound, single-carbon units in the most oxidized form, CO_2. This function is implemented by five biotin-dependent carboxylases and transcarboxylases[21] (Table 15.4) to which the biotin prosthetic group is attached by a single enzyme, biotin HCS.

The biotin-dependent carboxylases. Humans and other animals have five biotin-dependent carboxylases (Table 15.4). One (acetyl-CoA carboxylase 1) is cytosolic,

19. This is sometime called "**biotin holoenzyme synthetase**" and, in microorganisms, "**biotin protein ligase**."

20. Sealey, W.M., Teague, A.M., Stratton, S.L., et al., 2004. Am. J. Clin. Nutr. 80, 932.

21. **Transcarboxylase** has been called the "*Mickey Mouse enzyme*," as the electron micrograph image of the bacterial enzyme, with its large single subunit and two smaller flanking subunits, resembles the head of the famous rodent.

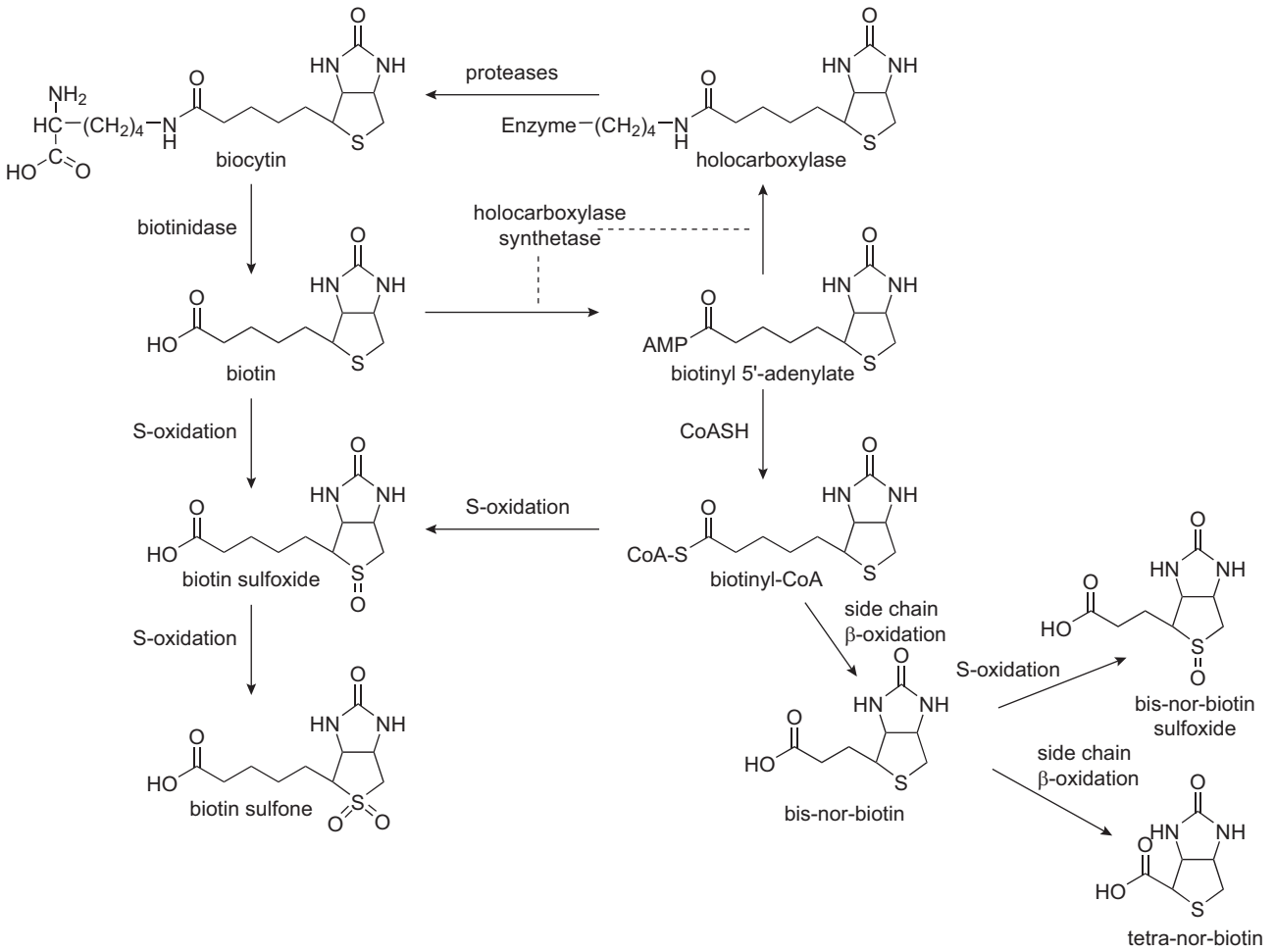


FIGURE 15.2 Biotin metabolism and recycling.

the others are mitochondrial. The catalytic action of each proceeds by a nonclassic, two-site, ping-pong mechanism, with partial reactions being performed by dissimilar subunits:

1. **Carboxylation** (at the carboxylase subsite)—involving the addition of a carboxyl moiety to the biotin ureido-N opposite the side chain, using the bicarbonate/ATP system as the carboxyl donor;
2. **Carboxyl transfer** (at the carboxyl transferase subsite)—involving transfer of the carboxyl group from carboxybiotin to the acceptor substrate.

In each enzyme, the carboxylase and carboxyl transfer two subsites appear to be spatially separated. The transfer of biotinyl CO_2 between those subsites is thought to be facilitated by movement of the prosthetic group back and forth through rotation of one or more of the 10 single bonds on the valeryl lysyl side chain.

Genetic disorders of biotin metabolism. Genetic defects in all of the biotin enzymes have been identified in humans (Table 15.5). These are rare, affecting infants and children, usually with serious consequences. Mutations in genes encoding the three proteins involved in biotin homeostasis (SMVT, biotinidase, HCS) can cause multiple carboxylase deficiencies. Biotinidase deficiency occurs at a frequency of 1 in 60,000 live births, with affected individuals having <30% of normal serum biotinidase activity, compromising their ability to release biotin from dietary proteins and recycle it from endogenous biotinylated proteins. They show the neurological and dermatological symptoms of biotin deficiency, with onset in the first year of life (sometimes within weeks). They also experience additional signs including hearing loss and optic atrophy, which has been interpreted as suggestive of other, still-unidentified functions of biotinidase. This can lead to irreversible neurologic damage but can be prevented by treatment with lifelong, high doses (5–20 mg/day) of biotin. Defects involving the absence of an apocarboxylase do not respond to supplements of the vitamin. They are treated by restricting dietary protein

TABLE 15.4 The Biotin-Dependent Carboxylases of Animals

Enzyme	Metabolic Function
Cytosol	
Acetyl-CoA carboxylase 1 (ACC1)	*Formation of malonyl-CoA from acetyl-CoA for carboxylase fatty acid synthesis in the cytoplasm*—ACC1 requires citrate; it catalyzes the incorporation of bicarbonate into acetyl-CoA to yield malonyl-CoA in the cytosol in the first committed step in the synthesis and elongation of fatty acids. Reduced ACC1 activity due to biotin deprivation impairs lipid synthesis
Mitochondria	
Pyruvate carboxylase (PC)	*Formation of oxaloacetate from pyruvate*—PC is a 130 kDa homologous tetramer. It requires acetyl-CoA and catalyzes the incorporation of bicarbonate into pyruvate to form oxaloacetate, a key step in gluconeogenesis. It also serves to replenish the mitochondrial supply of oxaloacetate to support the TCA (tricarboxylic acid) cycle and the formation of citrate for transport to the cytosol for lipogenesis. Reduced PC activity due to biotin deprivation can lead to fasting hypoglycemia, lactic acidosis, and ketosis
Acetyl-CoA carboxylase 2 (ACC2)	*Formation of malonyl-CoA from acetyl-CoA for carboxylase fatty acid synthesis in the mitochondria*—ACC2 requires citrate; it controls mitochondrial fatty acid oxidation by inhibitory effects of malonyl CoA, which it produces
Propionyl-CoA carboxylase (PCC)	Formation of methylmalonyl-CoA from propionyl-CoA produced by catabolism of some amino acids (e.g., isoleucine) and odd-chain fatty acids—PCC is a dimer with nonidentical subunits. It catalyzes the incorporation of bicarbonate into propionyl-CoA to form methylmalonyl CoA, which isomerizes to succinyl CoA and enters the TCA cycle for energy and glucose production
β-Methylcrotonyl-CoA carboxylase (βMCC)	*Part of the leucine degradation pathway*—**β**MCC is a dimer with nonidentical subunits. It catalyzes the degradation of leucine. Reduced activity due to biotin deprivation results in shunting the leucine degradation product β-methylcrotonyl-CoA through an alternate catabolic pathway to **3-hydroxyisovaleric acid**, which is excreted in the urine[22]

to limit the production of metabolites upstream of the metabolic lesion.

Gene Expression

Biotin has been shown to be necessary for the normal progression of cells through the cell cycle, with biotin-deficient cells arresting in the G1 phase. Proliferating lymphocytes increases their uptake of biotin, as well as their activities of β-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase. These effects would appear to involve effects on gene expression, as biotin has been shown to affect the expression of genes encoding enzymes involved in glucose metabolism (glucokinase, phosphoenolpyruvate carboxykinase, ornithine transcarbamylase), cytokines (IL-2, IL-2 receptor γ, IL-1β, interferon-γ, IL-4[23]), amino acid metabolism (βMCC), and regulation of biotin status (SMVT, HCS). Evidence indicates that biotin status affects the regulation of gene expression by affecting cell signaling and histones.

Signaling by biotin has been demonstrated as follows:

- Biotin deprivation increases the nuclear translocation, binding, and transcriptional activity of **NK-κB**. This increases nuclear levels of p50 and p65, and activities of IκB, which activates genes involved in suppression of apoptosis.[24]
- Biotin increases intracellular production of the key second messenger **nitrous oxide (NO)**, which activates a soluble guanylate cyclase to increase cellular levels of cGMP. Those increases stimulate protein kinase G and lead to phosphorylation and activation of proteins that increase transcription of genes encoding HCS and some carboxylases.[25] It also suppresses endo/sarcoplasmic reticular Ca^{2+}-ATPase 3 (SERCA3), which functions in the transport of Ca^{2+} from the cytosol into the endoplasmic reticulum, to increase cytosolic concentrations of Ca^{+2} and stimulate protein unfolding.[26] That this effect may be important in immune cell function was indicated by the results of a human trial showing biotin supplementation to markedly reduce the expression of SERCA3 in lymphocytes of healthy adults.[27]
- Biotin appears necessary for the expression of the transcription factors **Sp1** and **Sp3**, which are associated

22. Urinary 3-hydroxyisovalerate has, therefore, been proposed as a biomarker of biotin deficiency.
23. Unlike the other proteins listed, which depend on biotin for expression, IL-4 and SMVT are downregulated by biotin.
24. Rodriguez-Melendez, R., Schwab, L.D., Zempleni, J., 2004. Int. J. Vitaminol. Nutr. Res. 74, 209.
25. Solorzano-Vargas, R.S., Pacheco-Alvarez, D., Leon-Del-Rio, A., 2002. Proc. Nat. Acad. Sci. U.S.A. 99, 5325.
26. Griffen, J.B., Rodriguez-Melendez, R., Dode, L., 2006. J. Nutr. Biochem. 17, 272.
27. Weidmann, S., Rodriguez-Melendez, R., Ortega-Cuellar, D., et al., 2004. J. Nutr. Biochem. 15, 433–439.

TABLE 15.5 Genetic Disorders of Biotin Metabolism

Defect	Metabolic Basis	Physiological Effect	Treatment
SMVT (sodium-dependent vitamin transporter) deficiency	Recessive *SLC19A3* mutation	Biotin-responsive basal ganglia disease.[a] *Symptom*: childhood onset of subacute encephalopathy, ataxia, seizures, dystonia, quadriparesis, hyperreflexia	High-dose biotin plus thiamin
Propionyl-CoA carboxylase deficiency	Autosomal recessive lack of enzyme[b]	Propionate accumulation: acidemia, ketoacidosis, hyperammonemia; high urine citrate, 3-OH-propionate, propionyl glycine. *Symptoms*[c]: vomiting, lethargy, hypotonia, mental retardation, cramps	Restrict protein
Pyruvate carboxylase deficiency	Autosomal recessive lack of enzyme[d]	Changes in energy production, gluconeogenesis, and other pathways. *Symptoms*: metabolic acidosis (lactate), hypotonia, mental retardation	None
β-Methylcrotonyl-CoA carboxylase deficiency	Defective enzyme (basis unknown[e])	High urine β-CH_3-crotonylglycine and 3-hydroxyisovaleric acid. *Symptoms*: cramps	Restrict protein
Acetyl-CoA carboxylase deficiency	Lack of enzyme (basis unknown[f])	Aciduria. *Symptoms*: myopathy, neurologic changes	None
Multiple carboxylase deficiency Neonatal type	Autosomal recessive lack of HCS (holocarboxylase synthetase)[g]	Deficiencies of all biotin-containing holocarboxylases; acidosis and aciduria. *Symptoms*: vomiting, lethargy, hypotonia	High-dose biotin; varied results[h]
Juvenile type	Autosomal recessive lack of biotinidase[i]	Deficiencies of all biotin-containing holocarboxylases; acidosis and aciduria. *Symptoms*: skin rash, alopecia, conjunctivitis, ataxia, developmental anomalies, neurological signs	Massive doses of biotin

[a]20 cases have been reported.
[b]Incidence: 1 in 350,000.
[c]There is a wide variation in the clinical expression.
[d]Fewer than two dozen patients have been described.
[e]Three confirmed cases have been reported.
[f]One case has been described.
[g]Involves failure to link biotin to the apocarboxylases; some 30 HCS mutations have been reported.
[h]Response is related to residual HCS activity. Homozygous severe HCS deficiency is fatal unless diagnosed and treated early.
[i]Involves failure to release biotin from its bound forms in holocarboxylases; this reduces use of biotin in foods and blocks endogenous recycling of the vitamin.

with increased expression of cytochrome *P*450 1B1 and reduced expression of SERCA3.

- Biotin activates **jun/fos**[28] signaling which, in turn, activates AP1-dependent pathways.[29] It has been proposed that jun/fos signaling may increase the expression of SMVT, which gene has an AP1-like site.
- Biotin deficiency activates signaling by **tyrosine kinases**, which may contribute to increasing SMVT-mediated biotin uptake.
- Biotin status has been shown to affect the abundance of small, noncoding RNAs in at least some cells: miRNA-539 in cultured human fibroblasts and miRNA-153 in human kidney carcinoma cells.[30]

Histone biotinylation by HCS has been reported to silence several genes. Three histones (H3 and H4 primary targets; H1 to H2A to lesser extents) can be covalently modified by biotinylation at a dozen-specific lysyl residues to affect chromatin structure and stability of repeat regions and transposable elements.[31] Both biotinidase and HCS catalyze the biotinylation of proteins; however, only HCS has been localized in the nucleus, suggesting that it is the physiologically relevant factor in histone biotinylation. While debiotinylation of histones occurs, the mechanism is not clear; it may be catalyzed by an isoform of biotinidase. In humans, <0.1% of histones appear to be biotinylated[32]; nevertheless, the epigenetic impact is significant. One-third of H4 has been found to be biotinylated at lysyl residue

28. Protooncogenes that combine to form the AP-1 transcription factor.
29. Rodriguez-Melendez, R., Griffen, J.B., Zempleni, J., 2006. J. Nutr. 135, 1659.
30. Bao, B., Rodriguez-Melendez, R., Wijeratne, S.S., et al., 2010. J. Nutr. 140, 1546–1551.
31. Reduction of histone biotinylation by biotin-deprivation reduced life span and heat tolerance in *Drosophila melanogaster* (Camporeale, G., Giordano, E., Rendina, R., et al., 2006. J. Nutr. 136, 2735).
32. Bailey, L.M., Ivanov, R.A., Wallace, J.C., et al., 2008. Anal. Biochem. 373, 71.

12 in telomeric repeats,[33] and enrichments in H3 (at lysyl residues 9 or 18) and H4 (at lysyl residue 8) have also been found. While these interactions contribute to the condensation of nuclear chromatin, it has been argued that histone biotinylation may be secondary to HCS interactions with chromatin proteins,[34] placing it in physical proximity to histones, and that histone biotinylation may have little effect on gene repression.[35]

Other Biotin-Containing Proteins

It is possible that there may be unidentified biotin-dependent proteins and enzymes. A mass spectrometric screening of human embryonic kidney cells detected more than a hundred biotinylated proteins.[36] While that number is likely to include proteins biotinylated nonspecifically by HCS, some proteins appeared to have been overrepresented. Those include heat shock proteins and enzymes involved in glycolysis and protein synthesis.

8. BIOMARKERS OF BIOTIN STATUS

Biotin status can be assessed in two ways:

- **Blood and urinary metabolites**—One of the first indicators of biotin deficiency is increased by circulating concentrations of **3-hydroxyisovaleryl carnitine**, and its derivative **carnityl-3-hydoxyisovaleric acid**,[37] which changes as a result of the alternative metabolism of β-methylcrotonyl-CoA by enoyl-CoA hydratase with declining biotin-dependent β-methylcrotonyl-CoA carboxylase activity.[38] Plasma/serum concentrations of biotin and its metabolites are less informative, as they tend to remain stable under conditions of moderate deficiency.
- **Degree of biotin saturation of biotin-dependent enzymes**—This is a useful means of assessing biotin status. It takes advantage of the in vitro binding of biotin by lymphocyte propionyl-CoA carboxylase (PCC). Because biotin-adequate subjects typically have most PCC bound to biotin, the stimulation of PCC activity by added biotin indicates nonsaturation due to suboptimal biotin status. This can be expressed as an activity coefficient:
  - **PCC activity coefficient** = baseline PCC activity/PCC activity with added biotin.

33. Wijeratne, S.S., Camporeale, G., Zempleni, J., 2010. J. Nutr. Biochem. 21, 310.
34. e.g., DNA methyltransferase I; methyl-CpG-binding protein 2; eukaryotic histone-lysine methyltransferase 1.
35. Zemplini, J., Liu, D., Camara, D.T., et al., 2014. Nutr. Rev. 72, 369–376.
36. Xue, J., Zhou, J., Zempleni, J., 2013. Am. J. Physiol. Cell Physiol. 305, C1240–C1245.
37. Mock, D.M., Henrich-Shell, C.L., Carnell, N., et al., 2004. J. Nutr. 134, 317–320.
38. Stratton, S.L., Horvath, T.D., Bogusiewicz, A., et al., 2010. Am. J. Clin. Nutr. 92, 1399–1406.

9. BIOTIN DEFICIENCY

Because biotin is widely distributed among foods and feedstuffs and is synthesized by the gut microbiome, simple deficiencies in animals or humans are rare (Table 15.6). In fact, recommended intakes for biotin have not been established in the United States (Table 15.7). Biotin deficiency can be induced by antagonists, the most prominent is **avidin**, a biotin-binding protein found in egg whites.

Egg White Injury

In the mid-1930s, it was found that biotin supplements prevented the dermatitis and alopecia produced in experimental animals by feeding uncooked egg white. Subsequently, the damaging factor was isolated and named avidin. It is a water-soluble, basic glycoprotein with a molecular mass of

TABLE 15.6 General Signs of Biotin Deficiency

Organ System	Change/Signs
General	Decreased appetite, growth
Dermatologic	Dermatitis, alopecia, achromotrichia
Skeletal	Perosis
Vital organs	Hepatic steatosis FLKS (fatty liver and kidney syndrome in poultry)

TABLE 15.7 Recommended Biotin Intakes

The United States		FAO/WHO	
Age-Sex	AI[a], µg/day	Age-Sex	RNI[b], µg/day
0–6 months	5	0–6 months	5
7–11 months	7	7–11 months	6
1–3 years	8	1–3 years	8
4–8 years	12	4–6 years	12
9–13 years	20	7–9 years	20
14–18 years	25	10–18 years	25
>18 years	30	19+ year	30
Pregnancy	30	Pregnancy	30
Lactation	35	Lactation	35

[a]Adequate Intakes are given, as Recommended Dietary Allowances (RDAs) have not been established; Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.
[b]Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. Food Agriculture Org., Rome, 286 pp.

67 kDa. It is a homologous tetramer, each 128-amino acid subunit of which binds a molecule of biotin by linking to two to four tryptophan residues and an adjacent lysine in the subunit binding site. The binding of biotin to avidin is the strongest known noncovalent bond in nature[39]. Avidin is secreted by the oviductal cells of birds, reptiles, and amphibians and, thus, found in the whites of their eggs in which is thought to function as a natural antibiotic, as it is resistant to a broad range of bacterial proteases. It antagonizes biotin by forming with the vitamin a noncovalent complex[40] that is also resistant to pancreatic proteases, thus preventing the absorption of biotin.[41] The avidin–biotin complex is unstable to heat; heating to at least 100°C denatures the protein and releases biotin available for absorption. Therefore, although raw egg white is antagonistic to the utilization of biotin, the cooked product is without effect. The consumption of raw or undercooked whole eggs is probably of little consequence to biotin nutrition, as the biotin-binding capacity of avidin in the egg white is roughly comparable to the biotin content of the egg yolk. However, as a tool to produce experimental biotin deficiency, avidin in the form of dried egg white has been useful.[42]

Deficiency Signs in Humans

Clinical deficiency. Few cases of clinical manifestations of biotin deficiency have been reported in humans. Those have occurred in patients supported by TPN without biotin supplementation, in nursing infants whose mothers' milk contained inadequate supplies of the vitamin,[43] in infants born with congenital biotinidase deficiency, and in adults eating egg whites. Signs included periorificial dermatitis, ketolactic acidosis, conjunctivitis, alopecia, hypotonia, ataxia, seizures, developmental delays, and increased risk to skin infections. One case involved a child fed raw eggs for 6 years. The signs and symptoms included dermatitis, glossitis, anorexia, nausea, depression, hepatic steatosis, and hypercholesterolemia. The impairments of lipid metabolism responded to biotin therapy (Table 15.8).

TABLE 15.8 Effects of Biotin Treatment on Abnormalities in Serum Fatty Acid Concentrations in a Biotin-Deficient Human

Fatty Acid	Normal Values	Biotin-Deficient Patient Values	
		Before Biotin	After Biotin
18:2ω6	21.56 ± 6.65	9.85[a]	5.36[a]
18:3ω6	0.21 ± 0.27	0.45	0.40
20:3ω6	3.67 ± 1.39	8.66[a]	10.62[a]
20:4ω6	12.49 ± 3.79	9.26	11.72
22:4ω6	1.87 ± 1.01	0.52[a]	0.71[a]
20:3ω9	1.30 ± 1.25	1.05	1.67
18:3ω3	0.21 ± 0.19	0.33	0.18
Total ω6 acids	41.08 ± 5.86	29.42[a]	29.61[a]
Total ω3 acids	5.23 ± 2.16	5.24	4.97
Total ω9 acids	13.14 ± 3.98	17.59[a]	16.4

[a] $p > .05$.
Mock, D.M., Johnson, S.B., Holman, R.T., 1988. J. Nutr. 118, 342–348.

The frequency of **marginal biotin status** (deficiency without clinical manifestation) is not known. Studies with validated biomarkers of biotin status indicate that subclinical biotin deficiency may occur in as many as one-third of pregnancies. That pregnant women experience increased catabolism of the vitamin is indicated by their increased urinary excretion of bisnorbiotin, biotin sulfoxide, and other biotin metabolites. Increased urinary excretion of 3-hydroxyisovaleric acid in late pregnancy has been found to respond to biotin supplementation.[44] Relatively low levels of biotin (versus healthy controls) have been reported in the plasma or urine of patients with partial gastrectomy or other causes of achlorhydria, burn patients, epileptics,[45] elderly individuals, alcoholics,[46] and athletes. It has been suggested that vegetarians may be at risk for deficiency; however, studies have failed to support that hypothesis. In fact, both plasma and urinary biotin levels of strict

39. $K_a = 1015\,M$.
40. Two similar biotin-binding proteins have been identified, both of which show considerable sequence homology with avidin at the biotin-binding site: **streptavidin** from *Streptomyces avidinii* and an epidermal growth factor homolog in the purple sea urchin *Strongylocentrotus purpuratus*.
41. Some cultured mammalian cells (e.g., fibroblasts and HeLa cells) are able to absorb the biotin–avidin complex, using it as a source of the vitamin.
42. Other structural analogs of biotin are also antagonistic to its function: α-dehydrobiotin, 5-(2-thienyl)valeric acid, acidomycin, α-methylbiotin, and α-methyldethiobiotin; several of these are antibiotics.
43. The biotin content of human milk, particularly early in lactation, is often insufficient to meet the demands of infants for which reason it is recommended that nursing mothers take a biotin supplement. That practice substantially increases the biotin contents of their breast milk, e.g., a 3 mg/day supplement increases milk biotin from 1.2–1.5 μg/dL to >33 μg/dL.
44. Mock, D.M., Stadler, D.D., Stratton, S.L., et al., 1997. J. Nutr. 127, 710–716.
45. This may be due to anticonvulsant drug therapy, known side effects of which are dermatitis and ataxia. Some anticonvulsants (e.g., carbamazepine, primidone) are competitive inhibitors of biotin transport across the intestinal brush border.
46. About 15% of alcoholics have plasma biotin concentrations <140 pM, a level shown by only 1% of nonalcoholics.

vegetarians (vegans) and lactoovovegetarians[47] have been found to exceed those of persons eating mixed diets. That hemodialysis can deplete patients of biotin is suggested by the finding that biotin supplementation reduced the severity of muscle cramps in hemodialysis patients.[48]

Deficiency Signs in Animals

Avidin-induced biotin deficiency causes the syndrome originally referred to as **egg white injury**. The major lesions appear to involve impairments in lipid metabolism and energy production. In rats and mice, this is characterized by seborrheic dermatitis and **alopecia**, a hind limb paralysis that results in **kangaroo gait**. In mice and hamsters, it involves teratogenic effects indicated by congenital malformations: cleft palate, micrognathia,[49] micromelia.[50] Fur-bearing animals (mink and fox) show general dermatitis with hyperkeratosis, circumocular alopecia ("spectacle eye"), **achromotrichia** of the underfur, and unsteady gait. Pigs and kittens show weight loss, digestive dysfunction, dermatitis, alopecia, and brittle claws. Guinea pigs and rabbits show weight loss, alopecia, and achromotrichia. Monkeys show severe dermatitis of the face, hands, and feet; alopecia; and watery eyes with encrusted lids. The dermatologic lesions of biotin deficiency relate to impairments of lipid metabolism; affected animals show reductions in skin levels of several long-chain fatty acids (16:0,[51] 16:1, 18:0, 18:1 and 18:2) with concomitant increased in certain others (in particular, 24:1 and 26:1). All species show depressed activities of the biotin-dependent carboxylases, which respond rapidly to biotin therapy.

Biotin deficiency can be produced in chicks by dietary deprivation and seems to occur sporadically in practical poultry production, particularly in northern Europe.[52] This results in impaired growth and reduced efficiency of feed utilization, and is characterized by circumocular alopecia (Fig. 15.3), dermatitis mainly at the corners of the beak, but also of the footpad (Fig. 15.4).[53] In some instances, death occurs suddenly without gross lesions; this condition usually involves hepatic and renal steatosis with hypoglycemia, lethargy, paralysis, and hepatomegaly, and is thus referred to as **fatty liver and kidney syndrome** (**FLKS**). The etiology of FLKS appears to be complex, involving such other factors as choline, but seems to involve a marginal deficiency of biotin that impairs gluconeogenesis by limiting the activity of pyruvate carboxylase, especially under circumstances of glycogen depletion brought on by stress.

FIGURE 15.3 Loss of circumocular alopecia ("spectacle eye") in the biotin-deficient chick. *Courtesy, G.F. Combs, Sr.*

10. BIOTIN IN HEALTH AND DISEASE

Birth Defects

It has been suggested that marginal biotin status may be teratogenic. Fetal malformations have been produced in mice (Table 15.9) and poultry by feeding maternal diets containing marginal biotin levels, i.e., amounts that did not produce clinical signs of deficiency in the dams. Because humans appear to have relatively poor transport of biotin across the placenta,[54] it has been suggested that human fetuses may be predisposed to biotin deficiency when maternal intakes of the vitamin are marginal. Support for this hypothesis comes from observations that the production of arachidonic acid and prostaglandins, which depend on acetyl-CoA carboxylase and PCC activities, is required for normal palatal plate growth, elevation and fusion in mice, and skeletal development in chicks. That marginal biotin status may be prevalent was suggested by the finding that apparently healthy pregnant women had increased rates of biotin excretion and abnormally low activities of PCC.[55]

Sudden Infant Death Syndrome

It has been suggested that marginal biotin status may play a role in the etiology of sudden infant death syndrome (SIDS), which occurs in human infants at 2–4 months of age. In many ways, SIDS resembles FLKS in the chick, which is caused by biotin deprivation. Studies have shown

47. Individuals eating plant-based diets that include dairy products and eggs.
48. Oguma, S., Ando, I., Hirose, T., et al., 2012. Tohoku J. Exp. Med. 227, 217–223.
49. Underdevelopment of the (usually lower) jaw.
50. Undergrowth the limbs.
51. i.e., A 16-carbon fatty acid with no double bonds.
52. In that part of the world, barley and wheat, each of which has little biologically available biotin, are frequently used as major ingredients in poultry diets.
53. **Footpad dermatitis** caused by biotin deficiency is often confused with the dermatologic lesions of the foot caused by pantothenic acid deficiency. Unlike the latter, biotin deficiency lesions are limited to the footpad and do not involve the toes and superior aspect of the foot.
54. Schenker, S., Hu, Z., Johnson, R.F., et al., 1993. Alcohol Clin. Exp. Res. 17, 566.
55. Mock, D.M., Stadler, D.D., 1997. J. Am. Coll. Nutr. 16, 252.

FIGURE 15.4 Footpad dermatitis in the chick (affected, left; biotin-adequate control, right). *Courtesy, G.F. Combs, Sr.*

TABLE 15.9 Effect of Maternal Egg White Feeding on Fetal Malformations in Mice

	Dietary Egg White, %						
Malformation	0	1	2	3	5	10	25
Cleft palate	0.10±0.13	0.25±0.25	2±2	4±2	10±1	11±1	12±0.4
Micrognathia	0	0	0.2±0.2	3±2	9±1	11±1	12±1
Microglossia	0	0	0	0.5±0.5	2±1	6±2	9±3
Hydrocephaly	0	0	0	0.3±0.2	2±1	3±1	3±2
Open eye	0	0	0	0	0.8±0.5	4±1	5±2
Forelimb hypoplasia	0	0	0	7±2	9±2	11±1	12±0.4
Hind limb hypoplasia	0	0	0	5±2	9±2	10±0.8	12±0.4
Pelvic girdle hypoplasia	0	0	0	5±2	9±2	10±1	12±0.5

Mock, D.M., Mock, N.I., Stewart, C.W., et al., 2003. J. Nutr. 133, 2519–2526.

that infants who died of SIDS had significantly lower hepatic concentrations of biotin than did those who died of unrelated causes.[56]

11. BIOTIN TOXICITY

The toxicity of biotin appears to be very low. No cases have been reported of adverse reactions by humans to high levels (doses as high as 200 mg orally or 20 mg intravenously) of the vitamin, as are used in treating seborrheic dermatitis in infants, egg white injury, or inborn errors of metabolism. Animal studies have revealed few, if any, indications of toxicity, and it is probable that animals, including humans, can tolerate the vitamin at doses at least an order of magnitude greater than their respective nutritional requirements. Upper tolerable intakes have not been established for biotin.

56. Johnson, A.R., Hood, R.L., Emery, J.L., 1980. Nature 285, 159.

12. CASE STUDY

Instructions

Review the following case report, paying special attention to the diagnostic indicators on which the treatments were based. Then, answer the questions that follow.

Case

A 12-month-old girl had experienced malrotation[57] and midgut volvulus,[58] resulting in extensive infarction[59] of the small and large bowel at 4 months of age. Her bowel was resected, after which her clinical course was complicated by failure of the anastomosis[60] to heal, peritoneal

57. Failure of normal rotation of the intestinal tract.
58. Twisting of the intestine, causing obstruction.
59. Necrotic changes resulting from obstruction of an end artery.
60. An operative union of two hollow or tubular structures, in this case the divided ends of the intestine.

infection, and intestinal obstruction. After several subsequent surgeries, she was left with only 30 cm of jejunum, 0.5 cm of ileum, and approximately 50% of colon. By 5 months of age, she had lost 1.5 kg in weight and TPN[61] was initiated (providing 125 kcal/kg/day). By the third month of TPN, she had gained 2.9 kg; thereafter, her energy intake was reduced to 60 kcal/kg/day, which sustained her growth within the normal range. Soybean oil emulsion[62] was administered parenterally at least twice weekly in amounts that provided 3.9% of total calories as linoleic acid. Repeated attempts at feeding her orally failed because of vomiting and rapid intestinal transit; therefore, her only source of nutrients was TPN. She had repeated episodes of sepsis and wound infection; broad-spectrum antibiotics were administered virtually continuously from 4 to 11 months of age. Multiple enteroenteric and enterocutaneous fistulas[63] were formed; over 8 months, they provided daily fluid losses >500 mL.

During the third month of TPN, an erythematous[64] rash was noted on the patient's lower eyelids adjacent to the outer canthi.[65] Over the next 3 months, the rash spread became more exfoliative and exuded clear fluid. New lesions appeared in the angles of the mouth, around the nostrils, and in the perineal region.[66] This condition did not respond to topical application of various antibiotics, cortisone, and safflower oil.

During the fifth and sixth months of TPN, the patient lost all body hair developed a waxy pallor, irritability, lethargy, and mild hypotonia.[67] That she was not deficient in essential fatty acids was indicated by the finding that her plasma fatty acid triene-to-tetraene ratio was normal (0.11). During the period from the third to the sixth month, the patient was given parenteral zinc supplements at 7, 30, and 250 times the normal requirement (0.2 mg/day). Her serum zinc concentration increased from 35 to 150 µg/dL (normal, 50–150 µg/dL) and, finally, to greater than 2000 µg/dL without any beneficial effect. Intravenous zinc supplementation was then reduced to 0.4 mg/day. Biotin was determined by a bioassay using *Ochromonas danica*; urinary organic acids were determined by HPLC[68] and GC/MS[69]:

61. Feeding by means other than through the alimentary canal, referring particularly to the introduction of nutrients into veins.
62. e.g., Intralipid.
63. Passages created between one part of the intestine and another (an enteroenteric fistula) or between the intestine and the skin of the abdomen (an enterocutaneous fistula).
64. Marked by redness of the skin owing to inflammation.
65. Corners of the eye.
66. The area between the thighs extending from the coccyx to the pubis.
67. A condition of reduced tension of any muscle, leading to damage by overstretching.
68. High-performance liquid–liquid partition chromatography.
69. Gas–liquid partition chromatography with mass spectrometric detection.

Laboratory Results

Parameter	Patient	Normal Range
Plasma biotin	135 pg/mL	215–750 pg/mL
Urinary biotin excretion	<1 µg/24 h	6–50 µg/24 h
Urinary Organic Acid Excretion		
Methylcitrate	0.1 µmol/mg creatinine	<0.01 µmol/mg creatinine
3-Methylcrotonylglycine	0.7 µmol/mg creatinine	<0.2 µmol/mg creatinine
3-Hydroxyisovalerate	0.35 µmol/mg creatinine	<0.2 µmol/mg creatinine

Treatment with biotin (10 mg/day) was initiated and, after 1 week, the plasma biotin concentration increased to 11,500 pg/mL and organic acid excretion dropped to <0.01 µmol/mg creatinine. After 7 days of biotin supplementation, the rash had improved strikingly and the irritability had resolved. After 2 weeks of supplementation, new hair growth was noted, waxy pallor of the skin was less pronounced, and hypotonia improved. During the next 9 months of biotin therapy, no symptoms and signs of deficiency recurred. The patient's rapid transit time and vomiting did not improve.

Case Questions

1. What signs were first to indicate a problem related to biotin utilization by the patient?
2. What is the relevance of aciduria to considerations of biotin status?
3. How were problems involving essential fatty acids and zinc ruled out in the diagnosis of this condition as biotin deficiency?

13. STUDY QUESTIONS AND EXERCISES

1. Diagram the areas of metabolism in which biotin-dependent carboxylases are involved.
2. Construct a decision tree for the diagnosis of biotin deficiency in humans or an animal species.
3. What key feature of the chemistry of biotin relates to its biochemical function as a carrier of active CO_2?
4. What parameters might you measure to assess biotin status of a human or animal?

RECOMMENDED READING

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Chapter 16

Pantothenic Acid

Chapter Outline

Anchoring Concepts

1. Pantothenic acid is the trivial designation for the compound dihydroxy-β,β-dimethylbutyryl-β-alanine.
2. Pantothenic acid is metabolically active as the prosthetic group of coenzyme A (CoA) and the acyl carrier protein.
3. Deficiencies of pantothenic acid are manifested as dermal, hepatic, thymic, and neurologic changes.

A pellagrous-like syndrome in chicks has recently been obtained...in an experiment that was originally designed to throw added light upon an unusual type of leg problem occurring in chicks fed semi-synthetic rations....The data obtained in this experiment demonstrate the requirement in another species of the vitamin or vitamins present in autoclaved yeast, occasionally called vitamin B_2, vitamin G or the P–P factor, and indicate that the chick may be a more suitable animal than the white rat for delineating the quantities of this vitamin present in feedstuffs.

L.C. Norris and A.T. Ringrose[1]

LEARNING OBJECTIVES

1. To understand the chief natural sources of pantothenic acid.
2. To understand the means of absorption and transport of pantothenic acid.
3. To understand the biochemical functions of pantothenic acid as components of coenzyme A and the acyl carrier protein.
4. To understand the physiological implications of low-pantothenic acid status.

VOCABULARY

Acetyl CoA
Acyl carrier protein (ACP)
Acyl-CoA synthetase (ACS)
Burning feet syndrome
CoA synthetase
Coenzyme A (CoA)
Dephospho-CoA kinase
Dexpanthenol
Fatty acid synthetase
Malonyl CoA
ω-Methylpantothenic acid
Pantothenate kinase (PanK)
Pantothenic acid
Pantothenol
Pantetheine
Pantetheinase
Pantetheinase
Phosphopantetheine adenylytransferase
Phosphopantetheine-apo-ACP transferase
Phosphopantothenylcysteine decarboxylase
Phosphopantothenylcysteine synthase
4′-Phosphopantetheine

1. Leo Chandler Norris (1891–1986) was a pioneering American nutritionist and a founder of the nutrition programs at Cornell University. He did seminal work on "vitamin G" (later, riboflavin) and the "animal protein factor" (later, vitamin B_{12}), and discovered the essentiality of manganese. One of Norris's students, Arthur T. Ringrose (1908-?), went on to a career as a poultry nutritionist at the University of Kentucky. His students also included Milton L. Scott and Gerald F. Combs, the senior author's major professor and father, respectively.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00016-2

4′-Phosphopantothenic acid
Propionyl CoA
Sodium-dependent multivitamin transporter (SMVT)
Succinyl CoA

1. THE SIGNIFICANCE OF PANTOTHENIC ACID

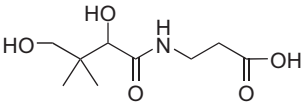
Pantothenic acid is widely distributed in many foods. Clinical deficiencies of the vitamin are rare. Pantothenic acid functions as the essential precursor of **coenzyme A (CoA),** which is used by many cellular enzymes, and **acyl carrier protein** (**ACP**). In these forms, pantothenic acid plays essential roles in the metabolism of fatty acids, amino acids, and carbohydrates, and has important roles in the acylation of proteins. Although pantothenic acid is required to produce CoA, rates of CoA synthesis are not affected by deprivation of the vitamin. From such observations, it can be inferred that the vitamin is recycled metabolically; however, definitive understanding of the mechanisms involved remains incomplete.

2. PROPERTIES OF PANTOTHENIC ACID

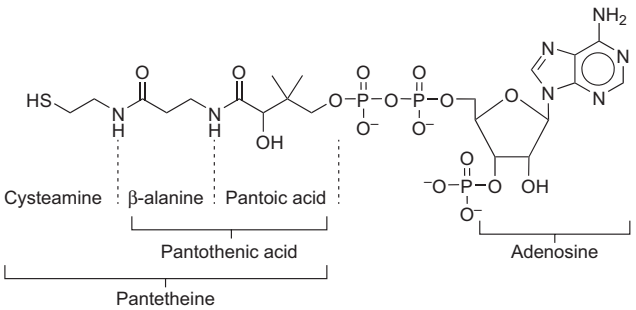
Pantothenic acid is the trivial designation for the compound dihydroxy-β,β-dimethylbutyryl-β-alanine.[2] It consists of β-alanine joined to 2,4-dihydroxy-3,3-dimethylbutyric acid by an amide linkage, and it is optically active. The vitamin has two metabolically active forms:

- **Coenzyme A (CoA)**[3] in which pantothenic acid has a phosphodiester linkage with adenosine-3′5′-diphosphate
- **ACP** in which pantothenic acid has a phosphodiester linkage with a serinyl residue of the protein.

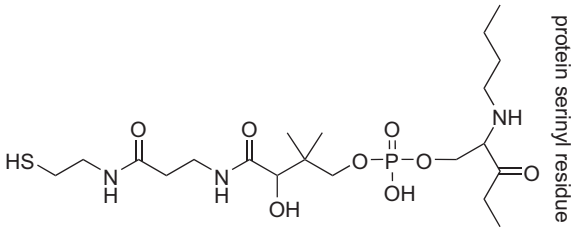
Chemical structures of pantothenic acid:



Pantothenic acid



Coenzyme A (showing its constituent parts)



Acyl-carrier protein

Pantothenic Acid Chemistry

Pantothenic acid has an asymmetric center; only the *R*-enantiomer, usually called **d-(+)pantothenic acid**, is biologically active and occurs naturally. Pantothenic acid is a yellow, viscous oil. Its calcium and other salts, however, are colorless and crystalline; calcium pantothenate is the main product of commerce. Neither form is soluble in organic solvents, but each is soluble in water and ethanol. Aqueous solutions of pantothenic acid are unstable to heating under acidic or alkaline conditions, resulting in the hydrolytic cleavage of the molecule (to yield β-alanine and 2,4-dihydroxy-3,3-dimethylbutyrate). The analog panthenol (in which the carboxyl group is replaced by a hydroxymethyl group) is fairly stable in solution. In dry form, the salts are stable to air and light; but they (particularly sodium pantothenate) are hygroscopic.

3. SOURCES OF PANTOTHENIC ACID

Hindgut Microbial Synthesis

Pantothenic acid can be produced by the microbiome of the colon. A genomic analysis of 256 representative organisms of the human gut microbiota found more than half capable of de novo synthesis of the vitamin.[4] However, the total synthetic capacity appeared to be low, e.g., <0.1% of the daily human need. While direct evidence is lacking for the absorption of pantothenic acid across the colon, one study found it necessary to use an antibiotic to produce signs of pantothenic acid deficiency in the mouse.[5] Pantothenic acid has been shown to be produced by some rumen microorganisms.[6] That pantothenic acid deficiency has not been reported in ruminants is consistent with their microbiome being a nutritionally significant source of the vitamin.

Distribution in Foods

As its name implies, pantothenic acid is widely distributed in nature (Table 16.1). It occurs mainly in bound forms

2. Formerly known as *pantoyl*-β-alanine.
3. Studies with liver slices in vitro have demonstrated a correlation between hepatic CoA content and lipid biosynthetic capacity, suggesting that CoA may be a limiting factor in lipogenesis.
4. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.
5. Stein, E.D., Diamond, J.M., 1989. J. Nutr. 119, 1973–1983.
6. *Escherichia coli* and *Streptococcus bovis* (Ford, J.E., Perry, K.D., Briggs, C.A.E., 1958. J. Gen. Microbiol. 18, 273–284; Porter, W.G., 1961. Vitamin synthesis in the rumen. In: Lewis, D. (Ed.), Digestive Physiology and Nutrition of the Ruminant. Butterworths, London, p. 226–233).

TABLE 16.1 Pantothenic Acid Contents of Foods

Food	Pantothenic Acid, mg/100 g
Dairy Products	
Milk	0.34–0.37
Cheeses	0.08–1.73
Meats	
Beef	0.31–0.67
Pork	0.40–1.71
Chicken giblets	2.97
Cereals	
Cornmeal	0.43
Rice, brown	0.38
Oats	1.35
Wheat flour	0.44
Wheat bran	2.18
Barley	0.14
Vegetables	
Asparagus	0.23
Broccoli	0.57
Cabbage	0.21
Carrots	0.27
Cauliflower	0.67
Lentils	2.14
Potatoes	0.56
Soybeans	0.15
Tomatoes	0.09
Fruits	
Apples	0.06
Bananas	0.33
Grapefruits	0.28
Oranges	0.26
Strawberries	0.13
Nuts	
Cashews	1.27
Peanuts	1.01
Walnuts	1.66
Other	
Eggs	1.53
Mushrooms	0.41–21.9
Bakers' yeast	13.5

From USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

(CoA, CoA esters, ACP). A glycoside has been identified in tomatoes. Therefore, it must be determined in foods and feedstuffs after enzymatic hydrolysis to liberate the vitamin from CoA. This is done in a two-step procedure using alkaline phosphatase followed by avian hepatic **pantetheinase**, yielding "total" pantothenic acid.

The most important food sources of pantothenic acid are meats (liver and heart are particularly rich). Mushrooms, avocados, broccoli, and some yeasts are also rich in the vitamin; however, cooking, canning, and freezing can produce losses of 35–80%. Whole grains are also good sources; however, the vitamin is localized in the outer layers, thus, it is largely (up to 50%) removed by milling. The most important sources of pantothenic acid for animal feeding are rice and wheat brans, alfalfa, peanut meal, molasses, yeasts, and condensed fish solubles. The richest sources of the vitamin in nature are cold water fish ovaries (>2.3 mg/g)[7] and royal jelly (>0.5 mg/g).[8]

Stability

Pantothenic acid in foods and feedstuffs is fairly stable to ordinary means of cooking and storage. It can, however, be unstable to heat and either alkaline (pH > 7) or acid (pH < 5) conditions.[9] Reports indicate losses of 15–50% from cooking meat and of 37–78% from heat-processing vegetables. The alcohol derivative, pantothenol, is more stable; for this reason, it is used as a source of the vitamin in multivitamin supplements.

Bioavailability

The biologic availability of pantothenic acid from foods and feedstuffs is a function of the efficiency of the enteric hydrolysis of its food forms and the absorption of those products. This area has not been well investigated. One study indicated "average" bioavailability of the vitamin in the American diet to be in the range of 40–60%;[10] similar results were obtained for maize meals in another study.[11]

4. ABSORPTION OF PANTOTHENIC ACID

Hydrolysis of Coenzyme Forms

Because pantothenic acid occurs in most foods and feedstuffs as CoA and ACP, the utilization of the vitamin in

7. Tuna, cod.
8. Royal jelly, the food responsible for the diet-induced reproductive development of the queen honeybee, is also the richest natural source of biotin.
9. Pasteurization of milk, because it occurs at neutral pH, does not affect its content of pantothenic acid.
10. Tarr, J.B., Tamura, T., Stokstad, E.L., 1981. Am. J. Clin. Nutr. 34, 1328–1337.
11. Yu, B.H., Kies, C., 1993. Plant Food Hum. Nutr. 43, 87–95.

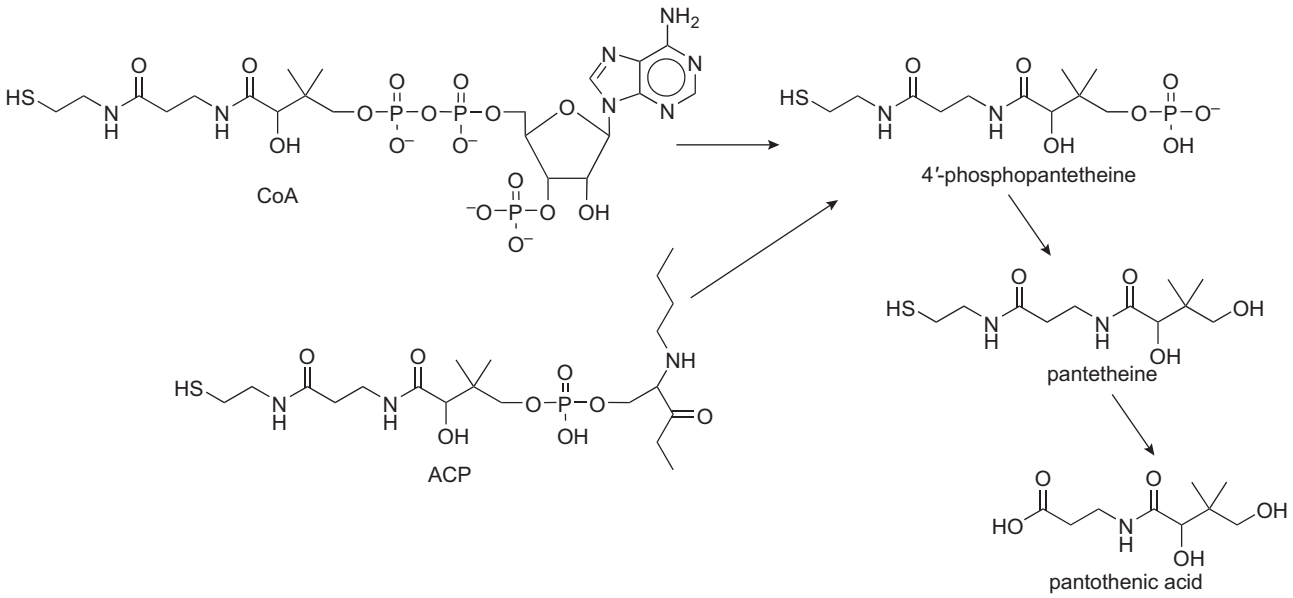


FIGURE 16.1 Liberation of pantothenic acid from coenzyme forms in foods.

foods depends on the hydrolytic digestion of these protein complexes to release the free vitamin. Both CoA and ACP are degraded in the intestinal lumen by hydrolases (pyrophosphatase, phosphatase) to release the vitamin as **4′-phosphopantetheine** (Fig. 16.1). That form is dephosphorylated to yield **pantetheine**, which is absorbed or converted to **pantothenic acid** by another intestinal hydrolase, **pantetheinase.**

Free pantothenic acid is absorbed by two mechanisms:

- **Active transport** at low luminal concentrations. Pantothenic acid[12] is absorbed by a saturable mechanism facilitated by **Na^+-dependent multivitamin transporter** (**SMVT**) located on the apical membrane of epithelial brush boarder, particularly villus, cells. This transporter has an apparent K_m of 10–20 μM[13] for pantothenic acid. It also facilitates the uptake of biotin and lipoic acid and can be inhibited by alcohol. The SMVT facilitates the exchange of the pantothenic acid for protons, driven by an extracellularly directed proton gradient. Pantothenic acid appears to be able to cross the enterocyte either in free solution or bound to SMVT via trafficking vesicles and the microtubule network and the motor protein dynein. It is thought that translocation of the vitamin across the basolateral membrane to the portal circulation involves another SMVT.
- **Passive diffusion** at higher luminal concentrations. The alcohol form, **panthenol,** which is oxidized to pantothenic acid in vivo, appears to be diffuse somewhat faster than the acid form.

12. Concentrations after a typical meal have been estimated at 1–2 μM.
13. Said, H.M., 2011. Biochem. J. 437, 357–372.

5. TRANSPORT OF PANTOTHENIC ACID

Plasma and Erythrocytes

Plasma contains the vitamin only in the free acid form. Erythrocytes carry 20–55% of the vitamin in the blood.[14]

Cellular Uptake

Pantothenic acid is taken into cells in its free acid form. In most tissues, this is mediated by the membrane SMVT; however, uptake by erythrocytes and the brain occurs by diffusion.[15] The active uptake of pantothenic acid results in its cellular concentrations being much greater (liver, 10–15 μM; heart ~100 μM) than those of plasma (1–5 μM). Upon cellular uptake, most of the vitamins combine with cysteamine,[16] adenine, and ribose-3′-phosphate, converting it to CoA, the predominant intracellular form, 70–90% of which is in the mitochondria. Erythrocytes metabolize the vitamin to 4′-phosphopantothenic acid, which, lacking the enzymes to produce CoA, they accumulate.

Tissue Distribution

The greatest concentrations of CoA are found in the liver, adrenals, kidneys, brain, heart, and testes.[17] Much of this (70% in liver, 95% in heart) is located in the mitochondria. Tissue CoA concentrations are not affected by deprivation of

14. For example, in the human adult, whole blood contains 1120–1960 ng/mL of total pantothenic acid; of that, plasma contains 211–1096 ng/mL. Blood pantothenic acid levels are generally lower in elderly individuals, e.g., 500–700 ng/mL.
15. Spector, R., 1986. Am. J. Physiol. 250, R292–R297.
16. i.e., Mercaptoethylamine.
17. The human liver typically contains ca. 28 mg of total pantothenic acid (ca.15 μM); that of heart is about 150 μM.

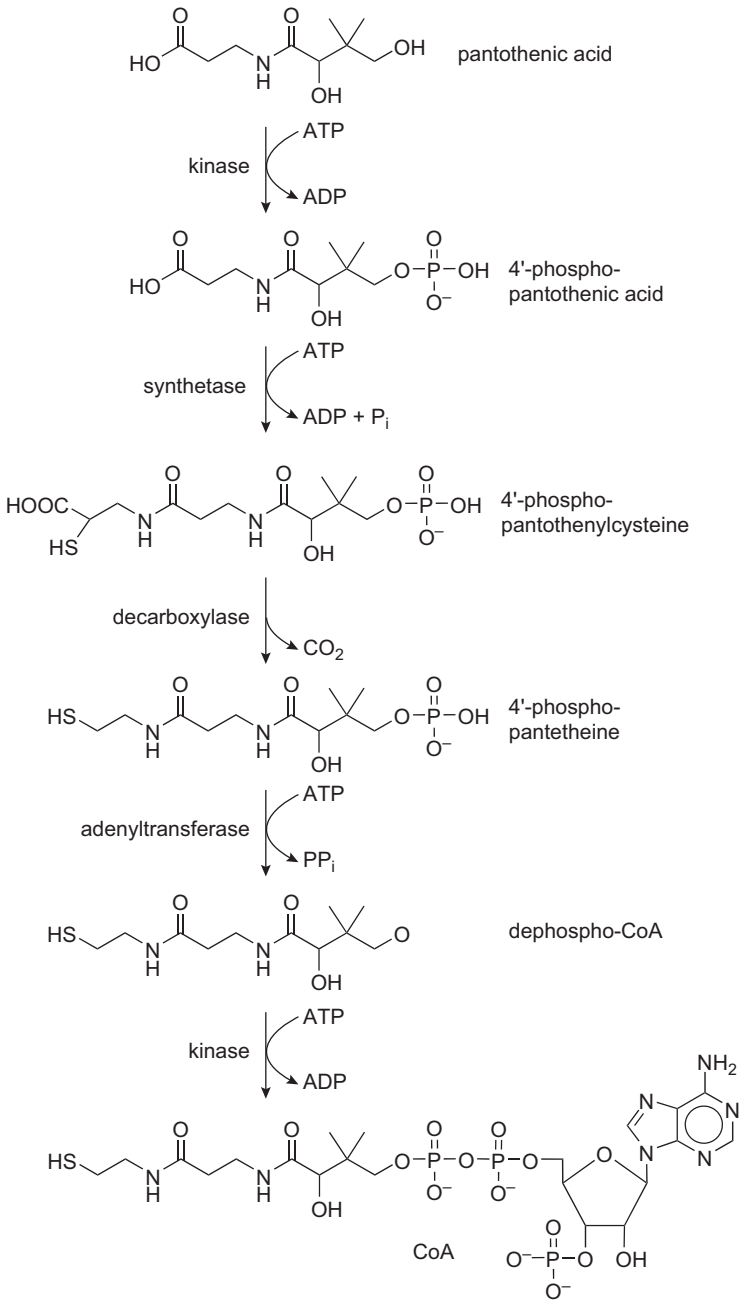


FIGURE 16.2 Biosynthesis of coenzyme A.

the vitamin. This surprising finding has been interpreted as indicating a mechanism for conserving the vitamin by recycling it from the degradation of pantothenate-containing molecules. Pantothenic acid is taken up in the choroid plexus by a specific transport process, which, at low concentrations of the vitamin, involves the partial phosphorylation of the vitamin. The cerebrospinal fluid, because it is constantly renewed in the central nervous system, requires a constant supply of pantothenic acid, which, as CoA, is involved in the synthesis of the neurotransmitter acetylcholine in brain tissue.

6. METABOLISM OF PANTOTHENIC ACID

CoA Synthesis

All tissues have the ability to synthesize CoA from pantothenic acid. At least in rat liver, all of the enzymes in the CoA biosynthetic pathway are found in the cytosol. Four moles of ATP are required for the biosynthesis of a mole of CoA from a single mole of pantothenic acid. The process (Fig. 16.2) is initiated in the cytosol and completed in the mitochondria.

In the cytosol,

1. **Pantothenate kinase (PanK)** catalyzes the ATP-dependent phosphorylation of pantothenic acid to yield **4′-phosphopantothenic acid.** This is the rate-limiting step in CoA synthesis; under normal conditions, it functions far below capacity. Four isoforms have been identified:[18]
   a. **PanK1** expressed primarily in the heart, liver, and kidney.[19]
   b. **PanK2** expressed in all tissues.[20]
   c. **PanK3** expressed (to a high degree) only in liver.
   d. **PanK4** expressed in most tissues, with highest concentrations in muscle.

   The PanKs are inducible[21] and feedback inhibited by 4′-phosphopantothenic acid, CoA esters and, more weakly, CoA and long-chain acyl CoAs all of which are allosteric effectors. Inhibition by CoA esters can be reversed by carnitine. The ethanol metabolite acetaldehyde inhibits the conversion of pantothenic acid to CoA.[22]
2. **Phosphopantothenylcysteine synthetase** catalyzes the ATP-dependent condensation of 4′-phosphopantothenic acid with cysteine to yield **4′-phosphopantothenylcysteine.**
3. **Phosphopantothenylcysteine decarboxylase** catalyzes the decarboxylation of 4′-phosphopantothenylcysteine to yield **4′-phosphopantetheine**, which is transported into the mitochondria.

   In mitochondria, two steps are catalyzed by a single bifunctional enzyme, **CoA synthetase**,[23] located in the inner membrane:
4. **Phosphopantetheine adenyltransferase** catalyzes the ATP-dependent adenylation of 4′-phosphopantetheine to yield **dephospho-CoA.** This reaction is reversible; therefore, at low ATP levels, dephospho-CoA can be degraded to yield ATP.
5. **Dephospho-CoA kinase** catalyzes the ATP-dependent phosphorylation of dephospho-CoA to yield CoA.

The mitochondrial concentration of nonacylated CoA determines the rate of oxidation-dependent energy production. CoA can also enter mitochondria by nonspecific membrane binding as well as by an energy-dependent membrane transporter.[24]

Acyl-CoA Synthesis

CoA serves to activate long-chain fatty acids for various key metabolic roles. These roles include regulation of enzymes and signaling pathways, oxidation to provide cellular energy, and incorporation into acylated proteins and complex lipids. The addition of long-chain fatty acids to CoA is catalyzed by a large family of **acyl-CoA synthetases (ACSs).** This energy-dependent esterification occurs in two steps:

1. Fatty acid + ATP → acyl-AMP + PPi
2. Acyl-AMP + CoASH → acyl CoA + AMP

More than two dozen isoforms of ACS have been identified in mammalian tissues. Most cells have multiple forms; hence, it has been suggested that each form may direct its fatty acid substrates along a specific metabolic route.

Acyl Carrier Protein Synthesis

In higher animals, ACP is associated with a large **fatty acid synthetase** complex composed of two 250 kDa subunits containing several functional domains.[25] The ACP domain is synthesized as an inactive apoprotein but is modified posttranslationally by the addition of the 4′-phosphopantetheine prosthetic group via a phosphoester linkage at a serinyl residue. This modification is catalyzed by **4′-phosphopantetheine-apo-ACP transferase** using CoA as the donor (Fig. 16.3). It is, therefore, likely that ACP synthesis serves as a regulator of intracellular CoA levels.

Pantothenic Acid Recycling

The pantothenic acid components of both CoA and ACP are released metabolically for reutilization. ACP is degraded by an ACP hydrolase that releases 4′-phosphopantetheine yielding apo-ACP. CoA can be catabolized by a nonspecific, phosphate-sensitive, lysosomal phosphatase to dephospho-CoA,

18. Of three distinct types of kinases that occur in various species, those of eukaryotes are grouped in the PanK2 class.
19. The mouse shows two (α, β) variants of PanK1, produced by alternate splicing of the same gene.
20. In humans, PanK2 is located in mitochondria, but its strong inhibition by acetyl CoA ($IC_{50} < 1\ \mu M$) would suggest that it functions at physiological concentrations of acetyl CoA, which exceed that level (Leonardi, R., Rock, C.O., Jackowski, S., et al., 2007. Proc. Natl. Acad. Sci. U.S.A. 104, 1494–1499). However, that PanK2 does, in fact, play a functional role is indicated by that fact that an autosomal recessive PanK2 mutation occurs in Hallervorden–Spatz syndrome, a neurodegenerative disorder presenting as dystonia and optic atrophy or retinopathy (Delgado, R.F., Sanchez, P.R., Speckter, H., et al., 2012. J. Magn. Reson. Imaging 35, 788–794).
21. PanK is induced by the antilipidemic drug clofibrate. Treatment with clofibrate increases hepatic concentrations of CoA, apparently owing to increased synthesis.
22. Alcoholics have been reported to excrete in their urine large percentages of the pantothenic acid they ingest, a condition corrected on ethanol withdrawal.
23. In plants and prokaryotes, these steps are catalyzed by separate enzymes.
24. Tahiliani, A.G., Neely, J.R., 1987. J. Mol. Cell. Cardiol. 19, 1161–1167.
25. Fatty acid synthase complex has several catalytic sites: acetyl transferase, malonyl transferase, 2-oxoacyl synthase, oxoacyl reductase, 3-hydroxyacyl dehydratase, enoyl reductase, and thioester hydrolase.

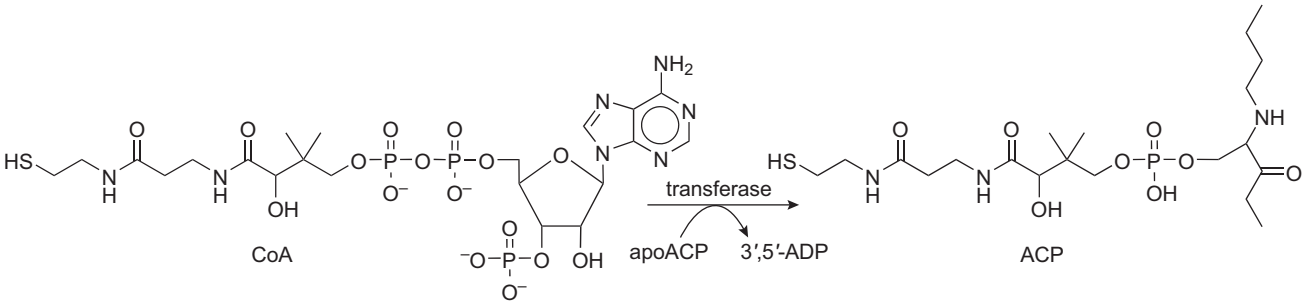


FIGURE 16.3 Coenzyme A provides 4-phosphopantetheine in the biosynthesis of the acyl carrier protein.

which is degraded to 4′-phosphopantetheine by a plasma membrane pyrophosphatase. 4′-Phosphopantetheine from either source is dephosphorylated by microsomal and lysosomal phosphatases to pantetheine, from which pantothenic acid is liberated by membrane **pantetheinases**.[26]

Excretion

Pantothenic acid is excreted mainly in the urine as free pantothenic acid and some 4′-phosphopantothenate; no catabolic products are known. The renal tubular secretion of pantothenic acid, probably by a mechanism common to weak organic acids, results in urinary excretion of the vitamin correlating with dietary intake. An appreciable amount (~15% of daily intake) is oxidized completely and is excreted across the lungs as CO_2. Humans typically excrete in the urine 0.8–8.4 mg of pantothenic acid per day. There appear to be two renal mechanisms for regulating the excretion of pantothenic acid:

- **Active transport** at physiological concentrations of the vitamin in the plasma, with pantothenic acid being reabsorbed by active transport;
- **Tubular secretion** at higher concentrations, tubular reabsorption appears to be the only mechanism for conserving free pantothenic acid in the plasma.

Disorders of Pantothenic Acid Metabolism.

- A polymorphism of PanK2 has been identified as the metabolic basis of an autosomal recessive neurodegenerative disorder, Hallervorden–Spatz syndrome. Affected subjects show dystonia and optic atrophy or retinopathy with the deposition of iron in basal ganglia.[27]

26. These are products of the so-called Vanin (i.e., *VNN1*) gene; they are widely expressed in all tissues, with greatest amounts in kidney, liver, intestine, and lymphoid cells and have significant sequence homology with, but not the activity of, biotinidases. Pantetheinases also appear to have roles in inflammatory responses via their product cysteamine. Studies with animal models in which pantetheinases were genetically deleted have shown reduced gut inflammatory reactions to drug and parasitic stimuli and failed cytokine responses to stress.

27. Gordon, N., 2002. Eur. J. Paediatr. Neurol. 6, 243–247.

7. METABOLIC FUNCTIONS OF PANTOTHENIC ACID

General Functions

Both CoA and 4′-phosphopantetheine in ACP function metabolically as carriers of acyl groups and activators of carbonyl groups in a large number of vital metabolic transformations, including the tricarboxylic acid cycle and the metabolism of fatty acids. In each case, the linkage with the transported acyl group involves the reactive sulfhydryl of the 4′-phosphopantetheinyl prosthetic group.

Different metabolic roles of active forms of pantothenic acid:

- **CoA**—in a broad array of acyl transfer reactions in oxidative energy metabolism and catabolism
- **ACP**—in synthetic reactions.

Acyl CoAs

Scope of functions. Acyl CoAs serve as essential cofactors for some 4% of known enzymes, including at least 100 enzymes involved in intermediary metabolism. CoA functions widely in metabolism in reactions involving either the carboxyl group (e.g., formation of acetylcholine, acetylated amino sugars, acetylated sulfonamides[28]) or the methyl group (e.g., condensation with oxaloacetate to yield citrate) of an acyl CoA. In these reactions, CoA forms high-energy thioester bonds with carboxylic acids, the most important of which is acetic acid, which can come from the metabolism of fatty acids, amino acids, or carbohydrates (Fig. 16.4).

Acetyl CoA, the "*active acetate*" group has many metabolic functions:

- **Acetylations** of alcohols, amines, and amino acids (e.g., choline, sulfonamides, *p*-aminobenzoate).

28. Coenzyme A was discovered as an essential factor for the acetylation of sulfonamide by the liver and for the acetylation of choline in the brain; hence, **coenzyme A** stands for **coenzyme for acetylations**.

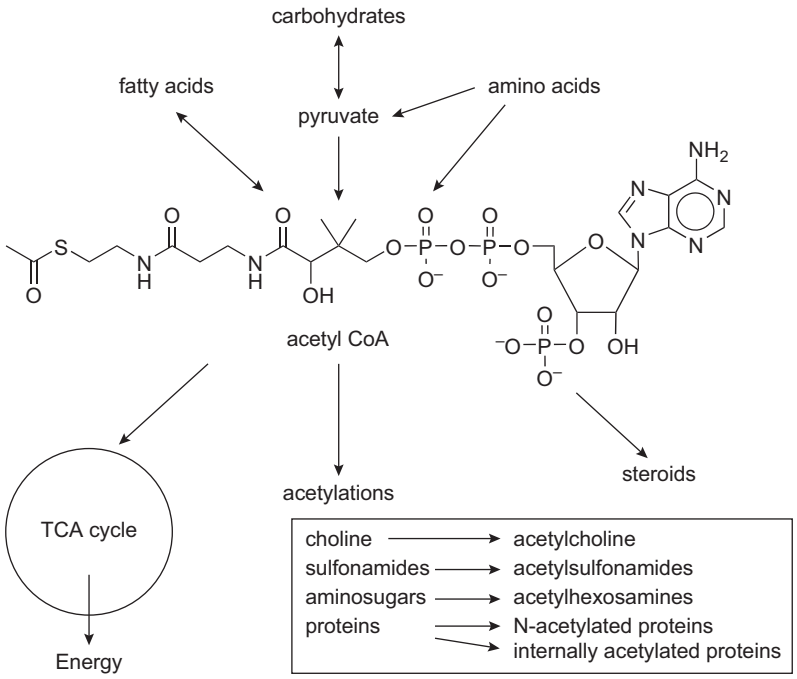


FIGURE 16.4 The central role of acetyl CoA in metabolism.

- **Activation of fatty acids** for incorporation into triglycerides, cholesterol, steroid hormones, prostaglandins, leukotrienes, membrane phospholipids, and regulatory sphingolipids.
- **Synthesis of fatty acids** by addition of 2-carbon fragments from acetyl CoA.
- **Transacylation to carnitine** to form energy-equivalent acylcarnitines capable of being transported into the mitochondria where β-oxidation occurs.[29]
- **Posttranslational long-chain acetylation of proteins,** an estimated half of proteins are acylated[30] at N-terminal residues (most frequently at terminal serinyl or alanyl residues) or internal lysyl residues. This includes the cotranslational processing of peptide hormones from their precursors (e.g., ACTH (adrenocorticotropic hormone) to α-melanocyte-stimulating hormone; β-lipotropin to β-endorphin). In most cases, acylation is without functional significance and can be reversed by NAD-dependent deacylases. In other cases, acylation is required for protein function: acylation of steroid hormone receptors and other regulatory proteins; acylation of α-tubulin, which stabilize microtubules;[31] acylation/deacylation of histones,[32] which affect chromatin packing and, thus, gene expression;[33] and S-acylation of Ras proteins,[34] conferring control of their subcellular trafficking.[35] Most acylations involve palmitic acid[36] (e.g., GTP-binding proteins, protein kinases, membrane receptors, cytoskeletal proteins, mitochondrial proteins) in reversible ester bonds; others involve myristic acid[37] in irreversible amide linkages.
- **Transcriptional regulation** via PPARs (peroxisome proliferator-activated receptors) α, β, and γ.
- **Production of the** "ketone body" acetoacetate derived from fat metabolism when glucose is limiting.

Regulation of acetyl CoA. The abundance of acetyl CoA reflects the energy state of the cell and can affect the activities of many enzymes. The amounts of nonacylated CoA reflect the activities of the various intracellular acyl-CoA thioesterases and acyltransferases in mediating cellular lipid metabolism. They also determine the rate of

29. This is the only means by which long-chain fatty acids can enter the mitochondria for energy production.
30. Stadtman, E.R., 1990. Biochemistry 29, 6323–6331.
31. Acetylation occurs in the a-tubulin after it has been incorporated into the microtubule. It can be induced by such agents as taxol. Acetylated microtubules are more stable to depolymerizing agents such as colchicines.
32. Acetylated histones are enriched in genes that are being actively transcribed.
33. Yasui, K., Matsuyama, T., Ito, T., 2005. Seikagaku 77, 498–504.
34. Ras proteins are located near cell membranes and participate in the regulation of cell division; abnormalities in these proteins can lead to uncontrolled cell division and tumorigenesis.
35. Smotrys, J,E., Linder, M.E., 2004. Annu. Rev. Biochem. 73, 559–587; Rowinsky, E.K., Windle, J.J., Von Hoff, D.D., 1999. J. Clin. Oncol. 17, 3631–3652.
36. *n*-Hexadecanoic acid, C16:0.
37. *n*-Tetradecanoic acid, C14:0.

oxidation-dependent energy production by mitochondria. Steady-state concentrations of CoA (20–150 μM in cytosol; ~2 mM in mitochondria) have been shown to respond to deprivation of food, glucose feeding, and insulin or glucagon treatment. These effects can be countered through the transfer of acyl CoA between subcellular compartments and the reduction of 4′-phosphopantetheine adenyltransferase and dephospho-CoA kinase through a catabolic activity of CoA synthetase.

Acyl Carrier Protein

ACP is a component of the multienzyme complex **fatty acid synthetase**.[38] In ACP, the cofactor functions in two domains, acetyl transferase and malonyl transferase, which transfer the respective acyl groups between 4´-phosphopantetheine at different active sites with successive cycles of condensations and reductions.[39] The nature of the fatty acid synthase complex varies considerably among different species. However, in each, 4´-phosphopantetheine is the prosthetic group for the binding and transfer of the acyl units to a 8.7 kD subunit during catalysis. The sulfhydryl group of the cofactor serves as the point of temporary covalent attachment of the growing fatty acid via a thiol linkage each time an acyl group is added by transfer to the cofactor. In this way, the cofactor appears to function as a swinging arm, allowing the growing fatty acid to reach the various catalytic sites of the enzyme.

8. BIOMARKERS OF PANTOTHENIC ACID STATUS

Pantothenic acid status can be assessed in two ways:[40]

- **Urinary metabolites**—The urinary excretion of pantothenic acid is considered the most reliable indicator of pantothenic acid status.
- **Blood metabolites**—Whole blood or plasma pantothenic acid levels reflect the level of intake of the vitamin. Healthy adults typically show whole blood levels in the range of 1.6–2.7 μM; values < 1 μM indicate suboptimal status.

38. Fatty acid synthase is the name used to identify the multienzyme complex on which the several reactions of fatty acid synthesis (condensations and reductions) occur. In higher animals, the complex is composed of two large (250 kDa) subunits.

39. The seven functional activities of the fatty acid synthase complex are acetyltransferase, malonyltransferase, 3-ketoacyl synthase, 3-ketoacyl reductase, 3-hydroxyacyl dehydratase, enoyl reductase, and thioester hydrolase.

40. A microbiological assay employing *Lactobacillus plantarum* is commonly used for the analysis of pantothenic acid. This requires enzymatic pretreatment of specimens to liberate free pantothenic acid.

TABLE 16.2 General Signs of Pantothenic Acid Deficiency

Organ System	Signs
General	Depressed appetite, growth
Vital organs	Hepatic steatosis, thymic necrosis, adrenal hypertrophy
Dermatologic	Dermatitis, achromotrichia, alopecia
Muscular	Weakness
Gastrointestinal	Ulcers
Nervous	Ataxia, paralysis

9. PANTOTHENIC ACID DEFICIENCY

Deficiencies Rare

Deprivation of pantothenic acid results in metabolic impairments including reduced lipid synthesis and energy production. Signs and symptoms of pantothenic acid deficiency vary among different species; most frequently, they involve the skin, liver, adrenals, and nervous system. Owing to the wide distribution of the vitamin in nature, dietary deficiencies of pantothenic acid are rare; they are more common in circumstances of inadequate intake of basic foods and vitamins and are often associated with (and mistakenly diagnosed as) deficiencies of other vitamins. Understanding of the presentation of pantothenic acid deficiency comes mostly from studies with experimental animals. These have shown a pattern of general deficiency signs (Table 16.2). Recommended intakes for pantothenic acid have been established (Table 16.3).

Antagonists

Pantothenic acid deficiency has been produced experimentally using purified diets free of the vitamin or by administering an antagonist. One antagonist is the analogue **ω-methylpantothenic acid**, which has a methyl group in place of the hydroxymethyl group of the vitamin; this change prevents it from being phosphorylated and inhibits the action of pantothenic acid kinase. Other antagonists include desthio-CoA, in which the terminal sulfhydryl of the active metabolite is replaced with a hydroxyl group, and hopantenate, in which the three-carbon β-alanine moiety of the vitamin is replaced with the four-carbon γ-aminobutyric acid.

Deficiency Signs in Humans

Pantothenic acid deficiency in humans has been observed only in severely malnourished patients and in subjects treated with the antagonist ω-methylpantothenic acid. In cases of the

TABLE 16.3 Recommended Pantothenic Acid Intakes

The United States		FAO/WHO	
Age Status	RDA[a], µg/day	Age Status	RNI[b], µg/day
0–6 months	[1.7][c]	0–6 months	1.7
7–11 months	[1.8][c]	7–11 months	1.8
1–3 years	2	1–3 years	2
4–8 years	3	4–6 years	3
9–13 years	4	7–9 years	4
>13 years	5	>9	5
Pregnancy	6	Pregnancy	6
Lactation	7	Lactation	7

[a]Recommended Dietary Allowances; Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.
[b]Recommended Nutrient Intakes; Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. WHO, Rome, 286 pp.
[c]RDA has not been established; adequate intake is presented.

former type, neurologic signs (paresthesia in the toes and sole of the feet) have been reported.[41] Subjects made deficient in pantothenic acid through the use of ω-methylpantothenic acid also developed burning sensations of the feet. In addition, they showed depression, fatigue, insomnia, vomiting, muscular weakness, and sleep and gastrointestinal disturbances. Changes in glucose tolerance, increased sensitivity to insulin, and decreased antibody production have also been reported.

There is some evidence of subclinical pantothenic acid deficiency. Urinary pantothenic acid excretion has been found to be low for pregnant women, adolescents, and the elderly compared with the general population.

Deficiency Signs in Animals

Pantothenic acid deficiency in most species results in reduced growth and reduced efficiency of feed utilization. In rodents, the deficiency presents as scaly dermatitis, achromotrichia, alopecia, and adrenal necrosis.[42] Congenital malformations of offspring of pantothenic acid-deficient dams have been reported. Excess amounts of porphyrins[43] are excreted in the tears of pantothenic acid-deficient rats in a condition called **blood-caked whiskers**. In the chick, deficiency presents as lesions at the corners of the mouth, swollen and encrusted eyelids, dermatitis of the entire foot (with hemorrhagic cracking),[44] poor feathering, fatty liver degeneration, thymic necrosis, and myelin degeneration of the spinal column with paralysis and lethargy.[45] Chicks produced from deficient hens show high rates of embryonic and posthatching mortality. In the dog, deficiency presents as hepatic steatosis, irritability, cramps, ataxia, convulsions, alopecia, and death.[46] In the pig, deficiency presents as dermatitis, acute encephalopathy, hypoglycemia, hyperammonemia, excessive lachrymation, colitis, spastic gait, hypertrophy, and steatosis of multiple organs (e.g., adrenals, liver, heart); and ovarian atrophy with impaired uterine development.[47]

Pantothenic acid deficiency would not be expected in ruminants if ruminal microbial synthesis of the vitamin is significant. In fact, deficiency has not been reported in ruminants, although one study found plasma pantothenic acid concentrations of cows to respond to dietary supplementation with the vitamin in a dose-dependent way.[48]

Marginal deficiency of pantothenic acid in the rat has been found to produce elevated serum levels of triglycerides and free fatty acids. The metabolic basis of this effect is not clear; however, it is possible that it involves a somewhat targeted reduction in cellular CoA concentrations, affecting the deposition of fatty acids in adipocytes (via impaired acyl-CoA synthetase) but not the hepatic production of triglycerides.

10. PANTOTHENIC ACID IN HEALTH AND DISEASE

Benefits have been reported for the use of supplements of pantothenic acid and/or metabolites.

Reduced Serum Cholesterol Level

High doses (500–1200 mg/day) of pantotheine, the dimmer of pantetheine, have been shown to reduce serum concentrations of total and LDL cholesterol and triglycerides, with increases in HDL cholesterol[49]. While the underlying mechanism is unclear, it is thought to involve roles of pantetheine

41. **Burning feet syndrome** was described during World War II in American prisoners held in Japan and the Philippines, who were generally malnourished. That large oral doses of calcium pantothenate provided some improvement suggested that the syndrome involved, at least in part, deficiency of pantothenic acid.
42. Pietrzik, K., Hesse, C.H., Zur, W., et al., 1975. Int. J. Vitam. Nutr. Res. 45, 153–162.
43. e.g., Protoporphyrin IX.
44. These lesions are often confused with the footpad dermatitis caused by biotin deficiency. Unlike the latter, in which lesions are limited to the footpad (i.e., plantar surface), the lesions produced by pantothenic acid deficiency also involve the toes and superior aspect of the foot. Prevention of footpad dermatitis is economically important in poultry production, the US–European market for chicken and duck feet has been estimated to exceed $300 M.
45. Gries, C.L., Scott, M.L., 1972. J. Nutr. 102, 1269–1285.
46. Noda, S., Haratake, J., Sasaki, A., et al., 1991. J. Neurol. Neurosurg. Psychiatr. 51, 582–585.
47. Nelson, R.A., 1968. Am. J. Clin. Nutr. 21, 495–501.
48. Bonomi, A., 2000. Rivista. Sci. dell'Aliment. 29, 321–338.
49. Binaghi, P., Cellina, G., Lo Cicero, G., et al., 1990. Minerva Med. 81, 475–479.

as a cofactor in shunting acetyl groups away from steroid synthesis to oxidative metabolism and/or in reducing triglyceride synthesis through inhibition of hydroxymethylglutaryl-CoA reductase.

Rheumatoid Arthritis

Patients with RA have been found to exhibit lower blood pantothenic acid levels than healthy controls. Nearly 50 years ago, an unblinded trial found relief of symptoms in 20 patients treated with pantothenic acid.[50] A subsequent randomized, controlled trial showed that high doses (up to 2 g/day) of calcium pantothenate reduced the duration of morning stiffness, the degree of disability, and the severity of pain for rheumatoid arthritis patients.[51]

Athletic Performance

While pantothenic acid deficiency is known to reduce exercise endurance in animal models, results of the few studies conducted in humans have been inconsistent. Some results showed improved efficiency of oxygen utilization and reduced lactate acid accumulation in athletes;[52] others showing no benefits.[53]

Wound Healing

Studies in animal models have found pantothenic acid, given orally or topically as pantothenol, to promote the closure of wounds of the skin by facilitating the recruitment of fibroblasts to the injured area.[54] Studies with humans given high, combined doses of pantothenic acid and ascorbic acid have shown no benefits,[55] although a derivative **dexpanthenol** has been found useful in reducing skin dehydration and irritation.[56]

Other Outcomes

It has been suggested that pantothenic acid may have value in treating the systemic autoimmune disease lupus erythematosus, the theoretical argument based on the observation that lupus can be caused by drugs that impair pantothenic acid metabolism. No relevant clinical data have been reported. It has also been proposed that pantothenic acid may have value in the prevention of graying hair.[57] That, too, is without substantiating evidence. There has been interest in the prospect of developing pantothenic acid antagonists for the treatment of malaria, as *Plasmodium falciparum* has been shown to require pantothenic acid, which it typically obtains from erythrocytes.

11. PANTOTHENIC ACID TOXICITY

The toxicity of pantothenic acid is negligible. No adverse reactions have been reported in any species following the ingestion of large doses of the vitamin. Massive doses (e.g., 10 g/day) administered to humans have not produced reactions more severe than mild gastrointestinal distress and diarrhea. Similarly, no deleterious effects have been identified when the vitamin was administered parenterally or topically. It has been estimated that animals can tolerate doses of pantothenic acid as great as at least 100 times their respective nutritional requirements for the vitamin. No upper tolerable intakes for pantothenic acid have been established.

12. CASE STUDY

Review the following experiment, paying special attention to the independent and dependent variables in the design. Then, answer the questions that follow.

Experiment

To evaluate the possible role of pantothenic acid and ascorbic acid in wound healing, a study was conducted assessing the effects of these vitamins on the growth of fibroblasts. Human fibroblasts were obtained from neonatal foreskin; they were cultured in a standard medium supplemented with 10% fetal calf serum and antibiotics.[58] The medium contained no ascorbic acid but contained 4 mg of pantothenic acid per liter. Cells were used between the third and ninth passages. Twenty-four hours before each experiment, the basal medium was replaced by medium supplemented with pantothenic acid (40 mg/L) or pantothenic acid (40 mg/L) plus ascorbic acid (60 mg/L). Cells (1.5×10^5) were plated in 3 mL of culture medium in 28-cm^2 plastic dishes. After incubation, they were collected by adding trypsin and then scraping; they were counted in a hemocytometer. The synthesis of DNA and protein was estimated by measuring the rates of incorporation of radiolabel from [^{3}H]thymidine and [^{14}C]proline, respectively. Total protein was measured in cells (lysed by sonication and solubilized in 0.5 *N* NaOH) and in the culture medium.

50. Subjects were given calcium pantothenate i.m. (Barton-Wright, E.C., Elliot, W.A., 1963. Lancet 2, 862–869).
51. U.S. Practitioner Research Group, 1980. Practitioner 224, 208–2015.
52. Litoff, D., 1985. Med. Sci. Sports Exerc. 17S, 287–294.
53. Nice, C., Reeves, A.G., Brinck-Johnson, T., et al., 1984. J. Sports Med. Phys. Fitness 24, 26–29.
54. Weimann, B.I., Hermann, D., 1999. Int. J. Vitam. Nutr. Res. 69, 113–119.
55. Vaxman, F., Olender, S., Lambert, A., et al., 1995. Eur. Surg. Res. 27, 158–166.
56. Biro, K., Thaci, D., Ochsendorf, F.R., et al., 2003. Contact Dermat. 49, 80–84.
57. That pantothenic acid deficiency can cause achromotrichia in rodents does not imply that graying of human hair is caused by low-pantothenic acid status, nor that supplemental pantothenic acid could have any effect. Indeed, no such effects have been demonstrated.
58. Gentamicin and amphotericin B (Fungizone).

Results After 5 Days of Culture

	Cells	^{3}H	^{14}C	Cell Protein	Protein in Medium
Treatment	($\times 10^5$)	(10^3 cpm)	(10^3 cpm)	(mg/dish)	(mg/mL)
Control	2.90 ± 0.16	11.6 ± 0.4	1.7 ± 1.0	10.0 ± 1.0	1.93 ± 0.01
+ Pantothenic acid	3.83 ± 0.14[a]	18.7 ± 0.5[a]	2.9 ± 0.1[a]	14.5 ± 0.9[a]	1.93 ± 0.02
+ Pantothenic acid and ascorbic acid	3.74 ± 0.19[a]	18.1 ± 0.8[a]	2.8 ± 0.1[a]	8.1 ± 0.9	2.11 ± 0.01[a]

[a]Significantly different from control value, $p < .05$.

Case Questions

1. Why were thymidine and proline selected as carriers of the radiolabels in this experiment?
2. Why were fibroblasts selected (rather than some other cell type) for use in this study?
3. Assuming that the protein released into the culture medium is largely soluble procollagen, what can be concluded about the effects of pantothenic acid and/or ascorbic acid on collagen synthesis in this system?
4. What implications do these results have regarding wound healing?

13. STUDY QUESTIONS AND EXERCISES

1. Diagram the areas of metabolism in which CoA and ACP (via fatty acid synthase) are involved.
2. Construct a decision tree for the diagnosis of pantothenic acid deficiency in humans or an animal species.
3. What key feature of the chemistry of pantothenic acid relates to its biochemical functions as a carrier of acyl groups?
4. What parameters might you measure to assess pantothenic acid status of a human or animal?

RECOMMENDED READING

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Kirby, B., Roman, N., Kobe, B., et al., 2010. Functional and structural properties of mammalian acyl-coenzyme thioesterases. Prog. Lipid Res. 49, 366–377.

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Chapter 17

Folate

Chapter Outline

Anchoring Concepts

1. Folate is the generic descriptor for folic acid (pteroylmonoglutamic acid) and related compounds exhibiting qualitatively the biological activity of folic acid. The term folates refers generally to the compounds in this group, including mono- and polyglutamates.
2. Folates are active as coenzymes in single-carbon metabolism.
3. Deficiencies of folate are manifested as anemia and dermatologic lesions.

Using Streptococcus lactis *R as a test organism, we have obtained in a highly concentrated and probably nearly pure form an acid nutrilite with interesting physiological properties. Four tons of spinach have been extracted and carried through the first stages of concentration…. This acid, or one with similar chemical and physiological properties, occurs in a number of animal tissues of which liver and kidney are the best sources…. It is especially abundant in green leaves of many kinds, including grass. Because of this fact, we suggest the name "folic acid" (Latin, folium—leaf). Many commercially canned greens are nearly lacking in the substance.*

Mitchell et al.[1]

1. Herschel K. Mitchell (1914–2000) was a biochemist at Stanford and Caltech Universities who did pioneering work in the field of molecular genetics. Esmond E. Snell (1914–2003) was a biochemist at the Universities of Wisconsin, Texas, and California–Berkeley whose work on the nutritional requirements of lactic acid bacteria led to his discoveries of pyridoxal and pyridoxamine; elucidation of the mechanisms of vitamin B_6-dependent enzymes; and to the development of microbiological assays for several vitamins, antivitamins, and growth factors. Roger J. Williams (1893–1988) was an Indian-born American biochemist at the Universities of Oregon, Oregon State and Texas. He is best known for discovering pantothenic acid and naming folic acid.

LEARNING OBJECTIVES

1. To understand the chief natural sources of folates.
2. To understand the means of absorption and transport of the folates.
3. To understand the biochemical functions of the folates as coenzymes in single-carbon metabolism and the relationship of that function to the physiological activities of the vitamin.
4. To understand the metabolic interrelationship of folate and vitamin B_{12} and its physiological implications.

VOCABULARY

p-Acetaminobenzoylglutamate
S-adenosylhomocysteine (SAH)
S-adenosylmethionine (SAM)
p-Aminobenzoylglutamate
Aminopterin
Antifolates
Arsenicosis
Betaine
Carboxypeptidase
Cerebral folate deficiency syndrome
Cervical paralysis
7,8-Dihydrofolate reductase
Dihydrofolic acid (FH_2)
Folacin
Folate
Folate-binding proteins (FBPs)
Folate export pump
Folate receptor (FRs)

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00017-4

Folic acid
Folyl conjugase (folyl γ-glutamyl carboxypeptidase)
Folylpolyglutamates
Folylpolyglutamate synthase
5-Formimino-FH_4
Formiminoglutamate (FIGLU)
5-Formyl-FH_4
10-Formyl-FH_4
γ-Glutamyl hydrolase
Hereditary folate malabsorption (HFM)
Homocysteine (Hcy)
Homocysteinemia
Leukopenia
Macrocytic anemia
Megaloblasts
5,10-Methenyl-FH_4
Methionine synthase
Methionine synthase reductase
Methotrexate
5-Methyl FH_4
Methylation index
5,10-Methylene-FH_4
5,10-Methylene-FH_4 dehydrogenase
5,10-Methylene-FH_4 reductase (MTHFR)
Methyl folate trap
Plasmodium
Methylmalonic acid (MMA)
Neural tube defects (NTDs)
Organic anion transporter (OAT)
Pernicious anemia
Proton-coupled folate transporter (PCFT)
Pteridine
Pterin ring
Pteroylglutamic acid
Pteroylmonoglutamic acid
Purines
Pyrazine nucleus
Reduced folate carrier (RFC)
Serine hydroxymethyltransferase
Single-carbon pool
Sulfa drugs
Tetrahydrofolate reductase
Tetrahydrofolic acid (FH_4)
Tetrahydropteroylglutamic acid
Thymidylate
Thymidylate synthase
Vitamin B_{12}

1. THE SIGNIFICANCE OF FOLATE

Folate is a vitamin that has only recently been appreciated for its importance beyond its essential role in normal metabolism, especially for its relevance to the etiologies of chronic diseases and birth defects. Widely distributed among foods, particularly those of plant foliar origin, this abundant vitamin is underconsumed by people whose food habits do not emphasize plant foods. Intimately related in function with vitamins B_{12} and B_6, its status at the level of subclinical deficiency can be difficult to assess and the full extent of its interrelationships with these vitamins and with amino acids remains incompletely elucidated. Folate deficiency is an important problem in many parts of the world, particularly where there is poverty and malnutrition. It is an important cause of anemia, second only to nutritional iron deficiency.

Evidence shows that marginal folate intakes can support apparently normal circulating folate levels while still limiting single-carbon metabolism. Thus, folate emerged as having an important role in the etiology of homocysteinemia, which was identified as a risk factor for occlusive vascular disease, cancer, and birth defects, particularly **neural tube defects** (**NTDs**). In 1998, the U.S. Food and Drug Administration mandated that folic acid be added to all "enriched" cereal grain products (breads, pastas, wheat flours, breakfast cereals, rice) to reduce the prevalence of NTDs. The food system-wide measure increased the folate intakes of Americans, more than doubled circulating levels of the vitamin and was expected to reduce both NTDs and coronary artery disease deaths, while also driving folate supplementation efforts in other countries.

More than two decades of population-based folate, supplementation has seen the prevalence of NTDs decline, indicating that this strategy has been successful. Still, concerns remain about potential risks of treating individuals who are not in need. The first of these was the prospective masking the **macrocytic anemia** of vitamin B_{12} deficiency, which will lead to neuropathy if not corrected. Additional concerns have been added in recent years with growing doubt about the role of **homocysteinemia** in the etiology of cardiovascular disease, with reports of enhanced cognitive impairment and colorectal cancer risk as a consequence of folate supplementation. For these reasons, it is important to understand the role of folate in nutrition and health.

2. PROPERTIES OF FOLATE

Folate Nomenclature

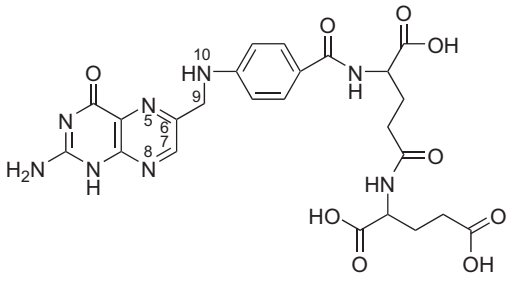
The term **folate** is the generic descriptor for **folic acid** (**pteroylmonoglutamic acid** or **pteroylglutamic acid**) and related compounds exhibiting the biological activity of folic acid. This group, often collectively referred to as **folacin**, **folic acids**, and **folates**, is comprised of large number of pteridine derivatives varying in degree of hydrogenation of pteridine nucleus and capable of binding single-carbon units to nitrogens at position 5 and/or 10. They also have one or more glutamyl residues linked via peptide bonds and are named for the number of glutamyl residues (*n*), using

TABLE 17.1 Key Members of the Folate Family

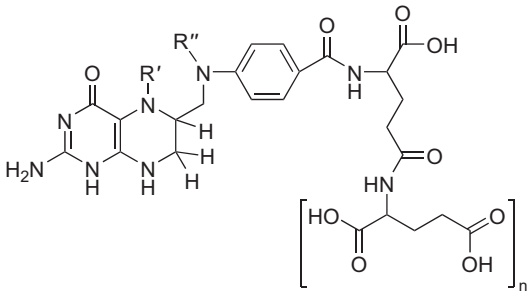
Vitamer	Abbreviation	R′ (at N-5)	R (at N-10)
Tetrahydrofolic acid	FH_4	H	H
5-Methyltetrahydrofolic acid	5-CH_3-FH_4	CH_3	H
5,10-Methenyltetrahydrofolic acid	5,10-CH^+-FH_4	-CH^+-(bridge)	
5,10-Methylenetetrahydrofolic acid	5,10-CH_2=FH_4	-CH_2=(bridge)	
5-Formyltetrahydrofolic acid	5-HCO-FH_4	HCO	H
10-Formyltetrahydrofolic acid	10-HCO-FH_4	H	HCO
5-Forminintetrahydrofolic acid	5-HCNH-FH_4	HCNH	H

such notations as **PteGlu*n***. The fully reduced compound **tetrahydropteroylglutamic acid** is called **tetrahydrofolic acid**; its single-carbon derivatives are named according to the specific carbon moiety bound. Key members of the folate family are listed in Table 17.1.

Chemical Structures of the Folate Group



Pteroylglutamic acid



Tetrahydrofolic acid and its derivatives

Folate Chemistry

The folates include a large number of chemically related species. With three reduction states of the **pyrazine nucleus**, six different single-carbon substituents on N-5 and/or N-10, and as many as eight glutamyl residues on the benzene ring, more than 170 different folates are theoretically possible. Not all of these occur in nature, but it has been estimated that as many as 100 different forms are found in animals. The compound called folic acid (pteroylmonoglutamic acid) is probably not present in living cells, rather an artifact of isolation of the vitamin. The folates from most natural sources usually have a single-carbon unit at N-5 and/or N-10; these forms participate in the metabolism of the **single-carbon pool**. The single-carbon units that may be transported and stored by folates can vary in oxidation state from the methyl (e.g., 5-CH_3-FH_4) to the formyl (e.g., 5-HCO-FH_4, 10-HCO-FH_4). Intracellular folates contain poly-γ-glutamyl chains usually of 2–8 glutamyl residues, sometimes extending to 12 in bacteria. Tissues contain enzymes called **conjugases** that hydrolytically remove glutamyl residues to release the monoglutamyl form, folic acid. The folylpolyglutamates are thought to be the active intracellular coenzyme forms. The monoglutamates, which can pass through membranes, appear to be transport forms.

Folates have an asymmetric center at C-6, which provides stereospecificity in the orientation of hydrogen atoms on reduction of the pteridine system; that is, they add to carbons 6 and 7 in positions below the plane of the pyrazine ring. The UV absorption spectra of the folates are characterized by the independent contributions of the pterin and 4-aminobenzoyl moieties; most have absorption maxima in the region of 280–300 nm.

Folic acid (pteroylmonoglutamic acid) is an orange-yellow crystalline substance that is soluble in water but insoluble in ethanol or less polar organic solvents. It is unstable to light, to acidic or alkaline conditions, to reducing agents, and, except in dry form, to heat. It is reduced in vivo enzymatically (or in vitro with a reductant such as dithionite) first to 7,8-dihydrofolic acid (FH_2) and then to FH_4; both of these compounds are unstable in aerobic environments and must be protected by the presence of an antioxidant (e.g., ascorbic acid, 2-mercaptoethanol). Two derivatives of folic acid, each having an amino group in the place of the hydroxyl at C-4, are folate antagonists of biomedical use: **aminopterin** (a rodenticide, 4-aminofolic acid)

and **methotrexate** (an antineoplastic agent, 4-amino-N^{10}-methylfolic acid).[2]

3. SOURCES OF FOLATE

Synthesis by the Gut Microbiome

The microflora of the hindgut, particularly *Bacteroides* spp., can synthesize folates in amounts that can contribute significantly to meeting daily needs.[3] A genomic analysis of 256 representative organisms of the human gut microbiota found 43% capable of de novo synthesis of the vitamin, with a total synthetic capacity equivalent to at least 37% of the daily human need.[4] Folate biosynthesis is affected by dietary factors that affect the gut microbiome, e.g., dietary fiber, oligosaccharides, probiotics. Folates can be absorbed across the human colon,[5] with an efficiency of some 46%.[6] Whether other species show similar hindgut absorptive capacities is not clear; pigs fed a prebiotic preferentially used by *Bacteroides* spp. markedly increased their colonic microbial biosynthesis of folate without affecting their circulating levels of the vitamin.[7]

Distribution in Foods

Folates (**folylpolyglutamates**) occur in a wide variety of foods of both plant and animal origin (Table 17.2). Liver, mushrooms, and green leafy vegetables are rich sources of folate in human diets; while oilseed meals (e.g., soybean meal) and animal by-products are important sources of folate in animal feeds. The folates in foods and feedstuffs are almost exclusively in reduced form as polyglutamyl derivatives of **tetrahydrofolic acid** (**FH_4**). Very little free folate (folyl monoglutamate) is found in foods or feedstuffs.

Analyses of foods have revealed a wide distribution of polyglutamyl folate derivatives, the predominant forms being **5-methyl-FH_4** and **10-formyl-FH_4**. The folates found in organ meats (e.g., liver and kidney) are about 40% methyl derivatives, whereas that in milk (and erythrocytes) is exclusively the methyl form. Some plant tissues also contain mainly 5-methyl-FH_4 (e.g., lettuce, cabbage, orange juice); others (e.g., soybean) contain relatively little of that form (~15%), the rest occurring as the 5- and 10-formyl derivatives. Most of the folates in cabbage are hexa- and heptaglutamates; whereas, half of those in soybean are monoglutamates. More than one-third of the folates in orange juice are present as monoglutamates and nearly half are present as pentaglutamates. Liver and kidney contain mainly pentaglutamates, and ~60% of the folates in milk are monoglutamates (with only 4–8% each of di- to hepta-glutamates).

Folate Fortification and Supplementation

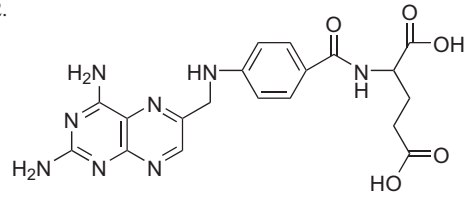
Since 1998, American law has mandated that all "enriched" cereal grain products (wheat flour, breads, pastas and breakfast cereals, and rice) be fortified with folic acid (140 µg/100 g); it has also permitted addition of folic acid to infant formulae, medical and special dietary foods, meal replacement products, and energy bars and drinks. More than 50 other countries have similar policies for folate fortification of grain products. The US folate fortification program increased folate intakes to medians of some 288 µg/day for adults,[8] and 489–656 µg/day for children 1–13 years[9] and more than doubled circulating levels of the vitamin (Table 17.3).

Human milk. The predominant form of folate in human milk is 5-methyltetrahydrofolate bound to **folate-binding proteins (FBPs)**, which stimulate the enteric absorption of the vitamin. Prenatal supplementation was found to increase folic acid as a percentage of breast milk total folates without increasing that total.[10]

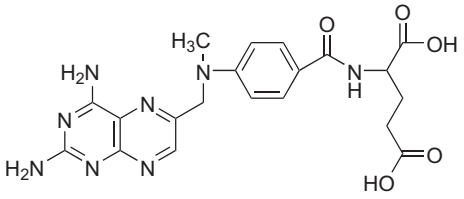
Stability

Most folates in foods and feedstuffs (that is, folates other than **folic acid**[11] and **5-formyl-FH_4**) are easily oxidized and,

2.



aminopterin



methotrexate

3. Rossi, M., Amaretti, A., Raimondi, S., 2011. Nutrients 3, 118–134.
4. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.
5. Aufreiter, S., Gregory, J.F., Pfeiffer, C.M., et al., 2009. Am. J. Clin. Nutr. 90,116–123; Lakoff, A., Fazili, F., Aufreiter, S., et al., 2014. Am. J. Clin. Nutr. 100, 1278–1286.
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9. Greater folate intakes of children reflect their greater consumption of fortified breakfast cereals; Bailey, R.L., Dodd, K., Gashche, J.J., et al., 2010. Am. J. Clin. Nutr. 91, 231–237.
10. West, A.A., Yan, J., Perry, C.A., et al., 2012. Am. J. Clin. Nutr. 96, 789–800.
11. Throughout this text, the term **folic acid** is used as the specific trivial name for the compound **pteroylglutamic acid**.

TABLE 17.2 Folate Contents of Foods

Food	Folate, μg/100 g
Dairy Products	
Milk	5
Cheese	5–65
Meats	
Beef	7–10
Chicken	3–9
Pork	0–12
Turkey	6–9
Beef liver	140–1070
Chicken liver	1810
Tuna	15
Cereals	
Barley	14
Cornmeal	25
Rice, white	3
Rice, brown	9
Wheat flour	44
Wheat bran	79
Vegetables	
Asparagus	149
Beans	33–106
Broccoli	63
Cabbage	43
Cauliflower	57
Peas	42
Soybeans	211
Spinach	194
Tomatoes	15
Fruits	
Apples	3
Bananas	20
Oranges	34
Others	
Eggs	47
Bakers' yeast	785

From USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

TABLE 17.3 Changes in Plasma Folate and Homocysteine Levels in the United States Since Implementation of Folate Fortification of Cereals

Year	Plasma Folate		Plasma Hcy	
	ng/mL	% ≤6.8 nM	μM	% ≤13 μM
1988–94	12.1 ± 0.3[a]	18.4	8.7 ± 0.1	13.2
1999–2000	30.2 ± 0.7[b]	0.8	7.0 ± 0.8	4.5
2001–02	27.8 ± 0.5[b]	0.2	7.3 ± 0.1	4.7

[a] *Mean ± S.E.*
[b] $p < .05$.
Ganji, V., Kafai, M.R., 2006. J. Nutr. 136, 153–158.

therefore, are unstable to oxidation under aerobic conditions of storage and processing. Under such conditions (especially in the added presence of heat, UVB light, and/or metal ions), FH_4 derivatives can be oxidized to the corresponding derivatives of **dihydrofolic acid** (**FH_2**) (partially oxidized) or folic acid (fully oxidized), some of which can react further to yield physiologically inactive compounds. For example, the two predominant folates in fresh foods, 5-methyl-FH_4 and 10-formyl-FH_4, are converted to 5-methyl-5,6-FH_2 and 10-formylfolic acid, respectively. For this reason, 5-methyl-5,6-FH_2 has been found to account for about half of the folate in most prepared foods. Although it can be reduced to the FH_4 form (e.g., by ascorbic acid), in the acidity of normal gastric juice, it isomerizes to yield 5-methyl-5,8-FH_2 which is completely inactive. It is of interest to note that, owing to their gastric acidosis, this isomerization does not occur in pernicious anemia patients, who are, thus, able to utilize the partially oxidized form by absorbing and subsequently activating it to 5-methyl-FH_4. Because some folate derivatives of the latter type can support the growth responses of test microorganisms used to measure folates,[12] some information in the available literature may overestimate the biologically useful folate contents of foods and/or feedstuffs. Substantial losses in the folate contents of food can occur as the result of leaching in cooking water when boiling (losses of total folates of 22% for asparagus and 84% for cauliflower have been observed), as well as oxidation, as described earlier. Due to such losses, green leafy vegetables can lose their value as sources of folates despite their relatively high natural contents of the vitamin. Photodegradation of folates in blood has been observed in patients with psoriasis given phototherapy with high cumulative doses of narrowband UVB

12. *Lactobacillus casei*, *Streptococcus faecium* (formerly, *Streptococcus lactis* R. and *Streptococcus faecalis*, respectively), and *Pediococcus cerevisiae* (formerly, *Leuconostoc citrovorum*) have been used. Of these, *Lactobacillus casei* responds to the widest range of folates.

irradiation.[13] Such degradation is accelerated by endogenous photosensitizers such as flavins and porphyrins but is suppressed by bilirubin and protein binding.

Bioavailability

The biological availability of folates in foods has been difficult to assess. Additional sources of error come from factors affecting the utilization of food folates:

- **antifolates** that bind to the food matrices or inhibit the intestinal brush border folyl conjugase.
- **inherent differences in folyl glutamates**.
- **nutritional status of the host**, e.g., deficiencies of iron and vitamin C can impair the utilization of dietary folate.[14] Vitamin C enhances the utilization of 5-methyl-FH_4 by preventing its oxidative degradation to 5-methyl-FH_2, which does not enter the folate metabolic pool.

Interactions of these factors complicate the task of predicting the bioavailability of dietary folates. This problem is exacerbated by the methodological difficulties in evaluating folate utilization, which has been approached with bioassays with animal models,[15] and with studies in humans using erythrocyte folate response, dilution of stable isotope labeled folate or urinary folate excretion. Each has limitations and sources of error.

The result is that estimates of the bioavailability of food folates (Table 17.4) show high interindividual variation but generally indicate that

- folic acid is virtually completely bioavailable;
- folic acid is similarly highly bioavailable from fortified foods;
- bioavailabilities of food folates appear to range from 10 to 98% of folic acid, although those of most are about 50%;[16] and
- mixed diets have aggregate bioavailability of dietary folates of 50–80%.[17]

TABLE 17.4 Individual Variability in Reported Bioavailability Values of Folates in Foods

Food/Feedstuff	Bioavailability, %[a]
Bananas	0–148
Cabbage	0–127
Eggs	35–137
Lima beans	0–181
Liver (goat)	9–135
Orange juice	29–40
Spinach	26–99
Tomatoes	24–71
Wheat germ	0–64
Brewers' yeast	10–100
Soybean meal	0–83

[a]*Results expressed relative to folic acid.*
Baker, H., Jaslow, F.P., Frank, D., 1978. J. Am. Geriatr. Soc. 26, 218–221; Babu, H., Srikantia, S.G., 1976. Am. J. Clin. Nutr. 29, 376–382; Tamura, T., Stokstad, E.R.L., 1973. Br. J. Haematol. 25, 513–532.

4. ABSORPTION OF FOLATE

The efficiency of absorption of dietary folates is about 50% but can vary considerably (10–90%). Folate absorption is a multistep process:

1. **Deconjugation of polyglutamyl folates**. Because the majority of food folates occur as reduced polyglutamates, their absorption depends on their cleavage to mono- or diglutamate forms. This is accomplished by mucosal folyl γ-glutamyl carboxypeptidases, commonly called **folyl conjugases** in the small intestine:[18]
   a. A 700 kDa **brush border exocarboxypeptidase** with an optimum of pH 6.5–7.0. Although present in relatively low amounts, it is most important for the hydrolysis of folylpolyglutamates. A genetic variant has been associated with low-serum folate concentrations and homocysteinemia.
   b. A 75 kDa **intracellular** (lysosomal) **carboxypeptidase** with a pH optimum of pH 4.5–50.

Loss of conjugase activity impairs folate absorption. This can be produced by zinc deficiency or by exposure to naturally occurring conjugase inhibitors in foods such as cabbage, oranges, yeast, beans (red kidney, pinto, lima, navy, soy), lentils, and black-eyed peas (Table 17.5).[19] This

13. El-Saie, L.T., Rabie, A.R., Kamel, M.I., et al., 2011. Lasers Med. Sci. 26, 481–485.
14. Some anemic patients respond optimally to oral folate therapy only when they are also given iron. Patients with scurvy often have megaloblastic anemia, apparently owing to impaired utilization of folate. In some scorbutic patients, vitamin C has an antianemic effect; others require folate to correct the anemia.
15. As with any application of information from studies with animal models, the validity of extrapolation is an issue important in assessing folate bioavailability. For example, the rat and many other species have little or no brush border conjugase activity, these species relying on pancreatic conjugase for folate deconjugation. This contrasts with the pig and human, which deconjugate folates primarily by brush border activity.
16. Brouwer, I.A., van Dusseldorp, M., West, C.E., et al., 2001. Nutr. Res. Rev. 14, 267–293; McNulty, H., Pentieva, K., 2004. Proc. Nutr. Soc. 63, 529–536; Mönch, S., Netzel, M., Netzel, G., et al., 2014. R. Soc. Chem. 6, 242–248.
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18. Conjugase activities have also been identified in bile, pancreatic juice, kidney, liver, placenta, bone marrow, leukocytes, and plasma, although the physiological importance of these activities is unclear. In the uterus, conjugase activity is induced by estrogen.
19. The conjugase inhibitors in beans and peas reside in the seed coats and are heat-labile.

TABLE 17.5 Inhibition of Jejunal Folyl Conjugase Activities In Vitro by Components of Selected Foods

	% Inhibition, by Conjugase Source	
Food	Pig	Human
Red kidney beans	35.5	15.9
Pinto beans	35.1	33.2
Lima beans	35.6	35.2
Black-eyed peas	25.9	19.3
Yellow cornmeal	35.3	28.3
Wheat bran	–2.0	0
Tomato	8.1	14.2
Banana	45.9	46.0
Cauliflower	25.2	15.3
Spinach	21.1	13.9
Orange juice	80.0	73.4
Egg	11.5	5.3
Milk	13.7	—
Cabbage	12.1	—
Whole wheat flour	0.3	—
Medium rye flour	2.2	—

Bhandari, S.D., Gregory, J.F., 1990. Am. J. Clin. Nutr. 51, 87–94.

is the basis for the low bioavailability of folate in orange juice. Folate absorption can also be reduced by certain drugs including cholestyramine (which binds folates), sal icylazosulfapyridine,[20] diphenylhydantoin,[21] aspirin and other salicylates, and chronic ethanol ingestion.[22]

2. **Active uptake by the enterocyte**. Dietary folates are absorbed in deconjugated form, i.e., as folic acid, 5-methyl-FH_4, and 5-formyl-FH_4.[23] These vitamers are actively transported across the brush border by processes facilitated by two transporters (Fig. 17.1):
   a. **Reduced folate carrier (RFC** or **SLC19A1)** is a member of the solute carrier 19 (SLC19) family of transporters.[24] It is found on the enterocyte apical membrane as a homodimer with independently acting monomers and 12 transmembrane domains. RFC in the enterocyte binds both reduced and oxidized forms of the vitamin with comparable affinities. Its bidirectional transport function is driven by a transmembrane pH gradient; activity is optimal at pH 7.4 and is stimulated by glucose. RFC binds antifolates with affinities at least an order of magnitude greater than those of folate binding. Its expression is upregulated by folate deficiency.
   b. **Proton-coupled folate transporter (PCFT or SLC46A1)** is a Na^+-dependent, high-affinity transporter also of the SLC19 family.[25] It has high affinities for folic acid, 5-methyl-FH_4 and 5-formyl-FH_4, and methotrexate. PCFT-facilitated transport is driven by a transmembrane pH gradient, but functions in the absence of such a gradient, being based on membrane potential and sensitive to folate gradient. Its activity is greatest under acidic conditions (pH optima 5–6) and is, thus, thought to play a major role in facilitating folate uptake in the acidic microenvironment of the jejunum. Loss-of-function mutations in PCFT results in **hereditary folate malabsorption (HFM)**,[26] which is readily corrected by high oral doses of 5-formyl-FH_4 or folic acid.
   c. **Multidrug resistance-associated protein 3 (MRP3)**[27] has been implicated in the enteric absorption of oxidized folates. Its genetic deletion reduced the transport of 5-formyl-FH_4 and 5-methyl-FH_4 across everted duodenal sacs.[28] This suggests that MRP3 may participate in moving folates across the enterocyte basolateral surface where the protein is located.
3. **Passive diffusion into the enterocyte** can account for 20–30% of folate absorption at high folate intakes. Folate diffusion is greatest under acidic conditions in which its molecular charge is reduced. This may be the basis of increased folate absorption observed in individuals with pancreatic exocrine insufficiency; their reduced excretion of bicarbonate and the resulting loss of buffering capacity render the luminal milieu more acidic. Under more basic conditions (pH > 6.0), folate absorption falls off rapidly.

20. Also called azulfidine and sulfasalazine, used to treat inflammatory bowel disorder.
21. Also called dilantin, an anticonvulsant.
22. Other factors may contribute to this phenomenon: enterocytes are known to be sensitive to ethanol toxicity; many chronic alcoholics can have inadequate folate intakes.
23. The dog appears to absorb folylpolyglutamates.
24. This family also includes two thiamin transporters, SLC19A2 and SLC19A3. RFC also transports the folate antagonist methotrexate.
25. PCFT was originally described as a low-affinity heme carrier protein (HCP1), although that is no longer regarded as its primary function. Hence, it is often referred to as PCFT/HCP1.
26. HFM has been reported in some 30 patients, presenting at 2–6 months of age as megaloblastic anemia, mucositis, diarrhea, failure to thrive, recurrent infections, and seizures.
27. The MRPs are members of the ATP-binding cassette (ABC) transporters, several of which (MRPs1–5 and ABCG2) have low-affinity, high capacity for binding folates.
28. Kitamura, Y., Kusuhara, H., Sugiyama, Y., 2010. Pharm. Res. 27, 665–672.

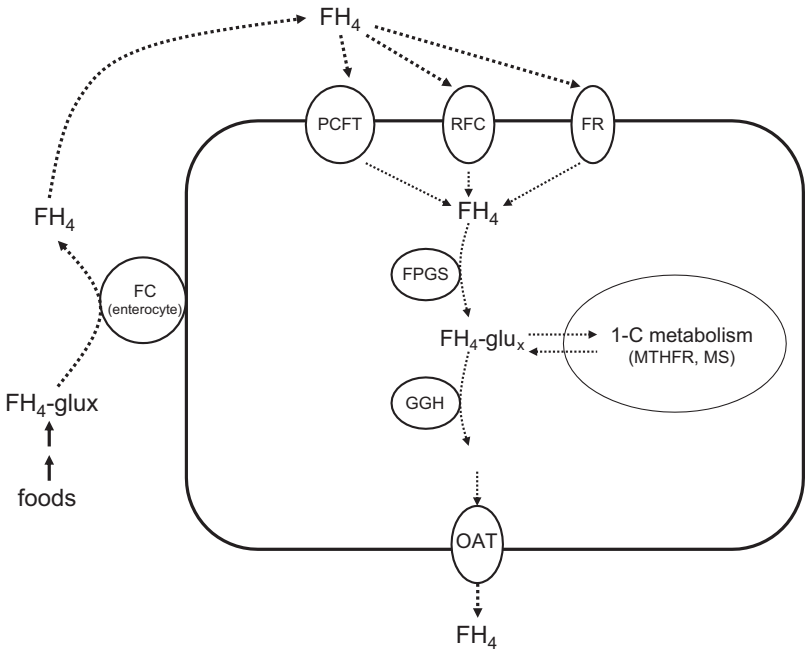

FIGURE 17.1 Facilitated Cellular Uptake and Utilization of Folate. *FC*, folyl conjugase; *FPGS*, folylpolyglutamate synthase; *FR*, folate receptor; *GGH*, γ-glutamyl hydrolase; *MS*, methionine synthase; *MTHFR*, methylenetetrahydrofolate reductase; *OAT*, organic anion transporter; *PCFT*, proton-coupled folate transporter; *RFC*, reduced folate carrier. *After Devos, L., Chanson, A., Liu, Z., et al., 2008. Am. J. Clin. Nutr. 88, 1149–1158.*

4. **Reconjugation**. Absorbed monoglutamyl folates are converted to higher-glutamyl forms by **folylpolyglutamate synthase**, preventing the vitamin being pumped out of the cell.
5. **Exportation**. Absorbed FH_4 can be exported without further metabolism to the portal circulation or after being first alkylated (e.g., by methylation to 5-methyl-FH_4). Both forms are exported across the basolateral cell membrane by a **folate export pump**, which is likely to be a member of the family of multispecific **organic anion transporters (OATs)**, one of which has been shown to transport the folate antagonist methotrexate. Because folate pumps do not transport polyglutamates, those forms are retained within the cell until being deconjugated.

Folates can be absorbed across the colon. This was demonstrated in humans using a $^{13}C_5$-formyl-FH_4 administered in caplets that did not dissolve before reaching the colon. $^{13}C_5$-formyl-FH_4 appeared in the plasma at the rate of 5.8 ± 1.2 nmol/h with a mean absorption of 46%.[29] It is likely that absorption across the colon occurs by the same process as in the small intestine. PCFT expression has been demonstrated in the colon.[30]

5. TRANSPORT OF FOLATE

Erythrocytes

Erythrocytes comprise the largest compartment of circulating folates, containing 670–1800 nM, depending on level of intake of the vitamin. The dominant share of this compartment is 5-methyl-FH_4, which comprises more than 80% of the total. Also present are the metabolite 4α-hydroxy-5-methyl-FH_4 and smaller amounts of FH_4, 5-formyl-FH_4, and 5,10-methenyl-FH_4.[31]

Free in Plasma

Plasma folate concentrations in most species are in the range of 10–30 nM most of which is in free solution. The predominant form in portal plasma is the reduced form, FH_4. This is taken up by the liver, which releases it to the peripheral plasma after converting it primarily to 5-methyl-FH_4 but also to 10-formyl-FH_4. The concentration of 10-formyl-FH_4 is tightly regulated;[32] whereas that of 5-methyl-FH_4 is not. Thus, the latter varies in response to recent folate intake. Therefore, most folate is transported to peripheral tissues in the form of monoglutamyl derivatives. The notable exception is the pig in which FH_4 is the predominant circulating

29. Lakoff, A., Fazili, Z., Aufreiter, S., et al., 2014. Am. J. Clin. Nutr. 100, 1278–1286.
30. Aufreiter, S., Gregory, J.F., Pfeiffer, C.M., et al., 2009. Am. J. Clin. Nutr. 90,116–123.
31. Hartman, B.A., Fazili, Z., Pfeiffer, C.M., et al., 2014. J. Nutr. 144, 1364–1369.
32. In humans, the plasma level is held at ~80 ng/dL.

form.[33] Erythrocytes contain relatively great concentrations of folate, typically 50–100 nM. These stores are accumulated during erythropoiesis; the mature erythrocyte does not take up folate.

Folate transport is reduced by cigarette smoking and chronic alcohol ingestion. Smokers show plasma folate levels that can be nearly half those of nonsmokers.[34] While serum folates have been found to be normal among consumers of moderate amounts of ethanol, more than 80% of chronic alcoholics show abnormally low-serum levels and some 40% show low-erythrocyte levels. This corresponds to a similar incidence (34–42%) of megaloblastosis of the bone marrow in alcoholic patients.[35]

Protein-Bound in Plasma

Folate is also bound to three proteins in the plasma: albumin and an FBP. The high-affinity FBP in plasma is thought to be a solubilized form of tissue FPBs. It is present in low concentrations (binding <10 ng folic acid per deciliter) but is elevated in folate deficiency and pregnancy. Most of FBP-bound plasma folate is likely to be folic acid, which FBP binds avidly and preferentially. Unmetabolized folic acid is detected in nearly all subjects, particularly in those given large (>~260 μg) or consecutive small oral doses (~100 μg) of folic acid.[36] It has been suggested that the presence of unmetabolized folic acid in the circulation reflects a mismatch of supply and cellular demand for the vitamin.

Cellular Uptake

Circulating monoglutamyl folates are taken up by same process by which they were taken up by enterocytes. This involves the folate transporters and receptors (Fig. 17.1):

- **RFCs** are the major transporters of folates into tissue in which they are ubiquitously expressed. In extraintestinal tissues, they bind reduced folates preferentially to nonreduced forms. In renal proximal tubules, RFCs expressed at the apical brush border membrane have antiporter function that favors the transport of folates into cells. In epidermal and dermal cells, their expression is upregulated by exposure to UV irradiation.[37]
- **PCFT** is highly expressed in liver, kidney, colon, brain, retina, and placenta. It functions to facilitate **folate receptor (FR)**-mediated endocytotic uptake of the vitamin.
- High-affinity **FRs** (also called **FBPs**) bind folic acid, 5-methyl-FH_4, and some antifolate drugs stoichiometrically and with high affinities, facilitating their uptake by endocytosis. They occur in three isoforms, some of which function in mediating folate uptake via endocytosis: FR-α (highly expressed in placental epithelia, renal tubules, retinal pigment epithelia, and choroid plexus) and FR-β (highly expressed by spleen, thymus, and CD34+ monocytes).[38] Regulation of FR expression is not well understood, but it is clear that extracellular folate concentration serves as an inverse stimulus to FR expression. That FRs and the folate transporters are localized on opposite aspects of polarized cells facilitate movement of folate across the apical membrane into the cell. In the renal tubule, FR-α functions in reabsorption, efficiently extracts folates from the glomerular filtrate and becoming saturated only at high plasma concentrations of the vitamin.[39] A neuropsychiatric disorder, **cerebral folate deficiency syndrome**, has been described; it involves blockade of folate transport across the blood–brain barrier due to high-affinity autoantibodies against membrane-bound FBPs on the choroid plexus.[40]
- **MRP2 and MRP3** appear to function in the efflux of folates from hepatocytes across their basolateral and canalicular membranes into hepatic sinusoids and bile ducts, respectively.[41]

Within cells, folates are stabilized and protected from oxidative degradation by binding to intracellular proteins. Folate-binding enzymes are typically present in micromolar amounts that exceed folate concentrations by several fold. They bind folates with high affinities, i.e., with dissociation constants in the nanomolar range. Therefore, intracellular concentrations of free folates are exceedingly low.

Tissue Distribution

In humans, the total body content of folate is 5–10 mg, half of which resides in the liver as tetra-, penta-, hexa- and heptaglutamates of 5-methyl-FH_4 and 10-formyl-FH_4.[42] The relative amounts of these single-carbon derivatives

33. The metabolic basis for this anomaly is not clear.
34. These findings probably relate to the inactivation of cobalamins by factors (cyanides, hydrogen sulfide, nitrous oxide) in cigarette smoke.
35. It is likely that effects of such magnitudes have several causes: inhibition of intestinal folyl conjugase activity; decreased urinary folate recovery; displacement of folate-containing foods by alcoholic beverages.
36. Pfeiffer, C.M., Sternberg, M.R., Fazili, Z., et al., 2015. J. Nutr. 145, 520–531.
37. Knott, A., Meilke, H., Koop, U., et al., 2007. Soc. Invest. Dermatol. 127, 2463–2466.
38. Mutations of FR-α are embryonically lethal, but deletion of FR-β produces no phenotype in the mouse. A third isoform, FR-γ, is found on natural killer and TGF-β-induced regulatory T cells.
39. Birn, H., Spiegelstein, O., Christensen, E.I., et al., 2005. J. Am. Soc. Nephrol. 16, 608–615.
40. Ramaekers, V.T., Rothenberg, S.P., Sequeira, J.M., et al., 2005. N. Engl. J. Med. 52, 1985–1991.
41. Kitamura, Y., Hirouchi, H., Kusuhara, H., et al., 2008. J. Pharmacol. Exp. Ther. 327, 465–473; Masud, M., I'isuka, Y., Yamazaki, M., et al., 1997. Cancer Res. 57, 3506–3510.
42. Hepatic reserves of folate should be sufficient to support normal plasma concentrations of the vitamin (>400 ng/dL) for at least 4 weeks. (Signs of megaloblastic anemia are usually not observed within 2–3 months of folate deprivation.) However, some evidence suggests that the release of folate from the liver is independent of nutritional folate status, resulting instead from the death of hepatocytes.

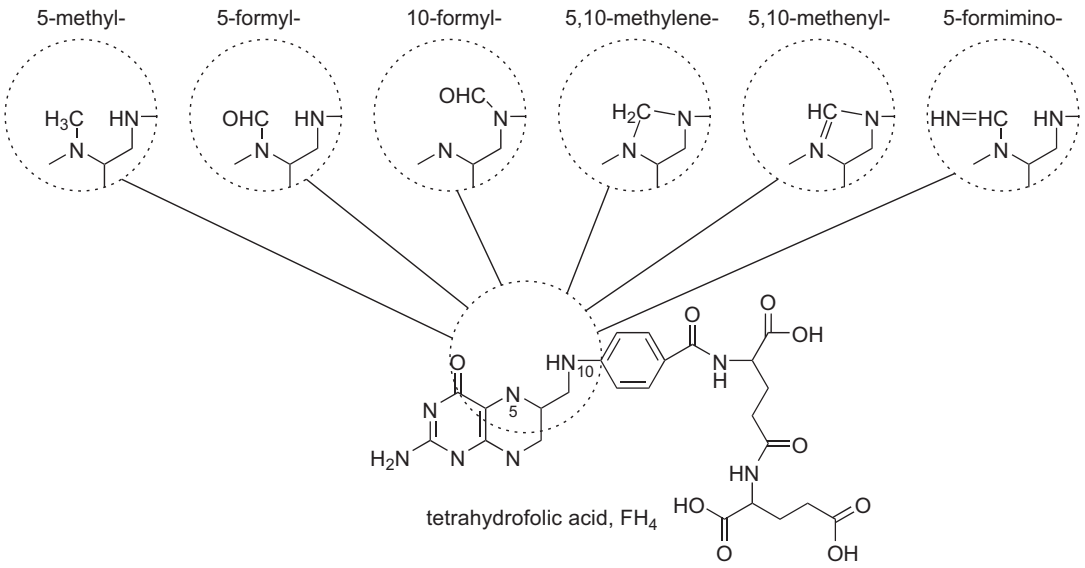

FIGURE 17.2 Single-carbon units carried by folate.

vary among tissues, depending largely on the rate of cell division. In tissues with rapid cell division (e.g., intestinal mucosa, regenerating liver, carcinoma), relatively low concentrations of 5-methyl-FH_4 are found, usually with concomitant elevations in 10-formyl-FH_4. In tissues with low rates of cell division (e.g., normal liver), 5-methyl-FH_4 predominates. Brain folate (mostly 5-methyl-FH_4) levels tend to be very low, with a subcellular distribution (penta- and hexaglutamates mostly in the cytosol and polyglutamates mostly in the mitochondria) the opposite of that found in liver. Folate-deficient animals show relatively low hepatic concentrations of shorter chain length folylpolyglutamates compared with longer chain length folates, suggesting that longer chain length metabolites are better retained within cells. In the rat, uterine concentrations of folates show cyclic variations according to the menstrual cycle, with maxima coincident with peak estrogen levels just before ovulation.[43]

6. METABOLISM OF FOLATE

There are three aspects of folate metabolism:

1. **Reduction of the pteridine ring system** from the two nonreduced states, folic acid and dihydrofolic acid (FH_2), to the fully reduced form tetrahydrofolic acid (FH_4). This form is capable of accepting a single-carbon unit by the action of the cytosolic enzyme **7,8-dihydrofolate reductase** (Fig. 17.2).[44] That activity is found in high amounts in liver and kidney and in rapidly dividing cells (e.g., tumors). It is inhibited by several drugs including the cancer chemotherapeutic drug **methotrexate**.[45]
2. **Reactions of the polyglutamyl side chain** by side chain hydrolysis to the corresponding monoglutamate. These conversions are catalyzed by two enzymes:
   - The ATP-dependent **folylpolyglutamate synthase**[46] catalyzes the conversion of 5-methyl-FH_4 to folylpolyglutamates by linking glutamyl residues to the vitamin by peptide bonds involving the γ-carboxyl groups.[47] The enzyme requires prior reduction of folate to FH_4 or demethylation of the circulating 5-methyl-FH_4 (by vitamin B_{12}-dependent methionine synthase). It is widely distributed at low concentrations in many tissues and is critical in converting the monoglutamyl transport forms of the vitamin to the metabolically active polyglutamyl forms. Mutational loss of the enzyme results in lethal folate deficiency. In most tissues, the activity is rate limiting for folate retention. Cells lacking the enzyme are unable to accumulate the vitamin.[48] Those lacking the mitochondrial enzyme cannot accumulate

43. On the basis of this type of observation, it has been suggested that estrogen enhancement of folate turnover in hormone-dependent tissues may be the basis of the effects of pregnancy and oral contraceptive steroids in potentiating low-folate status.

44. Also called **tetrahydrofolate dehydrogenase**, this 65-kDa NADPH-dependent enzyme can reduce folic acid to FH_2 and, of greater importance, FH_2 to FH_4. The enzyme is potently inhibited by the drug methotrexate.

45. Other inhibitors include the antimalarial drug, pyrimethamine and the antibacterial drug trimethoprim.

46. The mitochondrial and cytosolic forms of the enzyme are encoded by a single gene the transcription of which has alternate start sites and the mRNA of which has alternative translation sites.

47. This enzyme also catalyzes the polyglutamation of the anticancer folate antagonist methotrexate, which enhances its cellular retention. Tumor cells, which have the greatest capacities to perform this side chain elongation reaction, are particularly sensitive to the cytotoxic effects of the antagonist.

48. Because polyglutamation is also necessary for the cellular accumulation and cytotoxic efficacy of antifolates such as methotrexate, decreased folylpolyglutamate synthase activity is associated with clinical resistance to those drugs.

TABLE 17.6 Enzymes Involved in the Acquisition of Single-Carbon Units by Folates

Single-Carbon Unit	Folate Derivative	Enzymes
Methyl group (—CH_3)	5-Methyl-FH_4	5,10-Methylene-FH_4 reductase methionine synthase
Methylene group (=CH_2)	5,10-Methylene-FH_4	Serine hydroxymethyltransferase 5,10-Methylene-FH_4 dehydrogenase
Methenyl group (=CH)	5,10-Methenyl-FH_4	5,10-Methylene-FH_4 dehydrogenase
		5,10-Methenyl-FH_4 cyclohydrolase
		5-Formimino-FH_4 cyclohydrolase
		5-Formyl-FH_4 isomerase
Formimino group (—CH=NH)	5-Formimino-FH_4	FH_4 formiminotransferase
Formyl group (—CH=O)	5-Formyl-FH_4 10-Formyl-FH_4	FH_4:glutamate transformylase
		5,10-Methenyl-FH_4 cyclohydrolase
		10-Formyl-FH_4 synthase

the vitamin in that subcellular compartment; consequently, they show deficient mitochondrial single-carbon metabolism.

- Folylpolyglutamates are turned over in cells by hydrolysis to shorter chain derivatives by the action of soluble, lysosomal[49] γ-glutamyl carboxypeptidases, also referred to as **folyl conjugases**. Some are zinc metalloenzymes.

3. **Acquisition of single-carbon moieties** at the oxidation levels of formate, formaldehyde, or methanol[50] substituted at the N-5 and/or N-10 positions of the pteridine ring system (Fig. 17.2). The main source of single-C fragments is **serine hydroxymethyltransferase (SHMT)** (Table 17.6), which uses the dispensable amino acid serine[51] as the source of single-C. Each folyl derivative is a donor of its single-C unit in metabolism[52]; thus, by cycling through the acquisition/loss of single-C units, folyl derivatives deliver these species for different metabolic uses. Most single-C folate derivatives in cells are bound to enzymes or FBPs.

Methyl Folate Trap

The major cycle of single-C flux in mammalian tissues is the serine hydroxymethyl transferase/5,10-methylene-FH_4 reductase/methionine synthase cycle, in which the latter reaction is rate limiting (Fig. 17.3). The committed step (5,10-methylene-FH_4 reductase) is feedback-inhibited by **S-adenosylmethionine (SAM)** and product-inhibited by 5-methyl-FH_4. Methionine synthase depends on the transfer of labile methyl groups from 5-methyl-FH_4 to vitamin B_{12}, which, as methyl-B_{12}, serves as the immediate methyl donor for converting homocysteine (Hcy) to methionine. Without adequate vitamin B_{12} to accept methyl groups from 5-methyl-FH_4, that metabolite accumulates at the expense of the other metabolically active folate pools. This is known as the "**methyl folate trap**." The loss of FH_4 that results from this blockage in folate recycling blocks transfer of the histidine–formimino group to folate (as 5-formimino-FH_4) during the catabolism of that amino acid. This results in the accumulation of the intermediate formiminoglutamic acid (FIGLU). Thus, elevated urinary FIGLU levels after an oral histidine load are diagnostic of vitamin B_{12} deficiency.

Catabolism

Tissue folates turn over by the cleavage of the polyglutamates at the C-9 and N-10 bonds to liberate the pteridine and *p*-aminobenzoyl polyglutamate moieties. This results from chemical oxidation of the cofactor both in the intestinal lumen (dietary and enterohepatically recycled folates) as well as in the tissues. Once formed, *p*-aminobenzoylpolyglutamate is degraded, presumably by the action of folyl conjugase, and is acetylated to yield *p*-acetaminobenzoylglutamate and

49. Lysosomes also contain a folate transporter, which is thought to be active in bringing folypolyglutamates into that vesicle.

50. It should be noted that single carbons at the oxidation level of CO_2 cannot be transported by folates; fully oxidized carbon is transported by biotin and thiamin pyrophosphate.

51. Serine is biosynthesized from glucose in nonlimiting amounts in most cells.

52. Although the route of its biosynthesis is unknown, eukaryotic cells contain significant amounts of 5-formyl-FH_4. That folyl derivative, also called **leucovorin**, **folinic acid** and **citrovorum factor**, is used widely to reverse the toxicity of methotrexate and, more recently, to potentiate the cytotoxic effects of 5-fluorouracil.

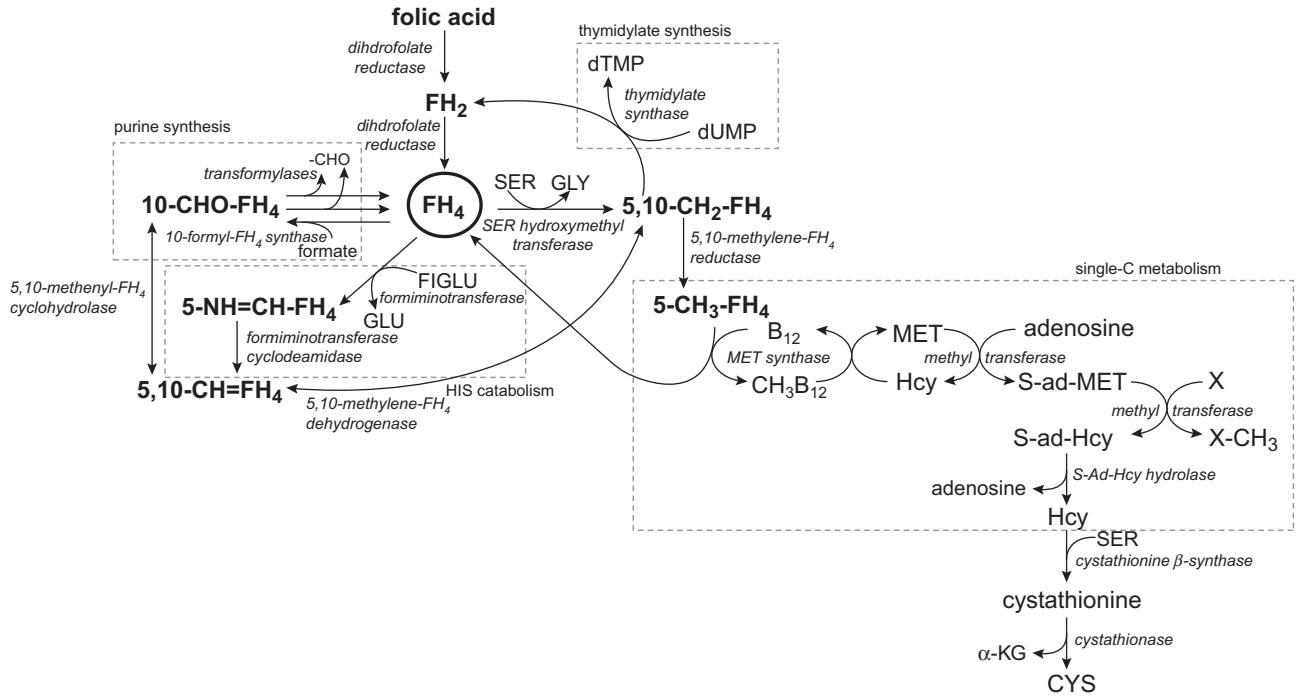

FIGURE 17.3 Folate roles in metabolism. Relationship with vitamin B_{12} and the basis of the "**methyl folate trap**" whereby folate accumulates as 5-CH_3-FH_4 when vitamin B_{12} is not available. *CYS*, cysteine; *dTMP*, deoxythymidine monophosphate; *dUMP*, deoxyuridine monophosphate; *FIGLU*, formiminolgutatmic acid; *GLU*, glutamic acid; *Hcy*, homocysteine; *HIS*, histidine; *MET*, methionine; *S-ad-Hcy*, S-adenosylhomocysteine; *S-ad-MET*, S-adenosylmethionine; *SER*, serine; *X*, methyl acceptor; *α-KG*, a-ketoglutarate.

p-acetamiobenzoate. The rate of folate catabolism is related to the rate of intracellular folate utilization, with urinary levels of *p*-acetamiobenzoate correlated with the total body folate pool. Studies of the kinetics of folate turnover using isotopic labels in nonpregnant women have indicated both fast- and slow-turnover body folate pools of which turn over faster with increasing folate intake (Table 17.7). At low-to-moderate intakes, 0.5–1% of total body folate appears to be catabolized and excreted per day. Folate breakdown is greatest under hyperplastic conditions (e.g., rapid growth, pregnancy[53]).

Excretion

Intact folates and the water-soluble side chain metabolites, *p*-acetaminobenzoylglutamate and *p*-acetamiobenzoate, are excreted in the urine and bile. The total urinary excretion of folates and metabolites is small (e.g., ≤1% of total body stores per day). Folate conservation is effected by the reabsorption of 5-methyl-FH_4 by the renal proximal tubule; renal reabsorption appears to be mainly a nonspecific process, although an FBP-mediated process has also been demonstrated. Fecal concentrations of folates are usually rather high—comparable to that excreted in the urine. They are mostly of intestinal microfloral origin, as enterohepatically circulated folates appear to be absorbed quantitatively.

Polymorphisms of Enzymes in Folate Metabolism

Genetic polymorphisms have been identified in most of the major enzymes involved in folate utilization (Table 17.8):[54]

- **Folyl conjugase**. Polymorphism involving a T→C substitution of base pair 1561 has been described.[55] Individuals with the T allele have higher circulating folate levels than those with the C allele.[56]
- **RFC**. Polymorphism involving a G→A substitution of base pair 80 has been described. Individuals homozygous for the G allele tend to have elevated circulating Hcy levels and reduced erythrocyte folate levels.[57]

53. This effect may be an important contributor to the folate-responsive megaloblastic anemia common in pregnancies in parts of Asia, Africa, and Central and South America (where rates as high as 24% have been reported) as well as in the industrialized world (where rates of 2.5–5% have been reported).

54. Polymorphisms of other enzymes involved in folate and single-carbon metabolism have also been described, although none has been shown to affect folate utilization. These include folylpolyglutamate synthase C303T and A2006G, methionine synthase G2756A, and folyl γ-glutamyl hydrolase upstream T→C and C→T polymorphisms.

55. The enzyme is encoded by the glutamate carboxypeptidase II gene (*GCPII*).

56. Afman, L.A., Trijbels, F.J.M., Blom, H.J., 2003. J. Nutr. 133, 75–77.

57. Chango, A., Emery-Fillon, N., de Courcy, G.P., et al., 2000. Mol. Genet. Med. 70, 310–315.

TABLE 17.7 Effect of Dietary Folate on Folate Turnover in Nonpregnant Women

Folate Intake n moles (µg)/day	Total Body Folate Pool µmoles	Catabolic Rate % Body pool/day	Residence Time Days
454 (200)	64.5 ± 2.3	0.47 ± 0.02[a]	212 ± 8[a]
680 (300)	71.5 ± 3.6	0.61 ± 0.04[b]	169 ± 12[b]
907 (400)	73.0 ± 2.4	0.82 ± 0.05[c]	124 ± 7[c]

$p < .05$, n = 5–6 per group; means with like superscripts are significantly different.
Gregory, J.F., Williamson, J., Liao, J.F., et al., 1998. J. Nutr. 128, 1896–1906.

TABLE 17.8 Polymorphisms of Proteins Related to Folate Absorption and Metabolism

Enzyme	Polymorphism		Genotype: Frequency	
MTHFR (5,10-methylene-FH_4 reductase)	T6557C	CC: 41%	TT: 18%	CT: 41%
	C1289A	AA: 53%	CC: 9%	AC: 37%
Folyl conjugase	T1561C	CC: 92%	TT: 0[a]	CT: 8%
Reduced folate transporter	G80A	AA: 35%	GG: 18%	AG: 47%
Methionine synthase	G2756A	AA: 59%	GG: 3%	AG: 38%
Methionine synthase reductase	G66A	AA: 28%	GG: 23%	AG: 49%

[a] *1 in 625 reported.*
Molloy, A.M., 2002. J. Vitam. Nutr. Res. 72, 46–52.

- **MTHFR** (methylenetetrahydrofolate reductase). Three polymorphisms have been identified in the human MTHFR: a C→T substitution of base pair 677, an A→C substitution of base pair 1298, and G1793A.
  - **C677T**. Some 89% of Americans have the C allele. Some 20% of Mexican Americans, 12% of non-Hispanic whites, but only 1% of non-Hispanic blacks are homozygous for the T variant (TT);[58] they have a form of MTHFR with 70% lower enzyme activity, lower affinity for the flavin cofactor, and lower thermal stability[59] than the C/C form of the enzyme (Fig. 17.4). They also show slightly lower plasma folate concentrations, elevated plasma 5,10-methylene-FH_4 concentrations, mild homocysteinemia,[60] and lower global DNA methylation than other genotypes (Table 17.9)—effects that appear to be exacerbated by low folate intakes. Compared to individuals with the CC genotype, those with the TT genotype have greater rates of synthesis of adenine[61] and Hcy,[62] as well as greater risks to colorectal cancer, unipolar depression,

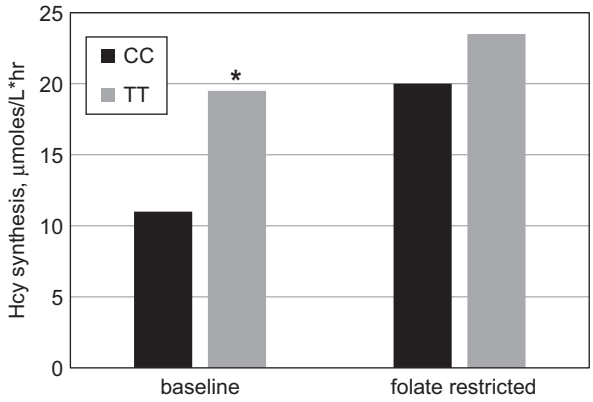

FIGURE 17.4 Effect of MTHFR (5,10-methylene-FH_4 reductase) C677T genotype on homocysteine synthesis by healthy women before and after folate restriction (115 µg/day for 7 weeks). *Difference between genotypes, $p < .05$. *After Davis, S.R., Quinlivan, E.P., Shelnutt, K.P., et al., 2005. J. Nutr. 135, 1045–1050.*

58. Yang, Q.H., Botto, L.D., Gallagher, M., et al., 2008. Am. J. Clin. Nutr. 88, 232–246.
59. For this reason the variant is frequently referred to as the "thermolabile enzyme."
60. Kauwell, G.P.A., Wilsky, C.E., Cerda, J.J., et al., 2000. Metabolism 49, 1440–1443; Hazra, A., Kraft, P., Lazarus, R., et al., 2009. Hum. Mol. Genet. 18, 4677–4687.
61. Quinlivan, E.P., Davis, S.R., Shelnutt, K.P., et al., 2005. J. Nutr. 135, 389–396.
62. Davis, S.R., Quinlivan, E.P., Shelnutt, K.P., et al., 2005. J. Nutr. 135, 1045–1050.

TABLE 17.9 Effect of MTHFR (5,10-methylene-FH_4 Reductase) C677T Genotype on Folate, Homocysteine, and Vitamin B_{12} Status in Humans

	MTHFR C677T Genotype		
Metabolite	CC	TC	TT
Plasma folate, nM	12.8 ± 6.5	12.8 ± 6.7	9.5 ± 3.1
Erythrocyte folate, nM	541 ± 188	517 ± 182	643 ± 186
Plasma Hcy, μM	13.4 ± 3.4	13.2 ± 3.1	17.1 ± 11.5
Plasma vitamin B_{12}, pM	246 ± 130	271 ± 121	233 ± 94

van der Put, N.M., Gabreels, F., Stevens, E.M., et al., 1995. Lancet 346, 1070–1071.

adult acute lymphoblastic leukemia, and ischemic stroke.[63] They also show the greater Hcy-lowering responses to folate supplements.

- **A1298C**. More than 90% of Americans have the A allele.[39] Having the C allele appears to be without significant physiological consequence unless combined with the MTHFR C677T polymorphism; doubly heterozygous individuals have been found to have MTHFR-specific activities of two-thirds those of doubly homozygous individuals, with lower circulating levels of folate and increased circulating levels of Hcy.[64]
- **G1793A**. This polymorphism is less frequent, about 4% of American women had the GG genotype. The functional significance of this polymorphism is unclear.

- **10-Formyl-FH_4 dehydrogenase**. Two intronic single-nucleotide variants have been identified; one has been associated with increased risk of breast cancer in postmenopausal women.[65] The enzyme is known to be epigenetically silenced in cancers.[66]
- **Dihydrofolate reductase**. Three variants have been identified: A 19 base pair deletion in the first intron,[60] a 5′ upstream 9 base pair repeat, and a polymorphism in the 3′-untranslated region. Individuals homozygous for the deletion have impaired tissue folate stores;[67] in mothers the trait is associated with a twofold increase in risk of spina bifida in their children.[68] Individuals with both the deletion and the repeat have lower plasma Hcy concentrations, but their circulating folate levels are unaffected.
- **Methionine synthase**. A polymorphism has been described involving A→G substitution of base pair 2756, which affects the domain involved in methylation of the vitamin B_{12} cofactor. The GG genotype has been associated with reduced plasma Hcy concentrations and increased risks of systemic lupus erythematosus, bipolar disorder, schizophrenia, congenital malformations of the face and spine, and Down syndrome.[69]
- **Methionine synthase reductase**.[70] A polymorphism has been described involving A→G substitution of base pair 66. It shows a prevalence of ~30%. The variant appears to be without physiologic consequence unless present with MTHFR 677TT, in which case serum Hcy concentrations are some 26% lower than those of other genotypes.

Effects of Drugs

Several drugs can impair folate metabolism. These have proven to have clinical applications ranging from the treatment of autoimmune diseases to cancer and malaria—all conditions in which cell proliferation can be suppressed through the inhibition of a folate-dependent step in single-C metabolism.

- **Methotrexate** (amethopterin), a folate analog differing from the vitamin by the presence of an amino group in lieu of the 4-hydroxyl group on the pteridine ring and a methyl group at the N-10 position, has greater affinity than the natural substrate for dihydrofolate reductase, resulting in its inhibition. Accordingly, the drug produces an effective folate deficiency, with reductions in thymidine synthesis and purine levels. This antiproliferative effect is the basis of its use in treating cancer, rheumatoid arthritis, psoriasis, asthma, and inflammatory bowel disease. Because its side effects include those of folate deficiency, methotrexate is usually used with accompanying and carefully monitored folate supplementation to reduce the incidence of side effects. Individuals with the MTHFR 677TT genotype have a higher risk of such adverse effects. In contrast, individuals with the MTHFR 1298CC genotype are at lower risk of side effects.

63. Carr, D.F., Whitely, G., Alfirevic, A., et al., 2009. Pharmacogenomics J. 9, 291–305.
64. Chango, A., Boisson, F., Barbe, F., et al., 2000. Br. J. Nutr. 83, 593–596.
65. Stevens, V.L., McCullough, M.L., Pavluk, A.L., et al., 2007. Cancer Epidemiol. Biomarkers Prev. 16, 1140–1147.
66. Oleinik, N.V., Krupenko, N.I., Krupenko, S.A., 2011. Cancer 2, 130–139.
67. Kalmbach, R.D., Choumenkovitch, S.F., Troen, A.P., et al., 2008. J. Nutr. 138, 2323–2327.
68. Carr, D.F., Whitely, G., Alfirevic, A., et al., 2009. Pharmacogenomics J. 9, 291–305.
69. Stover, P.J., 2011. J. Nutrigenet. Nutrigenomics 4, 293–305.
70. This enzyme that catalyzes the conversion of methionine synthase from its inactive to its active form by regenerating its methylcobalamin.

- **Other drugs** can impair folate status/metabolism. These include anticonvulsants (diphenylhydantoin, phenobarbital), antiinflammatory drugs (sulfasalazine), glycemic control drugs (metformin), and alcohol.

7. METABOLIC FUNCTIONS OF FOLATE

Folates function as enzyme cosubstrates in a network of reactions in the metabolism of amino acids and nucleotides, as well as the formation of the primary methyl donor for biological methylations, SAM. In each of these functions, the fully reduced form, tetrahydrofolic acid (FH_4), serves as an acceptor or donor of a single-carbon unit and is regenerated with the transfer such that continuous cycling can occur (Table 17.10).

Amino Acid Metabolism

Serine–glycine interconversion. FH_4 participates in the reversible interconversion of the dispensable amino acids, serine and glycine. That process is catalyzed by the pyridoxal phosphate-dependent enzyme, SHMT, which exists in three isoforms: SMHT1 and SMHT2α in the cytosol and nucleus, and SMHT2 in the mitochondria. FH_4 accepts a single-C unit from the serine C-3, binding it at the oxidation state of formaldehyde for form 5,10-methylene-FH_4 in the cytosol, and at the oxidation state of formate to form 10-formyl FH_4 in the mitochondria, subsequently entering the cytosol.

Histidine catabolism. Cytosolic formiminotransferase catalyzes the final reaction in the catabolism of histidine by transferring the formimino group from **formiminoglutamate** (**FIGLU**) to FH_4 to form 5-formimino-FH_4.

Methionine–cysteine transsulfuration. CYS can be produced by transfer of a methyl group from MET to form Hcy, and condensation of the latter with SER to form cystathionine from which α-ketoglutarate is cleaved to yield CYS. The rate-limiting step in this process of transsulfuration of MET to CYS is catalyzed by **MTHFR**, which is allosterically inhibited by SAM.[71]

Methionine regeneration. 5-Methyl-FH_4 is the ultimate methyl donor in the methylation of Hcy to form MET.[72] This occurs throughout the body by the action of the vitamin B_{12}-dependent enzyme MET synthase, which is transiently methylated at its corrin center before transferring the methyl group to Hcy. This process is blocked by deficiency of vitamin B_{12}, which causes secondary folate deficiency due to the accumulation of 5-methyl-FH_4 by the "methyl folate trap." This is the basis by which deficiency of either folate or vitamin B_{12} can cause macrocytic anemia.

Single-Carbon Metabolism

Formation of SAM occurs by moving single-C units from the nonessential amino acids SER and GLY, and the choline metabolites, sarcosine and dimethylglycine. Each is converted to formate, which in the cytoplasm condenses with FH_4 to form 10-formyl-FH_4. The utilization of those single-C units depends on 10-formyl-FH_4 being converted to 5-methyl-FH_4. That conversion is facilitated by low activities of 10-formyl-FH_4 dehydrogenase, which otherwise would deplete the single-C pool by catalyzing the conversion of 10-formyl-FH_4 to CO_2 and FH_4.[73] Ultimately, single-C units are needed in the form of 5-methyl-FH_4, which can pass them through MET to be activated as SAM, the donor of "labile" methyl groups[74] for more than 100 methyltransferases.

SAM-dependent methylations. SAM is the methyl donor for more than 100 methyltransferases, including the following:

- **Methylation of Hcy to regenerate Met** (see previous discussion). This process regenerates the methyl donor SAM from **S-adenosylhomocysteine (SAH)**, which inhibits methyltransferases through tight binding. Thus, the intracellular ratio of SAM:SAH, known as the "methylation index," determines methylation potential, affecting the availability of labile methyl groups to methyltransferases.
- **Methylation of chromatin** is a primary means of regulating gene expression and maintaining genomic integrity. It includes the methylation of DNA and histones catalyzed by methyltransferases that are particularly sensitive to cellular SAM:SAH ratio.[75] Hypomethylation of these factors is believed to alter chromatin structure in ways that affect transcription and can increase the rate of C→T transition mutation.[76]

71. It is also an allosteric inhibitor of 5,10-methylene-FH_4 reductase and an activator of the pyridoxal phosphate-dependent enzyme, cystathionine β-synthase, which catalyzes the condensation of Hcy and SER to form cystathionine.

72. Hcy can also be converted to MET in the liver and kidney by a folate-independent process, i.e., by betaine–homocysteine methyltransferase (BHMT), which using betaine (a product of choline oxidation) as the methyl donor.

73. Anguera, M.C., Field, M.S., Perry, C., et al., 2006. J. Biol. Chem. 281, 18335–18342.

74. Most SAM-dependent methylation reactions proceed via SN2 displacement mechanisms; however, some proceed via radical mechanisms that utilize a specialized iron–sulfur cluster to catalyze reductive cleavage yielding the highly reactive 5-adenosyl radical (Zhang, Q., Van der Donk, W.A., Liu, W., 2012. Acc. Chem. Res. 4, 555–564).

75. Waterland, R.A., Jirtle, R.L., 2004. Nutrition 20, 63–68.

76. Methylated CpG sites appear to be at particularly high risk for C→T changes, the most common type of mutational change, which are common in the p53 tumor suppressor gene.

TABLE 17.10 Metabolic Roles of Folate Coenzymes

Folate Coenzyme	Enzyme	Metabolic Role
5,10-Methylene-FH_4	Serine hydroxymethyltransferase	Receipt of a formaldehyde unit in SER catabolism (mitochondrial enzyme important in GLY synthesis)
	Thymidylate synthase	Transfers formaldehyde to C-5 of dUMP to form dTMP in pyrimidines
10-Formyl-FH_4	10-Formyl-FH_4 synthase	Accepts formate from TRY catabolism
	Glycinamide ribonucleotide transformylase	Donates formate in purine synthesis
	5-Amino-4-imidazolecarboxamide transformylase	Donates formate in purine synthesis
	10-Formyl-FH_4 dehydrogenase	Transfers formate for oxidation to CO_2 in HIS catabolism
5-Methyl-FH_4	Methionine synthase	Provides methyl group to convert Hcy to MET
	Glycine *N*-methyltransferase	Transfers methyl group from SAM to GLY in the formation of Hcy
5-Formimino-FH_4	Formiminotransferase	Accepts formimino group from HIS catabolism

- **DNA methylation** consists of the addition of a methyl group from SAM to cytosine bases within DNA CpG islands.[77] DNA hypermethylation is typically associated with gene silencing. Folate deprivation produces chromosomal breaks in megaloblastic bone marrow, reflecting DNA strand breaks and hypomethylation. Studies have found the MTHFR 677TT genotype, which reduces that activity by some 70%, to have reduced genomic DNA methylation.[78] One study found folate deprivation to produce hypomethylation of the *p53* gene and increased genome-wide DNA strand breakage. Such results suggest that folate deprivation may create fragile sites in the genome potentially inducing protooncogene expression. Another study found low-folate subjects with the MTHFR 677TT genotype to have hypomethylation of whole blood DNA, compared to those with the CC genotype; those differences were not apparent among folate-adequate subjects of both genotypes.[79] That folate status affects DNA integrity is supported by findings that circulating folate level is directly related to the length of peripheral lymphocyte telomeres, i.e., the capping chromosomal segments characterized by tandem repeats of DNA and associated proteins, dysfunction of which is associated with age-related disease.[80]
  - **Histone methylation** consists of the addition of a methyl group from SAM to lysyl and arginyl residues at the N-termini (i.e., the "histone tails") of histone proteins H3 and H4. This affects the binding of proteins, comprising a means of regulating gene expression and genomic stability. It can be associated with either active transcription or gene silencing. In this aspect of epigenetic control, folate serves both in the donation of the single-C unit as 5-methyl-FH_4, and as the acceptor as FH_4 for formaldehyde generated during oxidative demethylation of histone tails.
- **Transcription factor methylation** has been demonstrated as affecting transcriptional output.[81]
- **RNA methylation** is thought to protect from degradation by RNAses, extending the effective half-lives of RNAs. Evidence suggests that noncoding may also be subject to methylation.[82]
- **Methylation of phosphatidylethanolamine to produce phosphatidylcholine**, the predominantly phospholipid in membranes and lipoproteins. This methylation is dependent on SAM, consuming some 40% of the methyl donor in the liver. Thus, by reducing the flux of methyl groups from 5-methyl-FH_4, folate deficiency can also cause a secondary hepatic deficiency of choline.

77. These are regions of DNA in which the nucleotide composition has a high frequency of linear sequences of cytosine–guanine separated by a phosphate (5′C-p-3′G). In mammals, 70–80% of CpG sites are methylated. CpG islands typically consist of at least >300 base pairs more than half of which are CpGs. These are frequently located near the transcription starts of gene. Methylation of CpG sites within promoters can silence those genes.

78. Castro, R., Rivera, I., Ravasco, P., et al., 2004. J. Med. Genet. 41, 454–458.

79. Friso, S., Choi, S.W., Girelli, D., et al., 2002. Proc. Natl. Acad. Sci. U.S.A. 99, 5606–5611.

80. Paul, L., Catterneo, M., D'Angelo, A., et al., 2009. J. Nutr. 139, 1273–1278.

81. Stark, G.R., Wang, Y., Lu, T., 2011. Cell Res. 21, 375–380.

82. Huang, Y., Ji, L., Huang, Q., et al., 2009. Nature 461, 823–827.

Nucleotide Metabolism

Folate is required for the production of new cells through its functions in the synthesis of purines and thymidylate required for DNA synthesis. Folate deficiency results in the arrest of erythropoiesis prior to the latter stages of differentiation, resulting in apoptotic reduction of cells surviving to postmitotic, terminal stages in the condition called megaloblastic anemia. Anemia can have folate-responsive components in subjects with apparently normal plasma folate levels; therefore, addition of folate to iron supplements can improve the treatment of anemia in pregnancy as well as in undernourished individuals.

Folates are required for the synthesis of purines. While they are not required for the de novo synthesis of pyrimidines, they are required for the synthesis of thymidylate. Both roles are necessary for the de novo synthesis of DNA and, thus, for DNA replication and cell division. Disruption of these functions impairs cell division and results in the macrocytic anemia of folate deficiency.

- **Purine synthesis** (i.e., the synthesis of adenine and guanine) depends on the transfer of formate from 10-formyl-FH_4 to provide the C-2 and C-8 positions of the purine ring (Fig. 17.5). These reactions are catalyzed by aminoimidazolecarboxamide ribonucleotide transformylase and glycinamide ribonucleotide transformylase, respectively.
- **Thymidylate synthesis** depends on the transfer of the single-C unit from 5,10-methylene-FH_4 to deoxyuridine monophosphate, converting it to deoxythymidine monophosphate (dTMP). This step, catalyzed by **thymidylate synthase (TS)**,[83] is rate limiting to DNA replication and, thus, to the normal progression of the cell cycle. TS is expressed only in replicating tissues. During the S phase of the cell cycle TS, FH_2 reductase and SHMT enter the nucleus to effect the folate-dependent synthesis of thymidylate. TS activity limited by deprivation of folate, which results in uracil accumulation in DNA.

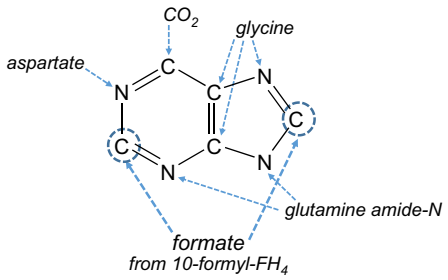

FIGURE 17.5 Sources of purine ring atoms.

83. The antifolate compound 5-fluorodeoxyuridylate complexes with the enzyme and its folate cosubstrate.

Regulation. The regulation of single-C metabolism is effected by the interconversion of oxidation states of the folate intermediates. In mammalian tissues, the β-C of SER is the major source of single-C units for these aspects of metabolism. That C-fragment is accepted by FH_4 to form 5,10-methylene-FH_4 (by **SHMT**), which has a central role in single-C metabolism. It can be used directly for the synthesis of thymidylate (by thymidylate synthase);[84] it can be oxidized to **5,10-methenyl-FH_4** (by **5,10-methylene-FH_4 dehydrogenase**) for the de novo synthesis of purines; or it can be reduced to 5-methyl-FH_4 (by MTHFR) for use in the synthesis of MET. The result is the channeling of single-C units in several directions: to MET, to thymidylate (for DNA synthesis), or to purine synthesis. Because folylpolyglutamates have been found to inhibit a number of the enzymes of single-C metabolism, it has been suggested that variation in their polyglutamate chain lengths (observed under different physiological conditions) may play a regulatory role.

Physiological Functions

Fetal development. Folate is required for normal embryonic growth and development. It plays an important role in promoting closure of the neural tube, defects of which results in malformations of the embryonic brain and/or spinal cord referred to as **NTDs**.[85] The role of folate in promoting neural tube closure likely involves its role in single-C metabolism, as exencephaly can be produced by knocking out SHMT, which is key to the synthesis of thymidylate and, thus, DNA[86] Folate may also function in preventing the misexpression of microRNAs,[87] which confer key roles in development by regulating the expression of certain target mRNAs. microRNAs show distinct expression patterns in the developing brain and are highly

84. This is the sole de novo path of thymidylate synthesis. It is also the only folate-dependent reaction in which the cofactor serves both as a single-carbon donor and as a reducing agent. Thymidylate synthase is the target of the anticancer drug 5-fluorouracil (5-FU); the enzyme converts the drug to 5-fluorodeoxyuridylate, which is incorporated into RNA and is a suicide inhibitor of the synthase, resulting in cellular accumulation of deoxyuridine triphosphate (dUTP) and incorporation of deoxyuridine (dU) into DNA. DNA with this abnormal base is enzymatically cleaved at sites containing it, leading to enhanced DNA breakage.

85. NTDs comprise the most common forms of congenital malformations, with an annual global incidence estimated at >300,000 new cases, >41,000 deaths, and the loss of 2.3 million disability-adjusted life years. These involve developmental failures of the neural structures (brain, spinal cord, cranial bones, vertebral arches, meninges, and overlying skin) formed from the embryonic neural tube in humans 20–28 days after fertilization. The most prominent NTDs are anencephaly and spina bifida. More than 95% of NTD pregnancies occur in families with no history of such defects; but women with one affected pregnancy or with spina bifida themselves face a risk of 3–4% of an NTD in a subsequent pregnancy.

86. Beaudin, A.E., Abarinov, E.V., Noden, D.M., et al., 2011. Am. J. Clin. Nutr. 93, 789–998.

87. miRNAs are small (ca. 22 nucleotides), noncoding transcripts that repress the expression of target mRNAs.

regulated by genomic methylation, deficiencies of which have also been proposed to contribute to NTDs. Folate deficiency does not produce neural tube changes in animal models; however, genetic deletion of methionine synthase reductase resulted in epigenetic changes manifest as congenital malformations in the mouse. Hcy treatment of chick and mouse embryos increases the frequencies of a wide range of congenital malformations including damage to cells of the neural crest.[88]

Homocysteinemia. Folate is required to maintain normal Hcy concentrations, elevations of which can occur through its overproduction from MET and, to a lesser extent, through its impaired disposal by transsulfuration to cystathionine. Both can have congenital causes[89] and can be affected by lifestyle factors[90] and nutritional status with respect to folate, vitamin B_6, and vitamin B_{12}.[91] Hcy can be converted to a thiolactone by methionyl tRNA synthase, in an error-editing reaction that prevents its incorporation into the primary structure of proteins; at high levels the thiolactone can react with protein lysyl residues. Protection against damage to high-density lipoproteins that results from homocysteinylation is effected by a Ca^{+2}-dependent Hcy-thiolactonase associated with those particles.[92] Homocysteinemia also causes displacement of protein-bound cysteine, which changes redox thiol status, probably via thiol–disulfide exchange and redox reactions. It has been suggested that pathogenesis may also involve SAH, which is a potent inhibitor of methyltransferases. SAH is reversibly converted with Hcy, the equilibrium favoring SAH. Therefore, homocysteinemia would be expected to lead to elevated levels of SAH, which appears to be present in the normal circulation at only low amounts (<0.2% of Hcy levels). Dietary intakes of polyunsaturated fatty acids (PUFAs) have been found to be a significant covariate with MTHFR C677T and A1298T genotype in affecting plasma Hcy level.[93] Individuals with 1298AA showed homocysteinemia only with consuming a high-PUFA diet; whereas, individuals with 677TT and the 1298C allele had low Hcy levels even on the high-PUFA diet. Circulating Hcy levels >13 μM[94] have been associated with dysfunction of several types:

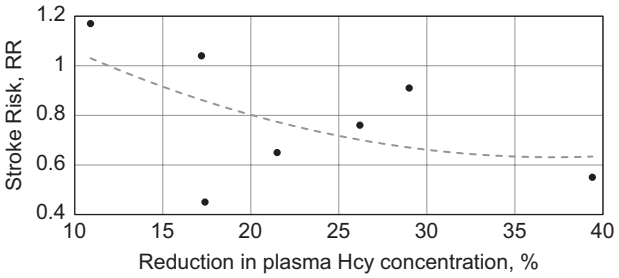

FIGURE 17.6 Results of seven randomized folate intervention trials showing relationship of homocysteine lowering and stroke risk. *After Wang, X., Qin, X., Demirtas, H., et al., 2007. Lancet 369, 1876–1882.*

- **Cardiovascular health**. Epidemiological associations have been made between moderately elevated plasma Hcy concentrations and risks of coronary, peripheral and carotid arterial atherosclerosis, venous thrombosis, carotid thickening, hypertension, and stroke (Fig. 17.6).[95] A meta-analysis of results of 27 cross-sectional and case–control studies[96] attributed 10% of total coronary artery disease to homocysteinemia.[97] That analysis suggested that a 5 μM increase in plasma Hcy level was associated with an increase in the risk of coronary artery disease comparable to a 0.5 mM (20 mg/dL) increase in plasma total cholesterol. A prospective, community-based study found plasma Hcy to be strongly inversely associated with plasma folate level and only weakly associated with plasma levels of vitamin B_{12} and pyridoxal phosphate (Table 17.11).[98] Other meta-analyses have concluded that folate supplementation can reduce risks for progression of carotid intimal thickening and stroke[99] but had no effect on risk of coronary revascularization.[100] Individuals with the MTHFR 677TT genotype are at risk for carotid intima-media thickening, itself a risk factor to vascular disease. They typically have lower levels of folate and higher levels of Hcy than other genotypes (Table 17.12).

88. Van Nil, N.H., Oosterbaan, A.M., Steegers-Theunisswen, R.P.M., 2010. Reprod. Toxicol. 30, 520–531.
89. Inherited deficiencies of cysteine β-synthase and 5,10-methylene-FH_4 reductase have been identified in humans. Genetic determinant of the Hcy response to MET has also been identified (Wernimont, S.M., Clark, A.G., Stover, P.J., et al., 2011. BMC Med. Genet. 12, 150–160; Lievers, K.J.A., Kluijtmans, L.A.J., Blom, J., et al., 2006. Eur. J. Hum. Genet. 14, 1125–1129.)
90. Hcy levels are increased by smoking and chronic alcohol use, and reduced by regular physical activity.
91. Chronic alcoholics have been found to have mean serum Hcy levels, about twice those of nonalcoholics. Chronic ethanol intake appears to interfere with single-C metabolism, and alcoholics are at risk of folate deficiency.
92. Jakubowski, H., 2000. J. Nutr. 130, 377S–381S.
93. Huang, T., Tucker, K.L., Lee, Y.C., et al., 2011. J. Nutr. 141, 654–659.
94. In the NHANES 2003–2006 cohort, such levels were found in 8% of adults ≥20 years and 19% of adults ≥60 years (Center for Disease Control and Prevention. Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population [2012]. CDC, Atlanta).
95. The low prevalence of coronary heart disease among South African blacks has been associated with their typically lower plasma Hcy levels and their demonstrably more effective Hcy clearance after methionine loading.
96. Boushey, C.J., Beresford, S.A., Omenn, G.S., et al., 1995. J. Am. Med. Assoc. 274, 1049–1057.
97. Defined as a plasma Hcy concentration >14 μM.
98. Selhub, J., Jacaques, P.F., Bostom, A.G., et al., 1996. J. Nutr. 126, 1258S–1265S.
99. Yang, H.T., Lee, M., Hong., K.S., et al., 2012. Eur. J. Intern. Med. 23, q745–q754; Qin, X., Xu, M., Zhang, Y., et al., 2012. Atherosclerosis 222, 307–313; Huo, Y., Qin, X., Wang, J., et al., 2012. Int. J. Clin. Pract. 66, 544–551; Wang, X., Qin, X., Demirtas, H., et al., 2007. Lancet 369, 1876–1882.
100. Qin, X., Fan, F., Cui, Y., et al., 2014. Clin. Nutr. 33, 603–612.

TABLE 17.11 Plasma Levels of Homocysteine and Vitamin in Elderly Subjects

Subject Age/Years	Hcy μM	% Elevated	Folate nM	Vitamin B_{12} pM	Pyridoxal Phosphate nM
Men					
67–74	11.8	25.3	9.3	265	52.6
75–79	11.9	26.7	9.3	260	49.6
80+	14.1	48.3	10.0	255	47.6
Trend, *P*	<0.001	<0.001	NS[a]	NS	NS
Women					
67–74	10.7	19.5	10.4	302	59.9
75–79	11.9	28.9	10.2	289	52.2
80+	13.2	41.1	9.7	290	52.1
Trend, *P*	<0.001	<0.001	NS	NS	NS

[a]*NS,* not significant.
From Selhub, J., Jacques, P.F., Wilson, P.W., et al., 1993. J. Am. Med. Assoc. 270, 2693–2698.

TABLE 17.12 Effects of Polymorphisms on Biomarkers of Folate Status

Polymorphism	Plasma Folate, nM	Erythrocyte Folate, nM	Plasma Hcy, μM
MTHFR 677C>T			
CC	12.4 (11.6, 13.2)	889 (851,929)	8.9 (8.6, 9.2)
CT	11.3 (10.6, 11.9)	821 (791, 852)	9.2 (9.0, 9.5)
TT	10.0 (9.2, 11.9)	652 (611, 695)	11.2 (10.5, 11.9)
SLC19A1 80G>A			
GG	11.9 (11.0, 12.9)	861 (815, 910)	9.6 (9.3, 10.0)
GA	11.4 (10.9, 12.0)	804 (775, 833)	9.4 (9.1, 9.7)
AA	10.9 (10.0, 11.9)	776 (733, 822)	9.2 (8.8, 9.6)

Means with 95% CI; means with like superscripts are not significantly different, $p < .05$.
n=120–394; Bueno, O., Molloy, A.M., Fernandez-Ballart, J.D., et al., 2016. J. Nutr. 146, 1–8.

Individuals of that genotype who are also of low riboflavin status are at risk to high blood pressure, presumably due to the lower affinity of the TT enzyme for its flavin cofactor.[101] A study of the NHANES III (1991–94) cohort found individuals with the TT genotype to have a 31% lower risk to cardiovascular disease mortality;[102] however, a meta-analysis of 53 studies showed the TT genotype to be associated with a 20% greater risk of venous thrombosis compared to the CC genotype.[103] Individuals with the 1298CC genotype have a relatively higher risk to cardiovascular disease than those carrying the 1298A allele.

- **Immune function**. Studies with human monocytes demonstrated that in vitro treatment with Hcy activated those cells to express inflammatory cytokines.[104] This suggests that homocysteinemia, by activating monocytes, may contribute to chronic inflammation involved in endothelial cell damage.

101. Yamada, K., Chen, Z., Rozen, R., et al., 2001. Proc. Natl. Acad. Sci. U.S.A. 98, 14853–14858.
102. Yang, Q., Bailey, L., Clarke, R., et al., 2012. Am. J. Clin. Nutr. 95, 1245–1253.
103. Den Heijer, M., Lewington, S., Clarke, R., 2005. J. Thromb. Haemostasis 3, 292–299.
104. Su, S.J., Huang, L.W., Pai, L.S., et al., 2005. Nutrition 21, 994–1002.

- **Bone health**. Homocysteinemia can affect the developing skeleton, producing knockvknees (*genu valgum*) and unusually high arches of the foot (*pes cavus*) in children, with subsequent development of marfanoid features (long limbs) and osteoporosis. Homocysteinemia induced in animals by feeding large amounts of MET or Hcy is accompanied with severe trabecular bone loss with attendant changes in microarchitecture and strength. An analysis of the NHANES 1999–2004 data found homocysteinemia (Hcy >13 μM) to be associated with a twofold increase is risk of lumbar spine osteoporosis.[105] These changes appear to ensue from ROS-dependent activation of osteoclastic bone resorption. Randomized trials using supplements of folate and other B-vitamins (B_6, B_{12}) have found no effects on biomarkers of bone turnover.[106] Some studies have found individuals with the 677TT genotype to have relatively high risks of fracture and relatively lower bone mineral density independent of plasma Hcy level.[107] However, other factors are likely to be involved; a study that found 677TT women to show reduced bone mineral density only if they also had relatively low intakes of folate, vitamin B_{12}, and riboflavin.[108]
- **Age-related decline in physical function**. Subjects in the highest quartile of plasma Hcy concentration had more than four times the risk of being in the worst quartile of decline in physical function.[109]
- **Neurologic function**. Hcy can be neurotoxic. This can occur in several ways: by SAH-inhibition of methyltransferases involved in catecholamine methylation, by Hcy oxidation products acting as agonists of the N-methyl-D-aspartate receptor to cause excitotoxicity, by ROS produced by Hcy oxidation, etc. Folate may also be directly involved in the regulation of neurotransmitter metabolism, as neuropsychiatric subjects with low-erythrocyte folate levels and homocysteinemia show low-cerebral spinal fluid levels of the serotonin metabolite 5-hydroxyindole acetic acid and reduced turnover of dopamine and noradrenaline.[110] It has been suggested that folate may act as a structural analog of tetrahydro biopterin,[111] an essential cofactor in the metabolism of monoamine neurotransmitters. MTHFR and dihydrofolate reductase are thought to function in tetrahydrobiopterin metabolism; the MTHFR 677TT genotype is associated with increased risk to neurological disorders. Thus, homocysteinemia can lead to several types of neurocognitive effects:
  - **Cognitive function**. Homocysteinemia is associated with age-related cognitive decline and risk of developing dementia; however, analyses of the NHANES data suggest that these outcomes are associated with low-vitamin B_{12} status and *not* low-folate status.[112] In fact, they suggest that high-folate status may exacerbate the neuropsychiatric effects of low-vitamin B_{12} status. Cross-sectional studies have indicated low-folate status to be a risk factor for low reading cognition in children[113] and for cognitive decline in aging;[114] and randomized clinical trials have found folic acid supplementation of older adults to improve domains of cognitive function that typically decline with age.[115] Meta-analyses of randomized clinical trials concluded that folic acid yielded no beneficial effects on measures of cognition within 3 years of supplementation.[116] However, supplementation with folic acid and vitamins B_6 and B_{12} reduced brain atrophy progression (a proxy for neuronal injury that would result in cognitive decline) particularly in subjects with higher plasma concentrations of ω-3 fatty acids.
  - **Alzheimer's disease**. Plasma Hcy level is directly associated with plasma level of amyloid β40, a protein associated with aging but not necessarily with Alzheimer's disease (AD). Relatively high intakes of folate have been associated with lower risk to AD[117]
  - **Depression**. Mood changes and other symptoms of depression have frequently been observed in folate deficiency. These symptoms are associated with homocysteinemia; it has been suggested that they reflect Hcy-induced cerebral vascular disease and

105. Bailey, R.L., Looker, A.C., Lu, Z., et al., 2015. Am. J. Clin. Nutr. 102, 687–694.
106. e.g., Green, T.J., McMahon, J.S., Skeaff, C.M., et al., 2007. Am. J. Clin. Nutr. 85, 460–464; van Wijgaarden, J.P., Swart, K.M.A., Enneman, A.W., et al., 2014. Am. J. Clin. Nutr. 100, 1578–1586.
107. Villadsen, M.M., Bunger, M.H., Carstens, M., et al., 2005. Osteoporos. Int. 16, 411–416; Abrahamsen, B., Jorgensen, H.L., Nielsen, T.L., et al., 2006. Bone 38, 215–219; Hong, X., Hsu, Y.U., Terwedow, H., et al., 2007. Bone 40, 737–742.
108. Abrahamsen, B., Madsen, J.S., Tofteng, C.L., et al., 2005. Bone 36, 577–583.
109. Kado, D.M., Bucur, A., Selhub, J. et al. (2002) Am. J. Med. 113, 537–542.
110. Many patients are likely to be exposed to anticonvulsants that antagonize folate metabolism, e.g., phenytoin, carbamazepine, primidone, phenobarbital, valproic acid products, pamotrigine.
111. Both contain a pterin moiety.
112. Selhub, J., Morris, M.S., Jacques, P.F., et al., 2009. Am. J. Clin. Nutr. 89, S702–S706.
113. Nguyen, C.T., Gracely, E.J., Lee, B.K., 2013. J. Nutr. 143, 500–504.
114. Kado, D.M., Karlamangla, A.S., Huang, M.H., et al., 2005. Am. J. Med. 118, 161–167.
115. Elias, M.F., Sullivan, L.M., D'Agostino, R.B., et al., 2005. Am. J. Epidemiol. 162, 644–653; Durga, J., van Boxtel, M.P.J., Schouten, E.G., et al., 2007. Lancet 369, 208–216.
116. Wald, D.S., Kasturiratne, A., Simmonds, M., 2010. Am. J. Med. 123, 522–527; Clarke, R., Bennett, D., Parish, S., et al., 2014. Am. J. Clin. Nutr. 100, 657–666.
117. Luchsinger, J.A., Tang, M.X., Miller, J., et al., 2007. Arch. Neurol. 64, 86–92.

neurotransmitter deficiency. Studies have shown that individuals of the MTHFR 677TT genotype have increased risks of depression.[118] A systematic review of randomized clinical trials suggested that folic acid may benefit the treatment of depression.[119] Victor Herbert[120] experienced depressive mood, irritability, insomnia, fatigue, and forgetfulness after consuming a folate-deficient diet for several months.[121] When he took a folate supplement, those symptoms resolved within 48 h.

- **Schizophrenia**.[122] Both depressed and schizophrenic patients responded to daily doses of 15 mg of 5-methyl-FH_4 with improved clinical and social outcomes compared with placebo controls.[123] Studies have shown that individuals of the MTHFR 677TT genotype have increased risks of schizophrenia.

8. BIOMARKERS OF FOLATE STATUS

Folate status can be assessed by analyses of blood (Fig. 17.7):[124]

- **Serum folate concentration**. Serum folate level is comprised mostly (>80%) of 5-methyl-FH_4. It is responsive to short-term changes in folate intake and is more responsive to intake of folic acid than to intakes of food folates. It also is affected by MTHFR 677 and SLC19A1 genotypes (Table 17.12); MTHFR 677CC individuals have been found to have 13% greater levels than those with the 677 TT genotype.[125] Levels <3 ng/mL are indicative of folate deficiency.

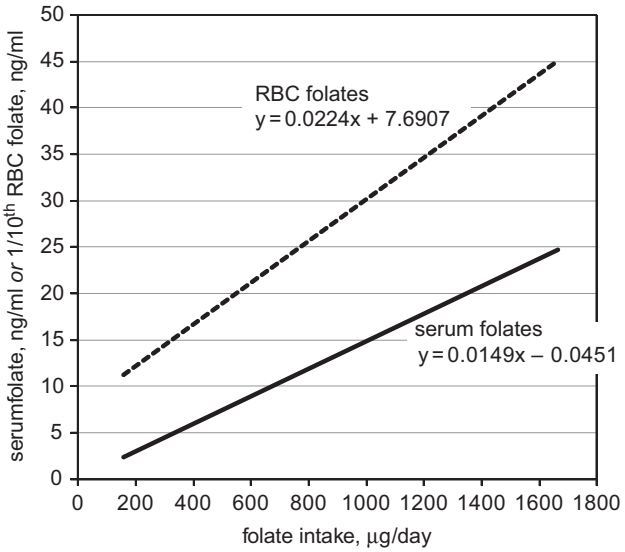

FIGURE 17.7 Relationships of folates in serum and erythrocytes (RBC) to dietary folate intake. Population data from National Health and Nutrition Examination Surveys (NHANES) III and annual NHANES from 1999 to 2004. Adapted from *Quinlivan, E.P., Gregory, J.F., 2007. Am. J. Clin. Nutr. 86, 1773–1779.*

- **Erythrocyte folate concentration**. Erythrocyte folate level indicates long-term folate status, reflecting folate status during erythropoiesis and, thus, particularly the preceding 120 days.[126] Responses to changes in folate intake tend to be greater for women that for men; half of this difference is explained on the basis of differences in body size. Erythrocyte folate levels correlate with hepatic folate levels; thus this biomarker is taken as an indicator of tissue folate stores.[127] Erythrocyte folate level is more responsive to intake of folic acid than to intakes of food folates. It also appears to be affected by MTHFR 677 genotype, as CC individuals have been found to have 16% greater levels than TT individuals. Erythrocyte folate concentrations <140 ng/mL (~320 nM) are indicative of folate deficiency.

Other biomarkers have been used to assess various aspects of folate status or function:

- **Plasma Hcy concentration**. This is not a specific indicator of folate status. While folate deficiency can produce elevated plasma Hcy concentration, that outcome can also be produced by deficiencies of vitamin B_{12} and/or MET, renal insufficiency, and some drugs. These factors must be considered in interpreting plasma Hcy level. In many populations exposed to food folate fortification, elevated plasma Hcy may be more likely to indicate suboptimal vitamin B_{12} status.

118. Almedia, O.P., McCaul, K., Hankey, G.J., et al., 2008. Arch. Gen. Psychiatr. 65, 1286–1294.
119. Taylor, M.J., Carney, S.M., Goodwin, G.M., et al., 2004. J. Psychopharmacol. 18, 251–256.
120. Victor Herbert, M.D. (1927–2002) was a prominent American nutrition scientist. He was the first to recognize that the anemia common among pregnant women was caused by folate deficiency; that alcoholics were frequently folate deficient; and that vitamin B_{12} deficiency produced the "methyl folate trap." Without missing a beat in his scientific career, he obtained a law degree and took on fraudulent healthy claims about putative nutrients (e.g., "vitamin B_{15}"). He served as President of the American Society for Clinical Nutrition and was the Chief of Hematology and Nutrition Laboratory of the Bronx Veterans Administration Hospital.
121. Herbert, V., Zalusky, R., 1962. J. Clin. Invest. 41, 1263–1276.
122. Nearly two-thirds of patients with megaloblastic anemia due to vitamin B_{12}/folate deficiency show neuropsychiatric complications.
123. Godfrey, P.S., Toone, B.K., Carney, M.W., et al., 1990. Lancet 336, 392–395.
124. Analyses of folates have been complicated by methodologic idiosyncrasies associated with the radioimmunoassay and a microbiological assay that have been used. The latter produced higher values, which are considered to be more accurate. This necessitates adjustment for that difference (Pfeiffer, C.M., Hughes, J.P., Lacher, D.A., et al., 2012. J. Nutr. 142, 886–893).
125. Tsang, B.L., Devine, O.J., Cordero, A.M., et al., 2015. Am. J. Clin. Nutr. 101, 1286–1294.
126. i.e., The half-life of erythrocytes.
127. Wu, A., Chanarin, I., Slavin, G., et al., 1975. Br. J. Haematol. 29, 469–478.

Homocysteinemia is typically marked by plasma Hcy concentrations >13 μM.

- **Serum unmetabolized folic acid concentration**. Circulating levels of unmetabolized folic acid are highly variable and reflect concentrations of soluble FBP to which that vitamer is preferentially bound. This biomarker typically correlates with serum total folates, particularly in individuals exposed to folic acid fortification/supplementation. It may be most valuable as an indicator of exposure to folic acid-fortified foods.
- **Urinary folates excretion**. Twenty-four hour urinary folate excretion can provide information about average daily folate status. This biomarker is subject to significant interindividual variation part of which has to do with little folate excretion at less than RDA levels of intake.
- **Urinary *p*-aminobenzoylglutamate and *p*-acetamidobenzoylglutamate concentrations**.[128] These oxidative metabolites of folate indicate folate turnover. Their concentrations in 24-h urine samples correlate with the concentrations of folates in serum and erythrocytes[129] but are less sensitive to changes in folate intake than those biomarkers.

9. FOLATE DEFICIENCY

Folate deficiency can have primary (privational) and secondary (nonprivational) causes (Fig. 17.11). Primary causes involve inadequate folate supply, i.e., dietary patterns that fail to provide folate in adequate amounts. Secondary causes relate to impaired absorption, metabolism, or metabolic function of the vitamin (Table 17.13):[130]

Malabsorption

- **Inflammatory bowel diseases** that cause persistent mucosal damage (Crohn disease, ulcerative colitis, tropical sprue, celiac disease).
- **Zinc (Zn) deficiency** reduces the absorption of folyl-polyglutamates (but *not* monoglutamates). This is thought to indicate a need for Zn by folate-metabolizing enzymes.
- **Several drugs**, the antiproliferative methotrexate; the anticonvulsants (diphenylhydantoin and phenobarbital); the antiinflammatory sulfasalazine; the diuretic triamterene; the glycemic control drug metformin.
- Chronic alcoholism.

128. Both metabolites can also be determined in plasma/serum.

129. Wolfe, J.M., Baailey, L.B., Herrlinger-Garcia, K., et al., 2003. J. Nutr. 77, 919–923.

130. Examples include *tropical sprue* (inflammation of the mucous membranes of the alimentary tract) and other types of enteritis that involve malabsorption and, usually, diarrhea.

TABLE 17.13 General Signs of Folate Deficiency

Organ System	Signs
General	Reduced appetite, growth
Dermatologic	Alopecia, achromotrichia, dermatitis
Muscular	Weakness
Gastrointestinal	Inflammation
Erythrocytes	Macrocytic anemia
Nervous	Depression, neuropathy, paralysis

Metabolic Impairments

- **Vitamin B_{12} and MET deficiencies**, patients with pernicious anemia[131] generally have low circulating folate levels due to accumulation of the vitamin as 5-methyl-FH_4 in the "methyl folate trap." These low-plasma folate levels cannot be corrected by supplements of MET, although that amino acid, via SAM, inhibits 5,10-methylene-FH_4 reductase to reduce the synthesis of 5-methyl-FH_4.
- **Chronic alcoholism** impairs hepatic MET metabolism by inhibiting the vitamin B_{12}-dependent transmethylation of Hcy.
- Dihydrofolate reductase inhibitors (e.g., methotrexate).
- **Genetic factors**, MTHFR deficiency; glutamate formiminotransferase deficiency; MTHFR polymorphisms.
- **Increased requirements**, hemodialysis, prematurity, pregnancy, lactation.

General Signs of Folate Deficiency

Deficiencies of folate result in impaired biosynthesis of DNA and RNA, and thus in reduced cell division, which is manifested clinically as anemia, dermatologic lesions, and poor growth in most species (Table 17.13). The anemia of folate deficiency is characterized by the presence of large, nucleated erythrocyte-precursor cells called macrocytes and of hypersegmented polymorphonuclear neutrophils,[132] reflecting decreased DNA synthesis and delayed maturation of bone marrow, i.e., megaloblastic erythropoiesis.[133] While clinical deficiency is usually detected as anemia, megaloblastic changes occur in other cells, reflecting growth arrest in the G2 phase of the cell cycle just prior to mitosis.

131. Pernicious anemia is caused by vitamin B_{12} deficiency, resulting from the lack of the intrinsic factor required for the enteric absorption of that vitamin (see Chapter 18).

132. Neutrophil hypersegmentation is defined as >5% five-lobed or any six-lobed cells per 100 granulocytes.

133. This distinguishes the macrocytic anemia of folate or vitamin B_{12} deficiencies from those macrocytic anemias caused by such factors as alcohol abuse, hypothyroidism, heavy smoking, chronic hemolytic anemia, which have normoblastic erythropoiesis.

TABLE 17.14 Recommended Folate Intakes

The United States		FAO/WHO	
Age/Sex	AI[a], µg/day	Age/Sex	RNI[b], µg/day
0–6 months	65	0–11 months	80
7–11 months	80		
1–3 years	150	1–3 years	160
4–8 years	200	4–6 years	200
9–13 years	300	7–9 years	300
>13 years	400	>9 years	400
Pregnancy	600	Pregnancy	600
Lactation	550	Lactation	500

[a]*Adequate Intakes are given, as RDAs have not been established. Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.*
[b]*Recommended Nutrient Intakes, Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. WHO, Rome, 286 pp.*

Affected cells have increased DNA content and DNA strand breakage, which is thought to result from the misincorporation of uracil into DNA in place of thymidylate—a potentially mutagenic situation. This sign, which is because it is a manifestation of impaired DNA synthesis, can also be caused by deficiency of vitamin B_{12}.

Severely anemic individuals show weakness, fatigue, difficulty in concentrating, irritability, headache, palpitations, and shortness of breath. Folate deficiency also affects the intestinal epithelium, where impaired DNA synthesis causes megaloblastosis of enterocytes. This is manifested clinically as malabsorption and diarrhea and is a contributor to the clinical picture of tropical sprue. Anemia can have folate-responsive components in subjects with apparently normal plasma folate levels; therefore, addition of folate to iron supplements can improve the treatment of anemia in pregnancy as well as in undernourished individuals.

Deficiency Signs in Humans

Adequate intakes for folate have been established (Table 17.14). Chronic intakes below those levels produce folate deficiency characterized by a sequence of signs, starting with nuclear hypersegmentation of circulating polymorphonuclear leukocytes[134] within about 2 months of deprivation of the vitamin. This is followed by megaloblastic anemia and, then, general weakness, depression, and polyneuropathy. In pregnant women, the deficiency can lead to birth defects or spontaneous abortion. Elderly humans tend to have lower circulating levels of folate, indicating that they may be at increased risk of folate deficiency. This finding appears to involve changes in food habits, which affect intake of the vitamin. Supplements of folate and iron[135] are recommended by the World Health Organization for use in many areas with endemic anemia among women of childbearing age.

Low-Folate Status

That folate-responsive homocysteinemia can be demonstrated in apparently healthy free-living populations suggests the prevalence of undiagnosed suboptimal vitamin status. For example, twice-weekly treatments of elderly subjects with folate (1.1 mg) in combination with vitamin B_{12} (1 mg) and vitamin B_6 (5 mg) have been shown to reduce plasma concentrations of Hcy by as much as half and also to reduce methylmalonic acid (MMA), 2-methylcitric acid, and cystathionine despite the fact that pretreatment plasma levels of those vitamins were not low.

Homocysteinemia. Folate supplementation has been shown to reduce homocysteinemia (Table 17.15), independently reverse endothelial dysfunction, reduce arterial pressure, and increase coronary dilation. It is likely that these effects involve the stimulation of nitric oxide production by 5-methyl-FH_4 and, perhaps, inhibition of lipoprotein oxidation by folic acid. Reduction of serum Hcy is linear up to daily folate intakes of about 0.4 mg, particularly for individuals with relatively high-serum Hcy levels (Fig. 17.8). Greater efficacy may be realized when folate is given in combination with vitamin B_{12}. A meta-analysis of 25 randomized controlled trials showed that daily intakes of 0.8 mg folic acid are required to realize maximal reductions in plasma Hcy levels.

NTDs. Low-maternal folate status is linked with increased risks of NTDs in infants, which affects 1 of every 33 births in the United States and 6% of all births worldwide.[136] NTDs are regarded as multifactorial disorders, but several clinical intervention trials have demonstrated that periconceptional supplemental folate can reduce NTD risk. One of these, a large, well-designed, multicentered trial conducted by the British Medical Research Council found that a daily oral dose of 4 mg of folic acid reduced significantly the incidence of confirmed NTDs among the pregnancies of women at high risk for such disorders.[137] Several subsequent

134. These cytological changes do not become manifest until well after circulating folate levels drop (by 6–8 weeks).

135. 60 mg Fe (as ferrous sulfate) and 2.8 mg folate.

136. CDC, 2015. Morb. Mortal. Wkly. Rep. 64, 1–5

137. The double-blind, randomized clinical trial involved 1817 women, each with a previous affected pregnancy, who were followed in 33 clinics in seven countries. Each subject was randomly assigned to a placebo or a supplement (vitamins A, D, C, B_6; thiamin; riboflavin; and nicotinamide) and/or a placebo or folic acid (4 mg/day) in a complete factorial design and the outcomes of their pregnancies were confirmed. Of 1195 completed pregnancies, 27 had confirmed NTDs; these included 21 cases in both groups not receiving folate, but only 6 cases in both folate groups (relative risk, 0.28; 95% CI, 0.12–0.71). The multivitamin treatment did not significantly affect the incidence of NTDs (MRC Vitamin Research Group, 1991. Lancet 338, 131–137).

TABLE 17.15 Effect of Folate Supplementation on Plasma Homocysteine Levels in Healthy Adults

Treatment	Homocysteine, μM		Change, %	*p*	% of Subjects With >25% Decrease
	Week 0	Week 13			
Placebo	14.6 (12.7, 16.8)[a]	15.3 (13.2, 17.7)[a]	4.8	.23	10.5
5-Methyl-FH_4	13.9 (12.1, 15.9)	11.2 (9.8, 12.8)	−19.4	.001	46.2
Folic acid	13.7 (12.5, 15.1)	10.7 (9.6, 11.9)	−21.9	<.0001	48.7

[a]*Mean (95% C.L.).*
Zappacosta, B., Mastroiacovo, P., Persichilli, S., et al., 2013. Nutrients 5, 1531–1543.

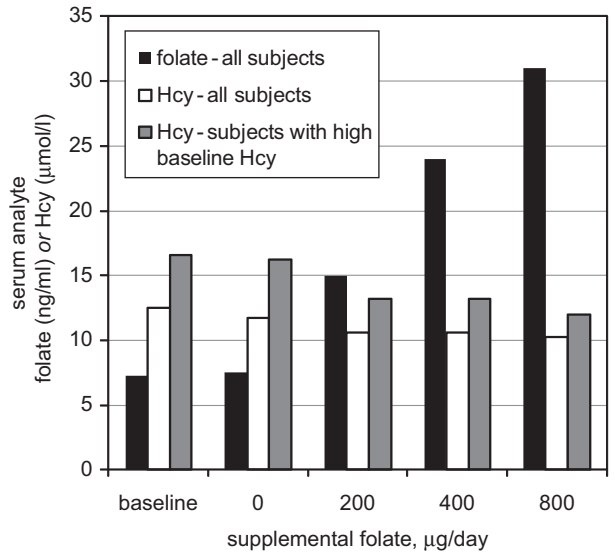

FIGURE 17.8 Effects of folate supplementation on serum levels of folate and homocysteine: results of a 26-week interventions. *After Tighe, P., Ward, M., McNulty, H., et al., 2011. Am. J. Clin. Nutr. 93,11–18.*

studies (Table 17.16) have supported those findings, showing folate supplements to reduce NTD risk by 50–70%. These have included trials conducted in the United States, which found folate supplements (400–4000 μg) effective in preventing NTDs in women with prior NTD pregnancies.[138] While folate supplements do not appear to affect NTD case fatality rates, reductions in NTD incidence are associated with reductions in neonatal deaths. A meta-analysis of eight observational studies indicated that folate supplementation was associated with a 46% reduction in NTD risk, which was associated with a 13% reduction in neonatal deaths.[139] Greatest benefits of folate supplementation have been found in countries with relatively high NTD rates (Fig. 17.9). A trial conducted in China with some 250,000 subjects showed that a daily supplement containing 400 μg folic acid consumed with ≥80% compliance during the periconceptional period was associated with reductions in NTD risk of 85% in a high NTD prevalence area and of 40% in a low prevalence area.[140] A systematic review of 14 folate intervention trials pointed out that not all NTD cases can be prevented by folate, i.e., 8–10 cases per 10,000 live births or abortions appear to be unaffected by increasing folate intake.[141]

Low folate status per se appears to be insufficient to cause NTDs. Instead, maternal low folate status appears to interact with fetal genes and other dietary factors affecting single-C metabolism. In animal models, this involves epigenetic marks on promoters essential for neurogenesis[142] and normal activity of SHMT.[143] In humans, this includes MTHFR 677C > T genotype: 677TT homozygosity has been associated with a fivefold increase in NTD risk for mothers not using multivitamin supplements.[144] A systematic review of clinical trials and observational studies found that erythrocyte folate concentrations in women are inversely associated with the presence of the 677T allele, i.e., CC > CT > TT.[145] Studies have also found DTD risk to be affected by maternal polymorphisms in the RFC (SLC19A1), methylene-FH_4 dehydrogenase, MET synthase, and Met synthase reductase.[146] Evidence suggests that NTDs in humans can result from tissue-specific

138. Centers for Disease Control and Prevention, 2000. Morb. Mortal. Wkly. Rep. 49, 1–4; Stevenson, R.E., Allen, R.E., Pai, G.S., et al., 2000. Pediatrics 106, 677–683.
139. Blencowe, H., Cousens, S., Modell, B., et al., 2010. Int. J. Epidemiol. 39:110–120.
140. Berry, R.J., Li., Z., Erickson, J.D., et al., 1999. N. Engl. J. Med. 341, 1485–1490.
141. Heseker, H.B., Mason, J.B., Selhub, J., et al., 2009. Br. J. Nutr. 102, 173–180.
142. i.e., *Hes*1 and *Neurog*2; Ichi, S., Costa, F.F., Bishof, J.M., et al., 2010. J. Biol. Chem. 285, 36922–36932.
143. Beaudin, A.E., Abarinov, E.V., Noden, D.M., et al., 2011. Am. J. Clin. Nutr. 93, 789–798; Beaudin, A.E., Abarinov, E.V., Malysheva, O., et al., 2012. Am. J. Clin. Nutr. 95, 109–114.
144. Botto, L.D., Moore, C.A., Khoury, M.J., et al., 1999. N. Engl. J. Med. 341, 1509–1519.
145. Tsang, B.L., Devine, O.J., Cordero, A.M., et al., 2015. Am. J. Clin. Nutr. 101, 1286–1294; Bueno, O., Molloy, A.M., Fernandez-Ballart, J.D., et al., 2016. J. Nutr. 146, 1–8.
146. Imbard, A., Benoist, J.F., Blom, H.J., 2013. Int. J. Environ. Res. Public Health 10, 4352–4389.

TABLE 17.16 Results of Placebo-Controlled, Clinical Intervention Trials of Folate Supplements in the Prevention of Neural Tube Defects (NTDs)

Trial	Folate Treatment	NTD Rates, Cases/Total Pregnancies		RR (95% CI)
		Placebo	Treatment	
1[a]	4 mg	4/51	2/60	0.42 (0.04–2.97)
2[b]	4 mg ± multivitamins	21/602	6/593	0.34 (0.10–0.74)
3[c]	0.8 mg + multivitamins	2/2104	0/2052	0.00 (0.00–0.85)

[a]*Lawrence, K.M., James, N., Miller, M., Campbell, H., 1981. Br. Med. J. 281, 1542–1511 (women with NTD histories).*
[b]*Milunsky, A., Jick, H., Jick, S.S., et al., 1989. J. Am. Med. Assoc. 262, 2847–2852 (women with NTD histories).*
[c]*Czeizel, A.E., Dudás, I., 1992. J. Am. Med. Assoc. 327, 1832–1835 (women without previous NTD births).*

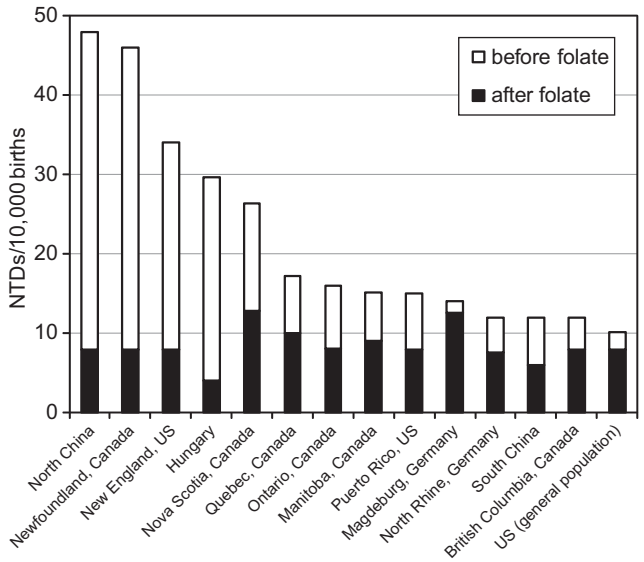

FIGURE 17.9 Reductions in NTD (neural tube defect) risk achieved in folate intervention trials and several countries. *After Heseker, H.B., Mason, J.B., Selhub, J., et al., 2009. Br. J. Nutr. 102, 173–180.*

differential hypermethylation of the fetal genes encoding the folate transporters FBP1 and RFC.[147]

Other effects on fetal growth. Evidence is inconsistent for associations of low-folate status and risks of reduced fetal growth or other congenital defects. Evidence suggests that MTHFR 677TT genotype elevates risk of Down syndrome in individuals also carrying a mutation in methionine synthase reductase.[148] Mothers and fetuses with the heterozygous 677CT genotype appear to have the best chances for viable pregnancies and live births.[149] Women with the MTHFR 1298CC genotype have lower chances of producing healthy infants than women with the 1298AA genotype.[150] The prenatal use of iron-folate supplements has reduced low birth weight in India and Nepal,[151] and the periconceptual use of folate has reduced preterm births in China.[152] A study found that high serum levels of folates and vitamin B_{12} were associated with greater success of assisted reproductive technologies;[153,154] however, a study in Sweden found no benefits of supplemental folate.

Carcinogenesis. Low-folate status has been associated with increased risk of cancers.[155] Women positive for human papilloma virus have a fivefold increase in risk of cervical dysplasia when they also have low serum folates.[156] Two large epidemiological studies have indicated that folate adequacy may reduce the effect of alcohol consumption in elevating breast cancer risk.[157] MTHFR polymorphisms have been related to risks to esophageal cancer, lymphocytic leukemia, and malignant lymphoma.[158] The 1298CC genotype has been associated with moderate reductions in colorectal cancer risk. The 677TT genotype does not appear to affect risk to colorectal adenoma unless folate status is low, in which case it is associated with an increase.[159] Studies in animal models have shown folate deprivation to promote colon carcinogenesis. Meta-analyses of cohort studies have

147. Farkas, S.A., Böttiger, A.K., Isaksson, H.S., et al., 2013. Epigentics 8 (3), 303–316.
148. Hobbs, C.A., Sherman, S.L., Yi, P., et al., 2000. Am. J. Hum. Genet. 67, 623–630.
149. Laanpere, M., Altmäe, S., Straveus-Evers, A., et al., 2009. Nutr. Rev. 68, 99–113.
150. Haggarty, P., McCallum, H., McBain, H., et al., 2006. Lancet 367, 1513–1519.
151. Balarajaan, Y., Subramanian, S.V., Fawzi, W., 2013. J. Nutr. 143, 1309–1315; Nisar, Y.B., Dibley, M.J., Mebrahtu, S., et al., 2015. J. Nutr. 145, 1873–1883.
152. Li, Z., Zhang, L., Ki, H., et al., 2014. Int. J. Epidemiol. 43, 1132–1139.
153. i.e., In vitro fertilization and intracytoplasmic sperm injection.
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found food–folate intakes to be associated with reductions in colorectal cancer risk.[160] However, meta-analyses of several randomized clinical trials concluded that folate supplementation is ineffective in reducing site-specific cancer risks, with the single exception of melanoma.[161]

Folate Supplementation and Fortification

Supplement use. The NHANES data have indicated that some 23% of American adults use folate-containing dietary supplements, whereas the majority (77%) of pregnant women do so.[162] These are typically in the form of a multivitamin/mineral supplement, which provides pregnant women more than 800 µg of the vitamin per day.

Food system-based fortification. In 1992 the U.S. Public Health Service issued a recommendation that all women of childbearing age consume 0.4 mg folic acid daily to reduce their risks of an NTD pregnancy. In the following year, the U.S. Food and Drug Administration (FDA) ruled that all cereal grain products be fortified with 140 µg folic acid per 100 g, and that additions of folic acid be allowed for breakfast cereals, infant formulae, medical and special dietary foods, and meal replacement products. Other countries developed similar policies; those that increased the folate in their food systems experienced significant reductions in the incidence of NTDs: the United States, by 19–31%; Costa Rica, by 63–87%; and Canada, by 47–54%.[163] The US folate-fortification program increased folate intakes and more than doubled circulating levels of the vitamin, reduced plasma Hcy levels, and reduced the incidence of NTDs (Fig. 17.10). Countries using folate fortification in 1999–2002 experienced significantly greater reductions in stroke incidence than those countries without such programs.[164]

Questions about high-folate intakes and cancer risk. That folate functions as a cofactor in nucleotide synthesis suggests the possibility that plentiful supplies of the vitamin may facilitate proliferation in dysplastic and malignant cells. Results of two studies appear to support the prospect that supranutritional folate intake may promote cancer. A prospective study involving 25,400 American women 55–74 years of age found the incidence of breast cancer to

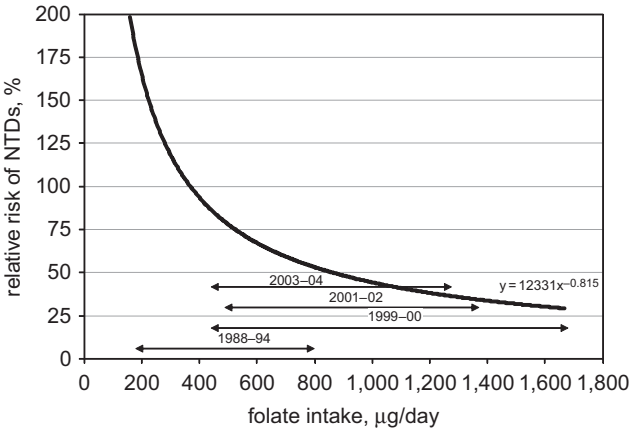

FIGURE 17.10 Relationships of estimated folate intake and risk of neural tube defects showing estimated ranges (10–90% of population) of folate intakes estimated in National Health and Nutrition Examination Surveys (NHANES III, 1988–94 and annual NHANES, 1999–2000, 2001–02, and 2003–04). *Based on combined population data from those surveys, Quinlivan, E.P., Gregory, J.F., 2007. Am. J. Clin. Nutr. 86, 1773–1779.*

be 20% greater for subjects reporting folate intakes ≥400 µg/day compared to those with lower intakes.[165] A placebo-controlled, randomized trial involving 1000 subjects with histories of colorectal adenomas found 2 years of supplementation with 1 mg folic acid to increase the risk of having a recurrent adenoma by 67% and to double the risk of having at least three adenomas.[166] That the institution of nationwide folate fortification in the United States and Canada corresponded with increasing colorectal cancer rates in those countries has been cited as evidence that increased folate intakes may be affecting cancer risk.[167] An analysis of colorectal cancer rates in the United States concluded that the increases observed in the 1990s were unlikely due to folate acid fortification,[168] and a meta-analyses of published data from randomized trials concluded that folic acid, at intakes greater than ~500 µg/day, does not increase cancer risk.[169] Still, those studies may not have included sufficient numbers of the subjects who might be at greatest risk to cancer promotion, i.e., those with prevalent malignancies.[170] This question is informed by a recent clinical study of postpolypectomy patients in which a high-level folate supplement was found to promote changes (increased folate content, reduced global DNA hypomethylation, and reduced DNA uracil misincorporation) in colonocytes adjacent to the polyp site (i.e., in the field that had produced

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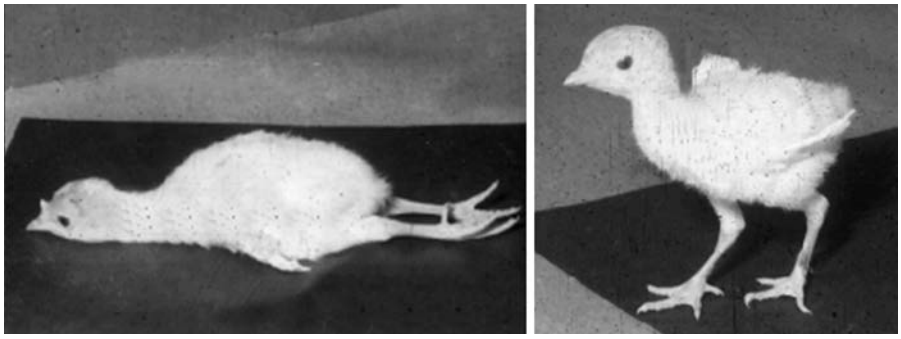

FIGURE 17.11 Cervical paralysis in a folate-deficient turkey poult (left); same poult 15 min after being treated (by injection) with folic acid (right). *Courtesy, G.F., Combs, Sr.*

an adenomatous poly) suggestive of reduced likelihood of mutagenesis and polyp formation.[171]

Deficiency Syndromes in Animals

Folate deficiency in animals is generally associated with poor growth, anemia, and dermatologic lesions involving skin and hair/feathers. In chicks, severe anemia is one of the earliest signs of the deficiency. The anemia is of the macrocytic (megaloblastic) type, involving abnormally large erythrocyte size (the normal range in humans is 82–92 μM^3) due to the presence of **megaloblasts**, which are also seen among the hyperplastic erythroid cells in the bone marrow. Anemia in folate deficiency is followed by **leukopenia** (abnormally low numbers of white blood cells), poor growth, very poor feathering, perosis, lethargy, and reduced feed intake.

Poultry with normally pigmented plumage[172] show achromotrichia due to the deficiency. Folate-deficient turkey poults show a spastic type of **cervical paralysis** in which the neck is held rigid (Fig. 17.11).[173] Folate-deficient guinea pigs show leukopenia and depressed growth; pigs and monkeys show alopecia, dermatitis, leukopenia, anemia, and diarrhea; mink show ulcerative hemorrhagic gastritis, diarrhea, anorexia, and leukopenia. The deficiency is not easily produced in rodents unless a **sulfa drug**[174] or folate antagonist is fed, in which case leukopenia is the main sign.[175] Folate-responsive signs (reduced weight gain, macrocytic anemia) can be produced in catfish by feeding them succinylsulfathiazole. Folate deficiency in the rat has been shown to reduce exocrine function of the pancreas, in which single-carbon metabolism is important.[176]

Folate deficiency is not expected in ruminants with functioning microflora, which produces the vitamin in amounts that are apparently adequate to meet the needs of the host. In fact, nearly all supplemental folate to a dairy diet acid appears to be degraded; high doses of the vitamin (e.g., 0.5 mg/kg of host body weight) are needed to increase serum and milk folate levels.

High-level folate supplementation of the diets of laying hens (e.g., 16 mg/kg of diet) has been an effective in producing eggs enriched in the vitamin for marketing purposes.

10. FOLATE IN HEALTH AND DISEASE

Pernicious anemia. High doses of folate (e.g., 400 µg/day intramuscular; 5 mg/day oral) have been shown to correct the **megaloblastic anemia** of pernicious anemia patients, which are deficient in vitamin B_{12}. This phenomenon renders megaloblastic anemia not useful for diagnosing either vitamin deficiency without accompanying metabolic measurements: FIGLU—elevations indicate folate deficiency, **MMA**—elevations indicate vitamin B_{12} deficiency.

Supplemental folate does not mask the irreversible progression of neurological dysfunction and cognitive decline of vitamin B_{12} deficiency; however, those signs develop over a longer period of time than the anemia produced by the same deficiency. In fact, folate supplementation has been shown to exacerbate the cognitive symptoms of vitamin B_{12} deficiency.[177] Because vitamin B_{12} deficiency affects an estimated 10–15% of the American population over 60 years of age, the amount of folate for the fortification of wheat flour (140 µg/100 g flour) was chosen to provide an amount of added folate (100 µg/person/day) sufficient for only a small proportion of the general population receiving a level (>1 mg/day) capable of masking vitamin B_{12} deficiency.

171. O'Reilly, S., McGlynn, A.P., McNulty, H., et al., 2016. J. Nutr. 146, 933–939.

172. Such breeds include the barred Plymouth Rock, the Rhode Island Red, and the Black Leghorn.

173. Poults with cervical paralysis may not show anemia; the condition is fatal within a couple of days of onset but responds dramatically to parenteral administration of the vitamin.

174. For example, sulfanilamide.

175. Although leukopenia was manifested relatively soon after experimental folate depletion, rats kept alive with small doses of folate eventually also develop macrocytic anemia.

176. Experimental pancreatitis can be produced in that species by treatment with ethionine, an inhibitor of cellular methylation reactions, or by feeding a diet deficient in choline.

177. Morris, M.S., Jacques, P.F., Rosenberg, I.H., 2007. Am. J. Clin. Nutr. 85, 193–200.

Cardiovascular Disease

The lowering of circulating Hcy levels effected by folate supplementation has not been found consistently to reduce risk to cardiovascular disease. A meta-analysis of eight trials involving 37,485 subjects randomized to folate and/or other B-vitamins were used as the intervention agents showed that a 25% reduction in circulating Hcy level for 5 years was not associated with any reductions in cardiovascular events (or death from any cause).[178] In one trial, cardiovascular disease patients who received a combined supplement of folate, vitamin B_6, and vitamin B_{12} showed increased risk for subsequent myocardial infarction.[179] Meta-analyses of trials that used folate as the single intervention agent yielded inconsistent results: one found the vitamin to improve flow-mediated dilatation, suggestive of enhanced vascular function;[180] the other found folate supplementation to be of no benefit in reducing stroke risk.[181] A placebo-controlled, randomized trial demonstrated that a combined supplement containing folate, vitamin B_{12}, and vitamin B_6 significantly reduced the progression of early stage subclinical atherosclerosis (carotid artery intima medial thickening) without homocysteinemia.[182]

Immune Function

That folate status may affect immune cell function is suggested by findings that folate deprivation of lymphocytes in vitro causes depletion of interleukin-2 and stimulates p53-independent apoptosis. Similar effects have not been observed in vivo. It has been noted that folate supplementation can stimulate natural killer (NK) cell cytotoxicity among subjects of low-folate status. Curiously, the opposite effect was observed among subjects consuming a high-folate diet, in whom NK cytotoxicity was inversely related to the plasma concentration of the nonmetabolized form of the vitamin, folic acid.[183] The MTHFR 677T allele has been associated with reduced risk to hepatitis B virus (HBV) infection in an HBV endemic area.[184]

Malaria

The malarial parasite, *Plasmodium falciparum*, requires folate for its own metabolism, including the DNA synthesis required for growth and proliferation.[185] The parasite is capable of synthesizing folate from *p*-aminobenzoic acid and L-glutamate; but it also uses exogenous supplies of folate, such as it finds within the host erythrocyte it invades, to continue its life cycle. Accordingly, antifolates[186] are first-line drugs in the treatment of malaria.[187] The host–parasite competition for folate contributes to the anemia observed in malarial patients, and malaria-induced hemolysis appears to increase the host's need for folate. While prenatal supplements of folate and iron have been found effective in reducing neonatal mortality in malaria-endemic regions, it is likely that those benefits may be limited to situations in which anemia is prevalent and the actual malaria prevalence is low. A robust study in a population with a high prevalence of malaria found supplementation with folate and iron to increase risk of severe illness and death in children who were not iron deficient.[188]

Arsenicosis

Studies in animals have shown folate status to be a determinant of the metabolism and tissue distribution of arsenic (As), which must be methylated to be excreted. Accordingly, urinary concentrations of dimethylarsenate of As-exposed subjects in Bangladesh were found to correlate positively with plasma folate level and negatively with plasma Hcy level; serum As levels were reduced by supplementation with folate.[189] An estimated 140 million people are exposed to As in drinking and irrigation waters in south Asia; many are also inadequately nourished and can be expected to be of low status with respect to folate, vitamin B_{12}, and MET.

Macular Degeneration

High-folate intake has been associated with reduction in risk to developing geographical atrophy, an advanced form of age-related macular degeneration.[190]

178. Clarke, R., Halsey, J., Bennett, D., 2011. J. Inherit. Metab. Dis. 34, 83–91.
179. In the Norwegian Vitamin Trial, 3749 men and women with histories of heart attack were randomized to four combinations of folate, and vitamin B_6 and B_{12} (Bønaa, K.H., Njølstadn I., Ueland, P.M., et al., 2006. N. Engl. J. Med. 354, 1578–1588).
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181. Lee, M., Hong, K.S., Chang, S.C., et al., 2010. Stroke 41, 1205–1212.
182. Hodis, H.N., Mack, W.J., Dustin, L., et al., 2009. Stroke 40, 730–736.
183. Troen, A.M., Mitchell, B., Sorenson, B., et al., 2006. J. Nutr. 136, 189–194.
184. Bronowicki, J.P., Abdelmouttaleb, I., Peyrin-Biroulet, L., 2008. J. Hepatol. 48, 532–539.
185. Globally, malaria causes an estimated 200 million morbid episodes and 2–3 million deaths each year. During pregnancy, the disease also contributes to low birth weight and intrauterine growth retardation.
186. e.g., Pyrimethamine, sulfadoxine.
187. Unfortunately, strains of *P. falciparum* have developed resistance through mutations in their dihydrofolate reductase.
188. Sazawal, S., Black, R.E., Ramsan, M., et al., 2006. Lancet 367, 133–14.
189. Gamble, M.V., Liu, X., Ashan, H., et al., 2005. Environ. Health Perspect. 113, 1683–1688.
190. Merle, B.M.J., Silver, R.E., Rosner, B., et al., 2016. Am. J. Clin. Nutr. 103, 1135–1144.

11. FOLATE TOXICITY

The toxicity of folic acid is negligible. No adverse effects of high oral doses of folate have been reported in humans or animals, although parenteral administration of pharmacologic amounts (e.g., 250 mg/kg, i.e., about 1000 times the dietary requirement) has been shown to produce epileptic responses and renal hypertrophy in rats. Inconsistent results have been reported concerning the effects of high-folate levels (1–10 mg doses) on human epileptics. Some have indicated increases in the frequency or severity of seizures and reduced anticonvulsant effectiveness;[191] whereas, others have shown no effects. Upper tolerable limits have been established for most age groups (Table 17.17).

Folate does have the potential for adverse effects, as it can exacerbate the consequences of vitamin B_{12} deficiency. By circumventing the methyl folate trap, high-folate intakes can provide folic acid directly for DNA synthesis, thus, correcting megaloblastic anemia caused by vitamin B_{12} deficiency. The loss of anemia as a sign of that deficiency can increase the likelihood of it progressing to the point of irreversible neurological damage. Folate supplementation in pregnant women with inadequate vitamin B_{12} intakes increased the risk of their having small-for-gestational-age infants.[192] High-folate intakes have also been found to increase circulating Hcy levels and impair the activities of MET synthase and methylmalonyl-CoA mutase.[193] Mice fed high levels of folic acid (10-fold recommended levels) in low-vitamin B_{12} diets showed reduced MTHFR expression and reduce fetal growth.[194]

TABLE 17.17 Recommended Upper Tolerable Intakes (ULs) of Folate

Ages, Years	UL, µg/day
1–3	300
4–8	400
9–13	600
14–18 females	1000
Males	800
>18 years	1000
Pregnancy	800
Lactation	1000

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12. CASE STUDY

Instructions

Review the following case report, paying special attention to the diagnostic indicators on which the treatments were based. Then, answer the questions that follow.

Case

A 15-year-old girl was admitted to the hospital because of progressive withdrawal, hallucinations, anorexia, and tremor. Her early growth and development were normal, and she had done average schoolwork until she was 11 years old, when her family moved to a new area. The next year, she experienced considerable difficulty in concentrating and was found to have an IQ of 60. She was placed in a special education program, where she began to fight with other children and have temper tantrums; when punished, she became withdrawn and stopped eating. A year earlier, she had experienced an episode of severe abdominal pain for which no cause could be found, and she was referred to a mental health clinic. Her psychologic examination at that time had revealed inappropriate giggling, poor reality testing, and loss of contact with her surroundings. Her verbal and performance IQs were then 46 and 50, respectively. She was treated with thioridazine[195] and, within 2 weeks, she ate and slept better and was helpful around the house. However, over the succeeding months, while she continued taking thioridazine, her functioning fluctuated and the diagnosis of catatonic schizophrenia was confirmed. Three months before the present admission, she had become progressively withdrawn and drowsy, and needed to be fed, bathed, and dressed. She also experienced visual hallucinations, feelings of persecution, and night terrors. On having a seizure, she was taken to the hospital. Her physical examination on admission revealed a tall, thin girl with fixed stare and catatonic posturing but no neurologic abnormalities. She was mute and withdrawn, incontinent, and appeared to have visual and auditory hallucinations. Her muscle tone varied from normal to diffusely rigid.

On the assumption that her homocystinuria was due to cystathionase deficiency, she was treated with pyridoxine HCl (300 mg/day, orally) for 10 days. Her homocystinuria did not respond; however, her mental status improved and, within 4 days, she was able to conduct some conversation and her hallucinations seemed to decrease. She developed new neurological signs: foot and wrist droop and gradual

191. High doses of folate appear to interfere with diphenylhydantoin absorption.
192. Dwarkanath, P., Barzilay, J.R., Thomas, T., et al., 2013. Am. J. Clin. Nutr. 98, 1450–1458.
193. Selhub, J., Morris, M.S., Jacques, P.F., et al., 2009. Am. J. Clin. Nutr. 89, 702S–706S.
194. Mikael, L.G., Deng, L., Paul, L., et al., 2013. Birth Defects Res. A 97, 47–52; Christensen, K.E., Mikael, L.G., Leung, K.Y., et al., 2015. Am. J. Clin. Nutr. 101, 646–658.
195. An antischizophrenic drug.

loss of reflexes. She was then given folate (20 mg/day orally) for 14 days because of her low-serum folate level. This resulted in a marked decrease in her urinary homocysteine and a progressive improvement in intellectual function over the next 3 months. She remained severely handicapped by her peripheral neuropathy, but she showed no psychotic symptoms. After 5 months of folate and pyridoxine treatment, she was tranquil and retarded but showed no psychotic behavior; she left the hospital against medical advice and without medication.

Laboratory Findings

Parameter	Patient	Normal Range
Electroencephalogram	Diffusely slow	
Spinal Fluid		
Protein (mg/dL)	42	15–45
Cells	None	None
Urine		
Homocysteine	Elevated	
Methionine	Normal	
Serum		
Homocysteine	Elevated	
Methionine	Normal	
Folate (ng/mL)	3	5–21
Vitamin B_{12} (pg/mL)	800	150–900
Hematology		
Hemoglobin (g/dL)	12.1	11.5–14.5
Hematocrit (%)	39.5	37–45
Reticulocytes (%)	1	~1
Bone marrow	No megaloblastosis	

The girl was readmitted to the hospital 7 months later (a year after her first admission) with a 2-month history of progressive withdrawal, hallucinations, delusions, and refusal to eat. The general examination was the same as her first admission, with the exceptions that she had developed hyperreflexia and her peripheral neuropathy had improved slightly. Her mental functioning was at the 2-year-old level. She was incontinent, virtually mute, and had visual and auditory hallucinations. She was diagnosed as having simple schizophrenia of the childhood type. Folate and pyridoxine therapy was started again; it resulted in her decreased Hcy excretion and gradual improvement in mental performance. After 2 months of therapy in the hospital, she was socializing, free of hallucinations, and able to feed herself and recognize her family. At that time, the activities of several enzymes involved in methionine metabolism were measured in her fibroblasts and liver tissue (obtained by biopsy).

Enzyme Activities

Enzyme	Specimen	Enzyme Activity[a]	
		Patient	Normal
Methionine adenyltransferase	Liver	20.6	4.3–14.5
Cystathionine-β-synthase	Fibroblasts	25.9	3.7–65.0
Betaine:Hcy methyltransferase	Liver	26.7	1.2–16.0
5-Methyl FH_4:Hcy methyltransferase	Fibroblasts	3.5	2.9–7.3
5,10-Methylene-FH_4 reductase	Fibroblasts	0.5	1.0–4.6

[a]*Enzyme units.*

Thereafter, she was maintained on oral folate (10 mg/day). She has been free of homocystinuria and psychotic manifestations for several years.

Case Questions

1. On admission of this patient to the hospital, which of her symptoms were consistent with an impairment in a folate-dependent aspect of metabolism?
2. What finding appeared to counterindicate an impairment in folate metabolism in this case?
3. Propose a hypothesis for the metabolic basis of the observed efficacy of oral folate treatment in this case.

13. STUDY QUESTIONS AND EXERCISES

1. Diagram the metabolic conversions involving folates in single-carbon metabolism.
2. Construct a decision tree for the diagnosis of folate deficiency in humans or an animal species. In particular, outline a way to distinguish folate and vitamin B_{12} deficiencies in patients with macrocytic anemia.
3. What key feature of the chemistry of folate relates to its biochemical function as a carrier of single-carbon units?
4. What parameters might you measure to assess folate status of a human or animal?
5. Detail the impact (positive and negative) of food fortification programs increasing the folate intake of populations.

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Chapter 18

Vitamin B_{12}

Chapter Outline

Anchoring Concepts

1. *Vitamin B_{12}* is the generic descriptor for all corrinoids (compounds containing the cobalt-centered corrin nucleus) exhibiting the biological activity of cyanocobalamin.
2. Deficiencies of vitamin B_{12} are manifested as anemia and neurologic changes, and can be fatal.
3. The function of vitamin B_{12} in single-carbon metabolism is interrelated with that of folate.

Patients with Addisonian pernicious anemia have…a "conditioned" defect of nutrition. The nutritional defect in such patients is apparently caused by a failure of a reaction that occurs in the normal individual between a substance in the food (extrinsic factor) and a substance in the normal gastric secretion (intrinsic factor).

W. B. Castle and T. H. Ham[1]

LEARNING OBJECTIVES

1. To know the chief natural sources of vitamin B_{12}
2. To understand the means of enteric absorption and transport of vitamin B_{12}
3. To understand the biochemical functions of vitamin B_{12} as a coenzyme in the metabolism of propionate and the biosynthesis of methionine
4. To understand the metabolic interrelationship between vitamin B_{12} and folate
5. To understand the factors that can cause low vitamin B_{12} status, and the physiological implications of that condition

VOCABULARY

Achlorhydria
Adenosylcobalamin
Aquocobalamin
Cobalamins
Cobalt
Corrinoid ring
Cubulin
Cyanocobalamin
Deoxyadenosylcobalamin
Gastric parietal cell
Haptocorrin
Helicobacter pylori
Holotranscobalamin (holoTC)
Homocysteinemia
Homocystinuria
Hydroxycobalamin
Hypochlorhydria
Intrinsic factor (IF)
IF receptor
IF–vitamin B_{12} complex
Imerslund–Gräsbeck syndrome
Lipotrope
Macrocyte
Megaloblastosis
Methionine synthase
Methionine synthase reductase

1. William B. Castle (1897–1990) was a physician and physiologist at Harvard University. He is known for transforming hematology into a dynamic interdisciplinary field with his early discovery of the gastric intrinsic factor, which ultimately led to the identification of vitamin B_{12}. Among his collaborators was a young physician, Thomas Hale Ham (1905–1987), who went on to join the faculty of Western Reserve University where he revolutionized the medical education curriculum with a model that was adopted nationwide.

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00018-6

Methylcobalamin
Methylmalonic aciduria
Methylmalonyl CoA mutase
Methylfolate trap
Methyl-FH_4 methyltransferase
Methylmalonic acid (MMA)
Methylmalonic acidemia
Methylmalonic aciduria
Methylmalonyl CoA mutase
Nitritocobalamin
Ovolactovegetarian
Pepsin
Peripheral neuropathy
Pernicious anemia
Perosis
Pseudovitamin B_{12}
R proteins
Schilling test
Transcobalamin (TC)
Transcobalamin receptor
Vegan
Vitamin B_{12} coenzyme synthetase.

1. SIGNIFICANCE OF VITAMIN B_{12}

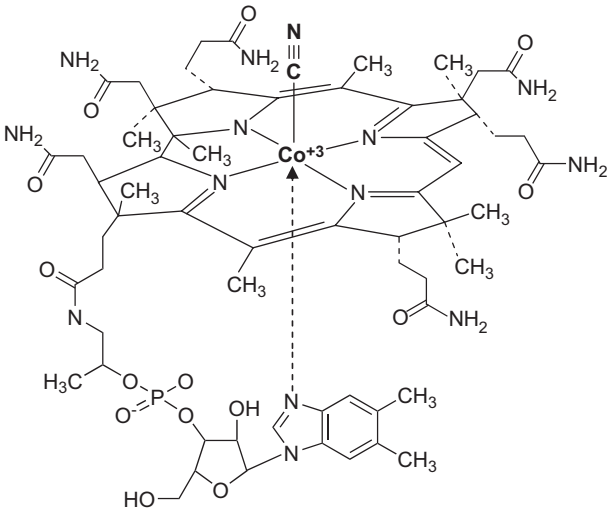
Vitamin B_{12} is synthesized by prokaryotic organisms. Animals require the vitamin for critical functions in cellular division and growth. Some animal tissues can store the vitamin in appreciable amounts that are sufficient to meet the needs of the organism for long periods (years) of deprivation. The vitamin is seldom found in foods derived from plants; therefore, noncoprophagous animals and humans that consume strict vegetarian diets are likely to have inadequate intakes of vitamin B_{12}. If prolonged, those will lead to anemia and peripheral neuropathy. Few humans are strict **vegans** (who exclude all foods of animal origin); most consume foods and/or supplements containing vitamin B_{12}. For this reason, frank vitamin B_{12} deficiency is not common. Nevertheless, low vitamin B_{12} status occurs, particularly in individuals with hereditary deficiencies in proteins involved in vitamin B_{12} transport and/or metabolism, or with compromised gastric parietal cell function. Low vitamin B_{12} status limits DNA synthesis, impairs the metabolic utilization of folate, and contributes to homocysteinemia, a risk factor for occlusive vascular disease.

2. PROPERTIES OF VITAMIN B_{12}

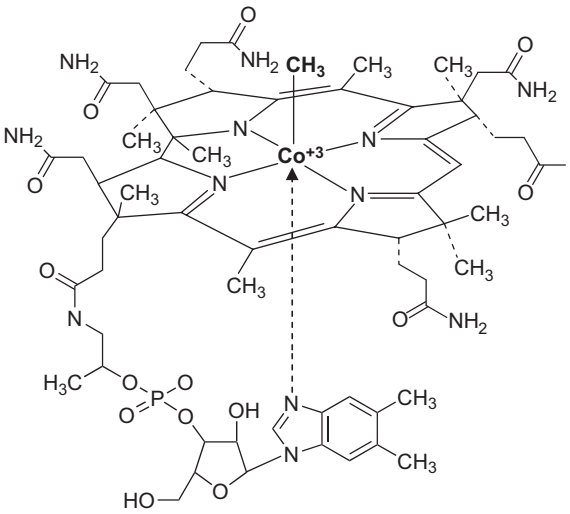
Vitamin B_{12} Nomenclature

The term **vitamin B_{12}** is the generic descriptor for all corrinoids (compounds containing the **corrin nucleus**) exhibiting the biological activity of **cyanocobalamin** (also **cobalamin**). The vitamers B_{12} are octahedral cobalt (Co) complexes consisting of a porphyrin-like, cobalt-centered macroring (the corrin nucleus), a nucleotide, and a second Co-bound group (e.g., CH_3, H_2O, CN^-). The corrin nucleus consists of four reduced pyrrole nuclei linked by three methylene bridges and one direct bond. The triply ionized cobalt atom (Co^{3+}) is essential for biological activity; it can form up to six coordinate bonds and is tightly bound to the four pyrrole nitrogen atoms. The central cobalt atom can also bind a small ligand above (α-position) and a nucleotide below (β-position) the plane of the ring system. For example, its α-position ligands include cyano (CN^-) (**cyanocobalamin**), methyl (**methylcobalamin**), 5′-deoxyadenosyl (**adenosylcobalamin**), or hydroxo (OH) (**hydroxocobalamin**[2]) groups. Those, and the unliganded form with a reduced cobalt center (**cob[I]alamin**), are found intracellularly. Other synthetic analogs with vitamin B_{12} activity include forms with aqua- (H_2O) (**aquacobalamin**[3]) or nitrite (**nitritocobalamin**[4]) ligands.

Chemical structures of vitamin B_{12}:

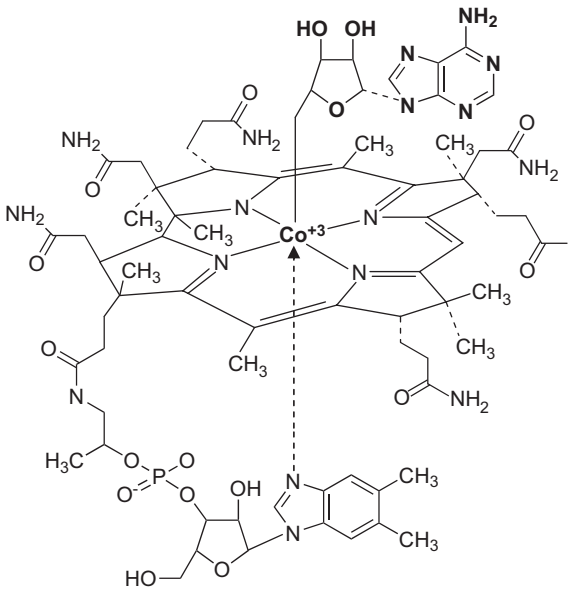


Cyanocobalamin

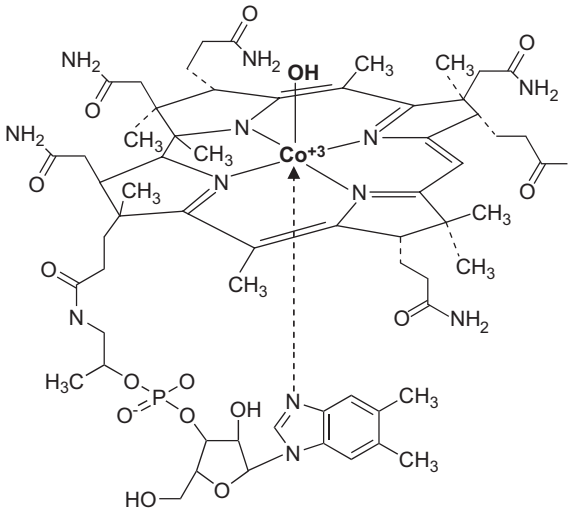


Methylcobalamin

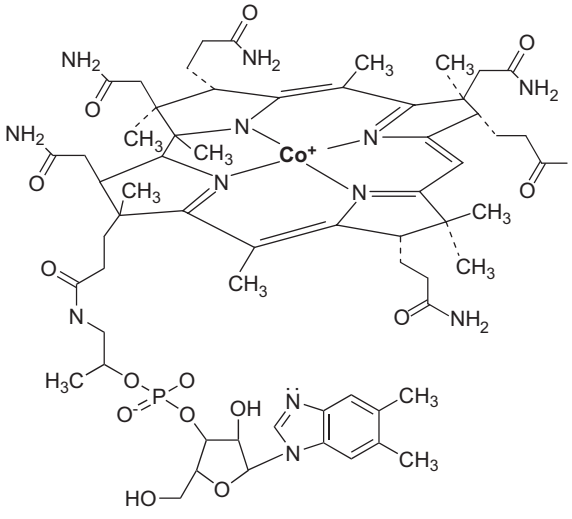
2. formerly, vitamin B_{12b}.
3. formerly vitamin B_{12a}.
4. formerly vitamin B_{12c}.



5′-Deoxyadenosylcobalamin



Hydroxocobalamin



Cob(I)alamin

Vitamin B_{12} Chemistry

The corrinoids are red, red–orange, or yellow crystalline substances that show intense absorption spectra above 300 nm owing to the π–π transitions of the corrin nucleus. They are soluble in water and are fairly stable to heat but decompose at temperatures above <210°C without melting. Vitamin B_{12} reacts with ascorbic acid, resulting in the reduction and subsequent degradation of the former, which releases its cobalt atom as the free ion. Cobalamins with relatively strongly bound ligands (e.g., cyano-, methyl-, and adenosylcobalamin) are less reactive and are therefore more stable in the presence of ascorbic acid. The cobalamins are unstable to light. Cyanocobalamin undergoes a photoreplacement of the CN^- ligand with water; the organocobalamins (methyl- and adenosylcobalamin) undergo photoreduction of the cobalt-carbon bond, resulting in the loss of the ligand and the reduction of the corrin cobalt. The vitamin can bind to the vitamin B_{12} enzymes through an imidazole nitrogen of a histidyl residue on the protein, which serves as the ligand to the lower axial position of the cobalt atom instead of the dimethylbenzimidazole grouping.

3. SOURCES OF VITAMIN B_{12}

Synthesis by the Gut Microbiome

Vitamin B_{12} is synthesized by some anaerobic microorganisms, particularly propionic acid bacteria.[5] That synthesis is dependent on an adequate supply of Co. A genomic analysis of 256 representative organisms of the human gut microbiota found 43% capable of de novo synthesis of the vitamin, with a total synthetic capacity equivalent to at least 31% of the daily human need.[6] However, it is not clear that vitamin B_{12} can be absorbed across the human colon, as only 19% of the corrinoids in human stool were found to be absorbable.[7] With sufficient Co from the diet,[8] the rumen microbial synthesis of vitamin B_{12} is substantial.[9] Not only do ruminant species have little, if any, need for preformed vitamin B_{12} in the diet but also their tissues tend to contain

5. *Pseudomonas denitrificans* is widely used for the commercial preparation of vitamin B_{12}. The vitamin is also produced in large amounts by *Propionibacterium freudenreichii* and *Propionibacterium shermanii*. Some lactic acid bacteria have been found to synthesize corrinoids; but they do not appear to release it and, thus, do not appear to contribute to hindgut microbial synthesis of the vitamin.

6. Magnúsdóttir, S., Ravchee, D., de Crécy-Lagard, V., et al., 2015. Front. Genet. 6, 148–166.

7. Albert, R.H., Stabler, S.P., 2008. Am. J. Clin. Nutr. 87, 1324–1335.

8. Beef cattle require 125–160 μg/kg dietary dry matter (typically added in a mineral premix or salt lick) to minimize their circulating levels of homocysteine and methylmalonic acid, but higher levels (235–255 μg/kg DM) to maximize vitamin B_{12} concentrations in plasma and liver (Stangl, G.I., Schwarz, F.J., Müller, H., et al., 2000. Br. J. Nutr. 84, 645–653).

9. In fact, ruminal infusion with a chelated form of Co has been shown to affect the saturation of cows' milk lipids, reducing the level of fatty acid desaturation. (Leskinen, H., Viitala, S., Mutikaninen, M., et al., 2016. J. Nutr. 146, 976–985.

substantial amounts of the vitamin, making them important potential dietary sources of vitamin B_{12} for meat-eating, nonruminant species.

Distribution in Foods

Because the synthesis of vitamin B_{12} is limited almost exclusively to anaerobic bacteria, the vitamin is found only in foods that have been bacterially fermented or derived from the tissues of animals that have obtained it from their ruminal or intestinal microflora, or ingested it either with their diet or coprophagously. Animal tissues that accumulate vitamin B_{12} are, therefore, excellent food sources of the vitamin (Table 18.1). The richest sources are liver[10] and kidney; other rich sources are dairy products, meats, eggs, fish, and shellfish. The amounts of the vitamin in the tissues of livestock species depend on the feeding management and cut of meat. The principal vitamers in foods are **methylcobalamin**, **deoxyadenosylcobalamin,** and **hydroxycobabalmin**. The richest sources of vitamin B_{12} for animal feedstuffs are animal by-products such as meat and bone meal, fish meal, and whey.

Only a few plant foods contain appreciable amounts of vitamin B_{12}. However, substantial amounts of the vitamin are found in some types of edible algae,[11] particularly green laver (*Enteromorpha* sp.), and purple laver (*Porphyra* sp., i.e., nori), which can contain 12–64 µg/100 g. The microalgae *Chlorella* sp. can vary in vitamin B_{12} content (0–200 + µg/100 g). The fruiting bodies of some mushrooms (*Craterellus cornucopioides*, *Cantharellus cibarius*, *Lentinula edodes*)[12] can contain >5 µg/100 g.[13] While soybeans contain little, if any, vitamin B_{12}, bacterially fermented soy products (e.g., tempeh, natto) can contain significant amounts (~0.75 µg/100 g). Tea leaves can contain vitamin B_{12} (0.1–1.2 µg/100 g). Trace amounts of the vitamin (e.g., 0.14 µg/100 g) have been found in spinach, broccoli, asparagus, and mung bean sprouts, apparently a result of uptake from vitamin B_{12}-containing organic fertilizers. Vitamin B_{12} has been found in cyanobacteria (*Spirulina*, *Aphanizomenon*, *Nostoc*) and a mushroom (*Hericium erinaceus*[14]); but these can also contain **pseudovitamin B_{12}** (7-adeninyl cyanocobamide),[15] which is biologically inactive and may antagonize the utilization of vitamin B_{12}.[16] Compounds with vitamin B_{12}-like activity have been found in bamboo cabbage, spinach, celery, lily bulb, bamboo shoots, and taro.

TABLE 18.1 Food Sources of Vitamin B_{12}

Foods	Vitamin B_{12} (µg/100 g)
Meats	
Beef	1.38–3.17
Beef brain	10.10
Beef kidney	24.9
Beef liver	83.13
Chicken	0.27–0.32
Chicken giblets	9.48
Ham	0.65–1.06
Pork	0.43–1.11
Turkey	0.36–1.65
Dairy Products	
Milk	0.38–0.5
Cheeses	0.29–2.28
Yogurt	0.75
Fish, Sea Food	
Herring	13.14
Salmon	3.26–4.48
Trout	6.3
Tuna	2.55
Clam	40.27
Oysters	16–19.13
Lobster	1.43
Shrimp	1.21–1.87
Vegetables, grains, fruits	None
Other	
Eggs, whole	0.89
Egg whites	0.09
Egg yolk	1.95
Tempeh	0.08

USDA National Nutrient Database for Standard Reference, Release 28 (http://www.ars.usda.gov/ba/bhnrc/ndl).

Breast Milk

The vitamin B_{12} concentration of human milk varies widely (330–320 pg/mL) and is particularly great in colostrum, which contains 10 times as much as mature milk. Most

10. Vitamin B_{12} was discovered as the antipernicious anemia factor in liver.
11. Studies have indicated that vitamin B_{12} is an essential metabolite for half of all algal species.
12. i.e., Black trumpet, golden chanterelle, and shiitake, respectively. Note: significant amounts of vitamin B_{12} were not found in porcini, parasol, oyster, or black morel mushrooms.
13. Watanabe, F., Yabuta, Y., Bito, T., et al., 2014, Nutrients 6, 1861–1873.
14. Lion's mane.
15. Pseudovitamin B_{12} differs from the vitamin by having an adenine moiety replacing the dimethylbenzimidazole.
16. Herbert, V., 1988. Am. J. Clin. Nutr. 48, 852–858.

of the vitamin (mainly methylcobalamin) is bound to R proteins.[17] Initial levels, 260–300 pM, decline to by half after the first 12 weeks of lactation. Breast milk vitamin B_{12} levels reflect the level of intake of the vitamin. Milk from strict vegetarian women contains reduced levels as compared to milk from women consuming mixed diets; levels tend to be inversely correlated with the length of time on the vegetarian diet. Oral supplementation with vitamin B_{12} can significantly increase the vitamin B_{12} contents of breast milk and, hence, the vitamin B_{12} intake of nursing infants.[18]

Stability

Vitamin B_{12} is very stable in crystalline form and aqueous solution. High levels of ascorbic acid have been shown to catalyze the oxidation of vitamin B_{12} in the presence of iron to forms that are poorly utilized.

Bioavailability

Vitamin B_{12} is bound to two proteins (enzymes and carriers) in foods. Therefore, its utilization depends on the nature of the food/meal matrix as well as the host's ability to release the vitamin and bind it to proteins that facilitate its enteric absorption. In practice, the bioavailability of vitamin B_{12} in foods is difficult to determine. Bioassays in animal models fed vitamin B_{12}-deficient diets leave questions about applicability to humans, and studies in nondeficient humans require the use of the vitamin labeled with an intrinsic tracer. Further, the microbiological assay commonly used to measure vitamin B_{12} in foods (i.e., *Lactobacillus delbrueckii* growth) appears to yield overestimates by ~30%, due to responses to nonvitamin corrinoids. With those caveats, the bioavailability of vitamin B_{12} from most foods appears to be moderate. Studies have found that about half of the vitamin in most foods is absorbed by individuals with normal gastrointestinal function (Table 18.2). Bioavailability declines at intakes (1.5–2 μg/day) that saturate the active transport of the vitamin across the gut; greater amounts depend on absorption by passive diffusion, a process with only 1% efficiency. Accordingly, about 1% of the vitamin is absorbed from vitamin B_{12} supplements.

TABLE 18.2 Bioavailability of Vitamin B_{12} in Common Foods

Food	Bioavailable (%)
Eggs	4–9
Fish meat	42
Chicken meat	61–66
Lamb meat	56–89
Milk	55–65

Watanabe, F., 2007. Exp. Biol. Med. 232, 1266.

4. ABSORPTION OF VITAMIN B_{12}

Digestion

The naturally occurring vitamin B_{12} in foods is bound in coenzyme form to proteins. The vitamin is released from such complexes on heating, gastric acidification and/or proteolysis (especially by the action of pepsin). Thus, impaired gastric parietal cell function, as in **achlorhydria** or with chronic use of proton pump inhibitors, impairs vitamin B_{12} utilization.

Protein Binding

Free vitamin B_{12} is bound to proteins secreted by the gastric mucosa:

- **R proteins**[19] are glycoproteins that bind vitamin B_{12} to these glycoproteins adventitiously. They are found in plasma, saliva, gastric juice, intestinal contents, tears, cerebrospinal fluid, amniotic fluid, breast milk, leukocytes and erythrocytes in humans, and probably only a few other species. R proteins are members of a family of proteins called **haptocorrins**. While the salivary R protein is the first to bind vitamin B_{12} released from food, it is normally digested by pancreatic proteases in the small intestine to release the vitamin. Patients with pancreatic exocrine insufficiency can have high concentrations of R proteins that render the vitamin poorly absorbed.
- **Intrinsic factor (IF)**[20] is a glycoprotein secreted by gastric parietal cells in the fundus and body of the stomach[21] in response to histamine, gastrin, pentagastrin, and the presence of food. IF is a relatively small protein with a molecular weight of about 50 kDa.[22] It is glycosylated (by fucose addition) posttranslationally. It binds the four cobalamins (methyl-, adenosyl-, cyano-, and aquocobalamin) with comparable, high affinities; but it does not bind cobamamides or cobinamides, which remain bound to R proteins and are not absorbed. The binding

17. This contrasts with cow's milk, which, containing no R proteins, typically shows lower concentrations of the vitamin, which is present mainly as adenosylcobalamin.
18. Duggan C., Srinivasan, K., Thomas, T., et al., 2014. J. Nutr. 144, 758–764.
19. These vitamin B_{12}-binding glycoproteins were named for their high electrophoretic mobilities: ***r**apid*.
20. IF was identified in the gastric mucosa that was necessary for the utilization of an "extrinsic factor" later identified as vitamin B_{12}.
21. i.e., The same cells that produce gastric acid.
22. e.g., Human 44–63 kDa, pig 50–59 kDa, according to the carbohydrate moiety isolated with the preparation.

of vitamin B_{12} by IF produces a complex with a smaller molecular radius than that of IF alone; this protects the vitamin from hydrolytic attack by pepsin and chymotrypsin, as well as from side chain modification of the corrin ring by intestinal bacteria.

Mechanisms of Absorption

Carrier-mediated active transport of vitamin B_{12} is efficient (>50%) and quantitatively important at low doses (1–2 μg). Such doses appear in the blood within 3–4 h of consumption. The active transport of vitamin B_{12} depends on the IF–vitamin B_{12} complex binding to a specific brush border receptor in the terminal portion of the ileum, a site it reaches after traveling the length of the small intestine. That receptor[23] consists of two components: the multiligand apical membrane protein cubilin,[24] which binds the IF–vitamin B_{12} complex; and a chaperone, amnionless (AMN), which contributes structure necessary for membrane anchorage in clathrin-coated pits,[25] trafficking to the plasma membrane, and signaling of endocytosis and receptor recycling.[26] After moving into the cell, the IF–vitamin B_{12} complex is thought to be degraded within lysosomes in which free vitamin B_{12} is released.

Deficiency of IF causes pernicious anemia. Patients have a severely limited ability to absorb vitamin B_{12}, excreting 80–100% of oral doses in the feces (vs. 30–60% fecal excretion rates in individuals with adequate IF). Individuals with loss of gastric parietal cell function may be unable to utilize dietary vitamin B_{12}, as these cells produce both IF and acid, both of which are required for the enteric absorption of the vitamin.[27] For this reason, geriatric patients, many of whom are hypoacidic, may be at risk of low vitamin B_{12} status. Mutations in the IF gene can result in failure of its expression or in expression of a defective protein incapable of binding vitamin B_{12}. Affected individuals show macrocytic anemia[28] within the first 3 years of life, which responds to large doses of vitamin B_{12} administered orally or by intramuscular injection. IF secretion can be affected by mutations in the gene encoding fucosyltransferase (FUT2) that catalyzes its posttranslational fucosylation.[29]

Passive diffusion of vitamin B_{12} occurs with low efficiency (~1%) throughout the small intestine and becomes significant only at higher doses. Such doses appear in the blood within minutes of consumption. This passive mechanism is utilized in therapy for pernicious anemia, in which patients are given high doses (>500 μg/day) of vitamin B_{12} per os. For such therapy, the vitamin must be given an hour before or after a meal to avoid competitive binding of the vitamin in food.

Enterohepatic Circulation of Vitamin B_{12}

A significant amount of vitamin B_{12} is released in the bile. In humans, this can be 0.5–5 μg each day,[30] depending on vitamin B_{12} status. Much of this is reabsorbed by the above mechanisms. This capacity to recycle the vitamin reduces dietary need.

5. TRANSPORT OF VITAMIN B_{12}

Transport Proteins

On absorption from the intestine, vitamin B_{12} is initially transported in the plasma as adenosylcobalamin and methylcobalamin bound to two proteins:

- **Plasma haptocorrin**,[31] a 60-kDa R protein, binds most (70–80%) of the vitamin B_{12} in plasma. Plasma haptocorrin is typically 80–90% saturated with its ligand, which turns over slowly (half-life, 9–10 days), becoming available for cellular uptake only over fairly long time frames. A minor variant of this protein, differing only in carbohydrate content, can also be found in plasma. Haptocorrin binds methylcobalamin preferentially, which, therefore, is the predominant circulating form of the vitamin. As most other species lack R proteins, their dominant circulating form is adenosylcobalamin.

Congenital defects in plasma haptocorrin are asymptomatic, suggesting that this form of the vitamin is not physiologically important. Affected individuals show normal absorption and distribution of vitamin B_{12} to their tissues;

23. Genetic defects in these proteins occur in Imerslund–Gräsbeck's syndrome, characterized by vitamin B_{12} malabsorption leading to megaloblastic anemia.
24. Cubilin is a large (460 kDa) membrane protein with no apparent transmembrane segment. It is expressed at high levels in the kidney where it appears to function in the reabsorption of several specific nutrient carriers including albumin, vitamin D-binding protein, transferrin, and apolipoprotein A.
25. Produced by lattices of three clathrin heavy chains and three clathrin light chains, these membrane vesicles facilitate transport of the IF–B_{12} complex across the plasma membrane into the epithelial cell.
26. Fyfe, J.C., Madsen, M., Højrup, P., et al., 2004. Blood 103, 1573–1579.
27. Individuals lacking IF are unable to absorb vitamin B_{12} by active transport. They can be given the vitamin by intramuscular injection (1 μg/day) or in high oral doses (25–2000 μg) to prevent deficiency. Randomized trials have shown that an oral dose regimen of 1000 μg daily for a week, followed by the same dose weekly and, then, monthly can be as effective as intramuscular administration of the vitamin for controlling short-term hematological and neurological responses in deficient patients Butler, C.C., Vidal-Alabell, J., Cannings-John, R., et al., 2006. Fam. Pract. 23, 279–285.
28. Anemia characterized by relatively low cell count with the presence of enlarged erythrocytes produced due to impaired cell division during hematopoiesis.
29. Chery, C., Hehn, A., Mrabet, N., et al., 2013. Biochimie 95, 995–1001.
30. El Kholty, S., Gueant, J.L., Bressler, L., et al., 1991. Gastroenterol. 101, 1399–1408.
31. Formerly, transcobalamin I.

however, they show low circulating levels of the vitamin and can be wrongly diagnosed as vitamin B_{12} deficient if other parameters [MMA, Hcy, FIGLU (formiminoglutatmic acid)] are not considered. The prevalence of plasma haptocorrin defects may be relatively high; one study noted that 15% of apparently healthy subjects had low plasma vitamin B_{12} levels.

Transcobalamin (TC)[32] binds most of the nonhaptocorrin-bound vitamin B_{12} in plasma, i.e., 10–20% of the total. TC is a 38–43 kDa β-globulin protein synthesized in the liver, intestinal mucosa, seminal vesicles, fibroblasts, bone marrow, and macrophages. It is filtered by the kidney and reabsorbed by the proximal tubules. It binds the vitamin stoichiometrically; within 3–4 h of ingestion of the vitamin, TC reaches is typically level of 10–20% saturation with the ligand. Movement of vitamin B_{12} from the intestinal mucosal cell into the plasma depends on the formation of the TC–vitamin B_{12} complex, i.e., **holotranscobalamin (holoTC)**, which turns over rapidly in the enterocyte (half-life c. 6 min). In the plasma, holoTC also turns over fairly rapidly (half-life, 60–90 min), rendering it the primary functional source of vitamin B_{12} for cellular uptake. Within hours, much of the vitamin originally associated with TC becomes bound to plasma haptocorrin[33] and, in humans, to other plasma R proteins.[34] Therefore, holoTC level can be a useful parameter of early-stage vitamin B_{12} deficiency.

Predominant transport forms of vitamin B_{12} differ among species, varying widely in concentration from only hundreds (humans) to thousands (rabbits) of pM. The major circulating vitamer in human plasma is methylcobalamin (60–80% of the total),[35] owing to the fact that haptocorrin and R proteins preferentially bind that vitamer (Table 18.3). However, the major circulating vitamer in other species is adenosylcobalamin, which is bound by TC with comparable affinity to methylcobalamin.

TABLE 18.3 Cobalamins in Normal Human Plasma

	Range (pM)
Total cobalamins	173–545
Methylcobalamin	135–427
Adenosylcobalamin	2–77
Cyanocobalamin	2–48
Aquocobalamin	5–67

Holotranscobalamin Receptor

Membrane-bound receptor proteins for holoTC occur in all cells. The **TC receptor**[36] is a 50-kDa glycoprotein in the low-density lipoprotein receptor family. It has a single holoTC binding site. It is thought that TC receptors mediate the endocytic uptake of holoTC (Fig. 18.1). A soluble form has been identified in human serum.

Intracellular Protein Binding

Upon cellular internalization, holoTC is degraded proteolytically in lysosomes and vitamin B_{12} is released for conversion to methylcobalamin in the cytosol. Virtually, all of the vitamin in the cell is ultimately bound to two vitamin B_{12}-dependent enzymes:

- **methionine synthetase** (also called **methyl-FH_4 methyltransferase**) in the cytosol
- **methylmalonyl CoA mutase** in mitochondria.

Congenital Disorders of Vitamin B_{12} Absorption and Transport

Congenital deficiencies in proteins involved in the absorption and transport of vitamin B_{12} have been described (Table 18.4). These result in tissue-level vitamin B_{12} deficiencies the effects of which are manifest within weeks to years after birth. Most can be managed with high, frequent doses of the vitamin administered intramuscularly or orally. IF gene mutations can result in either IF not being expressed, or in the expression of an IF protein that is functionally inactive or unstable. Affected individuals develop megaloblastic anemia within 1–3 years, i.e., when their maternal stores of vitamin B_{12} are exhausted. Dysfunction of the ileal IF receptor caused by defects in either cubulin or AMT occur in Imerslund–Gräsbeck syndrome,[37,38] a common cause of vitamin B_{12}-associated megaloblastic anemia. A single-nucleotide polymorphism in TC (776C→G) has also been identified. The G allele is most prevalent in Asians (56%) compared to whites (45%) and blacks (36%).[39] Individuals with GG genotype develop severe megaloblastic anemia within the first 5 years of life. They have low circulating levels of both apo- and holoTC, but because most circulating vitamin B_{12} is bound to haptocorrin, their plasma levels of the vitamin are typically normal such that this deficiency can easily

32. Formerly, transcobalamin II.
33. Only by this means does haptocorrin obtain vitamin B_{12}.
34. Due to their affinity for R proteins, the TCs are grouped in a heterogeneous class of proteins called *R binders*.
35. In pernicious anemia patients, methylcobalamin is lost in favor to others forms of the vitamins.
36. Also called CD320.
37. Also called autosomal recessive megaloblastic anemia.
38. Mutant cubilin has been found in Finnish patients; whereas mutant AMN has been found in Norwegian patients.
39. Bowen, R.A., Wong, B.Y., Cole, D.E., 2004. Clin. Biochem. 37, 128–133.

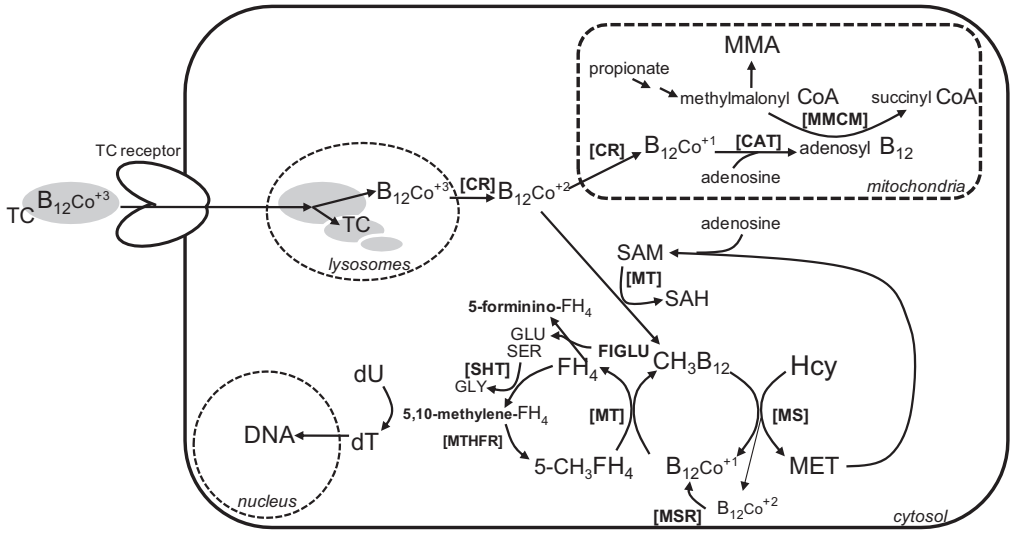


FIGURE 18.1 Uptake and metabolism of vitamin B_{12}, and its relationship with folate in single-carbon metabolism. *TC*, transcobalamin; *MMA*, methylmalonic acid; *SAM*, *S*-adenosylmethionine; *SAH*, *S*-adenosylhomocysteine; *Hcy*, homocysteine; *MET*, methionine; *FH4*, tetrahydrofolic acid; *CH3B12*, methylcobalamin; *5-CH3-FH4*, methyltetrahydrofolic acid; *FIGLU*, formiminoglutamic acid; *GLU*, glutamic acid; *SER*, serine; *dU*, deoxyuridylate; *dT*, deoxythimidylate; *CR*, cobalamin reductases; *CAT*, cobalamin adenosyl transferase; *MMCM*, methylmalonyl CoA mutase; *MT*, methyltransferases; *MS*, methionine synthase; *MSR*, methionine synthase reductase; *SHT*, serine hydroxymethyltransferase; *MTHFR*, methylenetetrahydrofolate reductase.

TABLE 18.4 Congenital Disorders of Vitamin B_{12} Absorption and Transport

Condition	Missing/Deficient Factor	Signs/Symptoms
Lack of intrinsic factor	IF	Megaloblastic anemia presenting at 1–3 years
Imerslund–Gräsbeck syndrome	IF receptor	Specific malabsorption of vitamin B_{12} presenting by 5 years
Lack of transcobalamin	TC	Severe (fatal) megaloblastic anemia presenting early in life
Lack of haptocorrin	Haptocorrin	None

be missed.[40] Patients respond to vitamin B_{12} administered in large doses by intramuscular injection, e.g., 1 mg three times per week. Congenital deficiencies in haptocorrin occur in some 15% of individuals. It is characterized by low circulating levels of vitamin B_{12}; however, that condition is without consequence as levels of the physiological transporter, TC-B_{12}, are unaffected. Still, it can lead to an erroneous impression of vitamin B_{12} deficiency.

Distribution in Tissues

Vitamin B_{12} is the best stored of the vitamins. Under conditions of adequate intake, the vitamin accumulates to appreciable amounts in the body, mainly in the liver (~60% of the total body stores) and muscles (~30% of the total). Body stores vary with the intake of the vitamin but tend to be greater in older subjects. Hepatic concentrations approaching 2 µg/g have been reported in humans; however, a total hepatic reserve of about 1.5 mg is typical.[41] Mean total body stores of vitamin B_{12} in humans are in the range of 2–5 mg. The greatest concentrations of vitamin B_{12} occur in the pituitary gland; kidneys, heart, spleen, and brain also contain substantial amounts; in humans, these organs each contain 20–30 µg of vitamin B_{12}. The great storage and long biological half-life (350–400 days in humans) of the vitamin provide substantial protection against periods of deprivation. The low reserve of the human infant (~25 µg) is sufficient to meet physiological needs for about a year.

40. The 776C>G polymorphism has also been linked to risks of spontaneous abortion and fetal developmental defects (Martinelli, M., Scapoli, L., Palmieri, A., et al., 2006. Hum. Mutat. 27, 294–301), as well as to the onset of Alzheimer's disease McCaddon, A., Blennow, K., Hudson, P., et al., 2004. Dement. Geriatr. Cogn. Disord. 17, 215–221.

41. i.e., c. 1 µg/g.

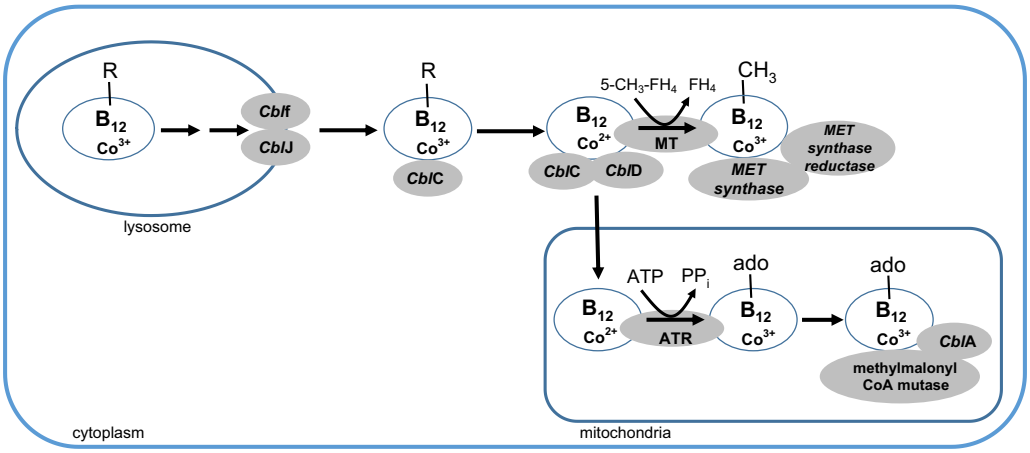


FIGURE 18.2 Intracellular trafficking of vitamin B_{12} by protein chaperones. Exit of the vitamin from the lysosome requires two membrane proteins, *Cbl*F and *Cbl*J. Upon entry into the cytoplasm, the β-axial liganded vitamin is thought to be bound to *Cbl*C [also called cobalamin reductase and MMACHC (for methylmalonic acid type C and homocystinuria)], which forms a complex with *Cbl*D [also called MMADHC (for methylmalonic acid type D and homocystinuria)]. *Cbl*D does not bind the cobalamin but is thought to assist its delivery by *Cbl*C to 5-methyl-FH_4:homocysteine methyltransferase (MT), which produces methylcobalamin that is bound by methionine synthase, which complexes independently with methionine synthase reductase. The mechanism is not clear whereby cobalamin enters the mitochondrion where it is adenosylated by the ATP-dependent cob(I)alamin adenosyltansferase (ATR, also CblB) and then transferred to methylmalonyl CoA mutase. Escape of 5′-adenosine from the mutase active site during catalysis is prevented by a G-protein chaperone *Cbl*A (also called MMAA, for methymalonic aciduria type A) using the binding energy of GTP. *After Gerashim, C., Hannibal, L., Rajabopalan, D., et al., 2013. Biochimie 95, 1023–1032; Gerashim, C., Lofgren, M., Banerjee, R., 2013. J. Biol. Chem. 288, 13186–13193.*

6. METABOLISM OF VITAMIN B_{12}

Intracellular Trafficking

Vitamin B_{12} is delivered to cells in the oxidized from, hydroxycob(III)alamin where it is reduced by thiol- and reduced flavin-dependent reduction of the cobalt center of the vitamin (to Co^{+}) to form cob(I)amin.[42] However, the vitamin is active in metabolism *only* as methyl or 5-deoxyadenosyl derivatives that have either respective group attached covalently to the cobalt atom. Therefore, vitamin B_{12} released from holoTC in lysosomes must enter the cytoplasm to be incorporated as methylcobalamin into methionine synthase, and traverse the cytoplasm to be incorporated as adenosylcobalamin into methylmalonyl CoA mutase. Several protein chaperones are essential to this trafficking (Fig. 18.2).[43]

Activation to Coenzyme Forms

The conversion to these coenzyme forms involves two different enzymatic steps:

- **Methylcobalamin—**Methylation of the vitamin is catalyzed by the cytosolic enzyme **5-methyl-FH_4:homocysteine methyltransferase**. This renders the vitamin, as methylcobalamin, a carrier for the single-C unit used in the regeneration of **methionine (MET)** from **homocysteine** (**Hcy**). Methylcobalamin is also produced by recharging the reduced vitamin (Co^{+1}) with a methyl group transferred from 5-methyl FH_4. This cycling risks the occasional oxidation of cobalamin–cobalt (to Co^{+2}), in which case it is reduced back to Co^{+1} by the enzyme **methionine synthase reductase**.
- **Adenosylcobalamin**—Adenosylation of the vitamin occurs in the mitochondrial due to the action of **vitamin B_{12} coenzyme synthetase**, which catalyzes the reaction of cob(II)amin with a deoxyadenosyl moiety derived from ATP. This step depends on the entry of hydroxycobalamin into the mitochondria and its subsequent reduction in sequential, one electron steps involving NADH- and NADPH-linked aquacobalamin reductases[44] to yield cob(II)alamin.

Catabolism

Little, if any, metabolism of the corrinoid ring system is apparent in animals, and vitamin B_{12} is excreted as the intact cobalamin. Apparently, only the free cobalamins (not the methylated or adenosylated forms) in the plasma are available for excretion.

Excretion

Vitamin B_{12} is excreted via both renal and biliary routes at the daily rate of about 0.1–0.2% of total body reserves (in humans this is 2–5 μg/day, thus constituting the daily

42. Also called vitamin B_{12s}.
43. Gerashim, C., Hannibal, L., Rajagopalan, D., et al., 2013. Biochimie 95, 1023–1032.
44. These activities are derived from a cytochrome b_5/cytochrome b_5 reductase complex, and from a cytochrome *P*-450 reductase complex and an associated flavoprotein.

TABLE 18.5 Categories of Congenital Disorders of Vitamin B_{12} Metabolism

Defect	Missing/Deficient Factor (Gene)	Signs/Symptoms
Mitochondrial		
B_{12}-Co^{+3} reduction to B_{12}-Co^{+2} Adenosyl-B_{12} production Production of ado-/methyl B_{12}	Mitochondrial cobalamin reductase Adenosyl transferase Cobalamin reductase	Methylmalonic aciduria Methylmalonic aciduria Homocysteinuria, Methylmalonic aciduria
B_{12} entry into mitochondria Isomerization of methylmalonyl CoA	B_{12} chaperone Methylmalonyl CoA mutase	Homocysteinuria, methylmalonic aciduria methylmalanic aciduria
Cytosolic		
Methionine synthase	Methyl transferase activity	Homocysteinemia, hypomethioninemia, megaloblastic anemia, developmental delay
B_{12}-Co^{+3} reduction to B_{12}-Co^{+2}	Methionine synthase reductase	Homocysteinemia, hypomethioninemia, megaloblastic anemia, developmental delay
Lysosomal		
Lysosome to cytosol B_{12} export	Lysosomal membrane protein	Developmental delay, homocysteinuria, methylmalonic aciduria

requirement for the vitamin).[45] Although it is found in the urine, glomerular filtration of the vitamin is minimal (<0.25 μg/day in humans), and it is thought that urinary cobalamin is derived from the tubular epithelial cells and lymph. Urinary excretion of the vitamin after a small oral dose can be used to assess vitamin B_{12} status; this is called the **Schilling test**. The biliary excretion of the vitamin is substantial, accounting in humans for the secretion into the intestine of 0.5–5 μg g/day. Most (65–75%) of this amount is reabsorbed in the ileum by IF-mediated active transport. This enterohepatic circulation constitutes a highly efficient means of conservation, with biliary vitamin B_{12} contributing only a small amount to the feces.

Congenital Disorders of Vitamin B_{12} Metabolism

Several congenital deficiencies in proteins involved in vitamin B_{12} metabolism, each an autosomal recessive trait, have been reported in humans (Table 18.5). Most present in early childhood as homocysteinemia and/or methylmalonic aciduria, frequently with neurological and psychiatric symptoms. Most can be managed with high, frequent doses of the vitamin administered intramuscularly or orally. Hereditary defects in the vitamin's intracellular protein chaperones can produce homocystinuria (*Cbl*C, *Cbl*D, *Cbl*E, *Cbl*G), methylmalonic aciduria (*Cbl*A, *Cbl*B, *Cbl*C) or combined homocystinuria, and methylmalonic aciduria (*Cbl*F, *Cbl*J, *Cbl*C) (see Fig. 18.2). Of these the most common defect is in cobalamin reductase activity (*Cbl*C); more than 40 mutations have been identified in that gene.[46]

7. METABOLIC FUNCTIONS OF VITAMIN B_{12}

Coenzyme Functions

Vitamin B_{12} functions in metabolism in two coenzyme forms: adenosylcobalamin and methylcobalamin. While several vitamin B_{12}-dependent metabolic reactions have been identified in microorganisms,[47] only these two have been discovered in animals. These play key roles in the metabolism of propionate, amino acids, and single carbon.

- **Adenosylcobalamin** is the coenzyme of **methylmalonyl CoA mutase**, which catalyzes the conversion of methylmalonyl CoA to succinyl CoA in the degradation of propionate formed from odd-chain fatty acids, which are particularly important as sources of energy source for ruminants. This reaction involves splitting a carbon–carbon bond of the coenzyme with the formation of a free radical on the coenzyme that can be transferred through an amino acid residue to the substrate. That the

45. Doets, E.L., in't Veld, P.H., Szczerińsk, A., et al., 2012. Ann. Nutr. Metab. 62, 311–322.

46. More than 400 patients have been described. Early onset (<1 year) patients present with severe neurological, hematological, renal, gastrointestinal, cardiac, and pulmonary symptoms. Late-onset patients present with slowly progressive neurological symptoms.

47. The following microbial enzymes require adenosylcobalamin: glutamate mutase, 2-methylene-glutarate mutase, L-β-lysine mutase, D-α-lysine mutase, D-α-ornithine mutase, leucine mutase, 1,2-dioldehydratase, glyceroldehydratase, ethanolamine deaminase, and ribonucleotide reductase; methylcobalamin is also required for the bacterial formation of methane and acetate.

propionic acid pathway is important in nerve tissue is suggested by the delayed onset of the neurological signs of vitamin B_{12} deficiency effected in animals by dietary supplements of direct (valine, isoleucine) or indirect (methionine) precursors of propionate.[48]

Methylmalonyl CoA mutase is a mitochondrial matrix enzyme, which forms a dimer that binds two adenosylcobalamin molecules. In humans, it is the first vitamin B_{12}-dependent enzyme to be affected by deprivation of vitamin B_{12}. With loss of this activity, vitamin B_{12}-deficient subjects show **methylmalonic aciduria**, especially after being fed odd-chain fatty acids. The accumulation of **MMA** can disrupt normal glucose and glutamic acid metabolism, apparently by inhibiting the tricarboxylic acid (TCA) cycle. Vitamin B_{12} deficiency can cause a reversal of propionyl CoA carboxylase activity, leading to the incorporation of the 3-C propionyl CoA in place of the 2-C acetyl CoA, and resulting in the production of small amounts of odd-chain fatty acids. Increased levels of methylmalonyl CoA can also lead to its incorporation in place of malonyl CoA, resulting in the synthesis of small amounts of methyl-branched chain fatty acids. It has been suggested that the neurological signs of vitamin B_{12} deficiency may result, at least in part, from the production of these abnormal fatty acids in neural tissues.

Several inborn metabolic errors result in decreased in methylmalonyl CoA mutase activities leading to methylmalonic aciduria. These include mutations in the gene that encodes the enzyme can block its expression or result in expression of a defective protein, and other mutations that reduce the synthesis of its cofactor adenosylcobalamin. Individuals with these defects respond to vitamin B_{12} treatment.

- **Methylcobalamin** is the coenzyme for **methionine synthase**, which catalyzes the methylation of Hcy to regenerate methionine (MET). In this reaction, methylcobalamin serves as the methyl group carrier between the donor 5-methyltetrahydrofolate (5′-methyl-FH_4) and the acceptor Hcy. This reaction is a simple transfer of the single-C moiety. Because of diminished methionine synthase activity, vitamin B_{12}-deficient subjects show reduced availability of MET, which is essential for the synthesis of proteins and polyamines, and is the precursor of **S-adenosylmethionine (SAM)**. SAM is the primary donor of "labile" methyl groups for more than 100 enzymatic reactions with critical roles in metabolism.[49]

SAM also serves as a key regulator of the transsulfuration and remethylation pathways, which involve the folate-dependent methylenetetrahydrofolate reductase. Losses of SAM lead to impairments in the synthesis of creatine, phospholipids, and the neurotransmitter acetylcholine, all of which have broad impacts on physiological function. Low vitamin B_{12} status, thus, results in the accumulation of both Hcy and 5′-methyl-FH_4 (via the methyl folate trap), the latter resulting in the loss of FH_4, the key functional form of folate. Methionine synthase can also catalyze the reduction of nitrous oxide to elemental nitrogen; in doing so, it generates a free radical that inactivates the enzyme. Methionine synthase expression is induced by vitamin B_{12} by the vitamin binding to a *trans*activating protein, inducing a conformational change that allows it a bind to an internal site on the methionine synthase mRNA, thus enhancing ribosomal recruitment and promoting translation.[50]

Genetic defects in methionine synthase and the production of its cofactor methylcobalamin result in homocysteinemia and, commonly, megaloblastic anemia. Individuals with these defects do not respond to vitamin B_{12} treatment, but their anemia can respond to folate supplementation. A 2756A>G polymorphism in methionine synthase has been described; women with the AG genotype have been found to experience double the risk of having a child with NTDs and a 3.5-fold increase in the risk of having a child with Down syndrome.[51] A 66A>G polymorphism of methionine synthase reductase, also involved in this functioning pathway, has been associated with similar effects.

Interrelationships With Folate

The major cycle of single-C flux in mammalian tissues is the serine hydroxymethyltransferase/5,10-methylene-FH_4 reductase/methionine synthase cycle. In this cycle, the committed step (5,10-methylene-FH_4 reductase) is feedback inhibited by SAM and product inhibited by 5-methyl-FH_4; but methionine synthase is rate limiting (Fig. 18.1). It depends on the transfer of labile methyl groups from 5-methyl-FH_4 to vitamin B_{12}. Methyl-B_{12} serves as the immediate methyl donor for converting Hcy to MET. Without adequate vitamin B_{12} to accept methyl groups from 5-methyl-FH_4, that metabolite accumulates at the expense of the other metabolically active folate pools. This is known as the "**methyl folate trap**" a blockade resulting in the accumulation of the intermediate FIGLU.[52] These interrelated

48. In bacteria, levels of adenosylcobalamin are controlled by regulating the genes responsible for its synthesis and import; this is effected by an adenosylcobalamin riboswitch, i.e., a regulatory segment of mRNA Johnson, J.E., Reyes, F.E., Polaski, J.T., et al., 2012. Nature 492, 133–137.
49. By loss of the flux of methyl groups via 5-methyl-FH_4:Hcy methyltransferase, folate deficiency causes a secondary hepatic choline deficiency.
50. Oltean, S. and Banerjee, R., 2005. J. Biol. Chem. 280, 32,662–32,668.
51. Doolin, M.T., Barbaux, S., McDonnel, M., et al., 2002. Am. J. Hum. Genet. 71, 1222–1226; Bosco, P., Guéant-Rodriguez, R.M., Anello, G., et al., 2003. Am. J. Med. Genet. 121A, 219–224.
52. Thus, elevated urinary FIGLU level after an oral histidine load is diagnostic of vitamin B_{12} deficiency.

metabolic pathways are affected by vitamin B_{12} deprivation in two ways:

- **Reduced MET regeneration** by the loss of methionine synthase activity, which results in a secondary folate deficiency due to the accumulation of 5-methyl-FH_4 by the "methyl folate trap" (the basis by which deficiency of either folate or vitamin B_{12} can cause macrocytic anemia) and the accumulation of Hcy manifest as homocysteinemia.
- **Reduced DNA methylation** by the reduced availability of single-C units. Hypomethylation of DNA cytosine bases and histone proteins alters chromatin structure in ways that affect transcription and can increase C→T transition mutation.[53] Thus, suboptimal status with respect to vitamin B_{12} (or folate) can affect gene expression and stability. Accordingly, chromosomal aberrations have been reported for some patients with pernicious anemia.[54] A cross-sectional study showed that the vitamin B_{12} levels of buccal cells were significantly lower in smokers and nonsmokers and that elevated levels of the vitamin were associated with reduced frequency of micronucleus formation.[55]

Physiological Functions

By participating in the regeneration of MET, vitamin B_{12} functions in the regulation of Hcy and, thus, the prevention of homocysteinemia, which can cause various adverse metabolic effects (discussed more extensively in Chapter 17). A prospective, community-based study found plasma Hcy to be weakly associated with plasma vitamin B_{12} concentration.[56] Circulating Hcy levels >13 μM have been associated with dysfunction that has been related specifically to vitamin B_{12} status:

- **Hematological development**. Vitamin B_{12} supports in hematopoietic cell division in the bone marrow by providing single-C units via the methionine synthase for the synthesis of thymidylate, which is required for normal DNA synthesis.
- **Neurological function**. Vitamin B_{12} has essential neurological functions including the synthesis of functional myelin sheaths and the synthesis of choline, the precursor of the neurotransmitter acetylcholine. These functions support both peripheral and cerebral–spinal aspects of neurological function, including cognition, sensation, and muscular coordination.
- **Fetal development**. It is likely that vitamin B_{12} has a role in supporting normal early fetal development. Studies have shown lower vitamin B_{12} levels in amniotic fluid from NTD pregnancies compared to healthy ones, even though the vitamin B_{12} contents of mothers' serum in both cases were in the normal range.[57] This suggests a limitation in the maternal capacity to provide the fetus with an adequate supply of the vitamin. Because women with NTD pregnancies are more likely to have the methionine synthase 66AG genotype, which presumably produces an aberrant enzyme, it is possible that compromised vitamin B_{12} function may be involved in the residual incidence of NTDs not prevented by folate supplementation.
- **Bone health**. An analysis of the NHANES 1999–2004 data found homocysteinemia to be associated with a twofold increase is risk of lumbar spine osteoporosis.[58] This was found to be more prevalent among elderly Dutch women of marginal or deficient vitamin B_{12} status than those adequate with respect to the vitamin (Table 18.6). Studies have shown positive associations of serum vitamin B_{12} level and bone mineral density, markers of bone turnover, and risks of osteoporosis and hip fracture.[59] Most randomized trials using supplements of vitamin B_{12} and other B vitamins (folate, vitamin B_6) have found no effects on biomarkers of bone turnover,[60] although one found that prevention of homocysteinemia with a combined supplement of vitamin B_{12} and folate significantly reduced hip fracture risk.[61]

TABLE 18.6 Relationship of Vitamin B_{12} Status and Osteoporosis Risk Among Elderly Women

Plasma Vitamin B_{12} (pM)	n	Relative Risk
>320	34	1.0
210–320	43	4.8 (1.0–23.9)[a]
<210	35	9.5 (1.9–46.1)

[a]*95% confidence interval.*
Dhonukshe-Rutten, R.A.M., Lips, M., de Jong, N., et al., 2003. J. Nutr. 133, 801–807.

53. Methylated CpG sites appear to be at particularly high risk for C→T changes, the most common type of mutational change which are common in the p53 tumor suppressor gene.
54. Jensen, M.K., 1977. Mutat. Res. 45, 249–252.
55. Piyathilke, C.J., Macaluso, M., Hine, R.J., et al., 1995. Cancer Epid. Biomakers Prev. 4, 751–758.
56. Selhub, J., Jacques, P.F., Bostom, A.G., et al., 1996. J. Nutr. 126, 1258–1265S.
57. Ray, J.G. and Blom, H.J., 2003. Quart. J. Med. 96, 289–295.
58. Bailey, R.L., Looker, A.C., Lu, Z., et al., 2015. Am. J. Clin. Nutr. 102, 687–694.
59. Dhonukshe-Rutten, R.A., van Dusseldorp, M., Schneede, J., et al., 2005. Eur. J. Nutr. 44, 341–347; Tucker, K.L., Hannan, M.T., Qiao, N., et al., 2005. J. Bone Min. Res. 20, 152–158; Dhonukshe-Rutten, R.A., Pluijim, S.M., de Groot, L.C., et al., 2005. J. Bone Min. Res. 20, 921–927.
60. e.g., Green, T.J., McMahon, J.S., Skeaff, C.M., et al., 2007. Am. J. Clin. Nutr. 85, 460–464; van Wijgaarden, J.P., Swart, K.M.A., Enneman, A.W., et al., 2014. Am. J. Clin. Nutr. 100, 1578–1586.
61. Sato, Y., Honda, Y., Iwamoto, J., 2005. JAMA 293, 1082.

- **Hearing**. Low vitamin B_{12} status has been found in patients with tinnitus.[62,63] Such a relationship might indicate effects of homocysteinemia on the vascular or bone systems of the ear.

8. BIOMARKERS OF VITAMIN B_{12} STATUS

Vitamin B_{12} status can be assessed by analyses of blood:

- **Serum vitamin B_{12} concentration** is the most widely used tool to assess vitamin B_{12} status.[64] Normal values are in the 150–665 pM range; values <194 pM indicate deficiency as defined by significant risk of elevated serum MMA.[65] This biomarker is limited by the fact that it measures two different pools of the vitamin that turn over at different rates. Most (70–80%) serum vitamin B_{12} is bound to haptocorrin with a half-life of up to 10 days; whereas, the physiologically significant pool, i.e., vitamin B_{12} as holoTC, is smaller (10–20% of the serum total) with a much faster turnover (60–90 min). Therefore, short-term deprivation of vitamin B_{12} can reduce holoTC without affecting the haptocorrin-bound pool and, thus, not having a detectable effect on total vitamin B_{12} concentration. Therefore, the effects of short-term deprivation of the vitamin can easily be missed. Serum vitamin B_{12} level can be depressed in subjects expressing variants of the fucosyltransferase that influences the gastric secretion of IF,[66] and taking the antidiabetic drug metformin.[67] Markedly elevated serum vitamin B_{12} levels can occur in subjects with antibodies to holoTC given the vitamin intramuscularly and in patients with overproduction of haptocorrin due to myeloproliferative disease. Elevated serum vitamin B_{12} is among the diagnostic criteria for polycythemia vera[68] and hypereosinophilic syndrome.[69]
- **Serum holoTC concentration**[70] is a sensitive indicator of vitamin B_{12} recent absorption and status. It has been found to decline within a week after damage to the posterior ileum even though total serum concentrations of the vitamin were unaffected. Values <30 pM are considered indicative of deficiency; such levels have been found to be prevalent in many elderly subjects as well as in AIDS patients. HoloTC level does not, however, reflect declining vitamin B_{12} stores in subjects with adequate recent intakes of the vitamin.
- **Plasma/serum or urinary MMA**. Circulating and urinary MMA levels increase in vitamin B_{12} deficiency due to the diminished activity of MMA mutase. This can be tested after a meal of odd-chain fatty acids or a propionate load. Interpretation of MMA results necessitates evaluating renal function and diabetes status. Renal insufficiency can lead to elevated plasma MMA, which also occurs in patients with type 2 diabetes despite their normal serum vitamin B_{12} levels.[71]

Other biomarkers have been used to assess various aspects of vitamin B_{12} status or function but are not specific for vitamin B_{12}:

- **Plasma/serum Hcy concentration**. This is not a specific indicator of vitamin B_{12} status. While vitamin B_{12} deficiency can elevate plasma Hcy that outcome can also be produced by deficiencies of folate and/or MET, renal insufficiency, and some drugs. These factors must be considered in interpreting plasma Hcy level. Homocysteinemia (plasma Hcy >13 μM) is most likely to indicate suboptimal vitamin B_{12} status in populations exposed to folate-fortified foods.
- **Urinary FIGLU**. Deficiency of vitamin B_{12} increases urinary FIGLU excretion; but this is not a specific indicator of vitamin B_{12} status, as it is also caused by deprivation of folate.
- **Plasma/serum MET**. Serum MET levels are highly correlated with those of vitamin B_{12} in vitamin B_{12}-deficient subjects. About half of subjects with either vitamin B_{12} or folate deficiencies, and more than half of those with the combined deficiencies, show subnormal plasma MET concentrations.[72]

Distinguishing Deficiencies of Vitamin B_{12} and Folate

Macrocytic anemia and other clinical signs can result from deficiencies of either vitamin B_{12} or folate. This is based on their common participation in the regulation of the FH_4 pool. Deprivation of either vitamin will reduce that folate directly; vitamin B_{12} indirectly, via the methyl folate trap. In either case, the yield of 5,10-methylene-FH_4 is reduced.

62. i.e., The perception of sound within the ear in the absence of corresponding external sound.
63. Houston, D.K., Johnson, M.A., Nozza, R.J., et al., 1999. Am. J. Clin. Nutr. 69, 564–571; Shemesh, Z., Attias, J., Ornan, M., et al., 1993. Am. J. Otalaryngol. 2, 94–99.
64. Formerly determined by a microbiological growth assay (e.g., *Lactobacillus leichmannii*) or radioisotope dilution technique, this is now accomplished using enzyme-linking immunoassays.
65. Bailey, R.L., Durazo-Arvizu, R.A., Carmel, R., et al., 2013. Am. J. Clin. Nutr. 98, 460–467.
66. Chery, C., Hehn, A., Mrabet, N., et al., 2013. Biochimie 95, 995–1001.
67. Greib, E., Trolle, B., Bor, M.V., et al., 2013. Nutrients 5, 2475–2482.
68. A bone marrow disorder characterized by overproduction of erythrocytes.
69. A disease characterized by persistent, elevated eosinophil count (>1500/mm^3).
70. The ligand saturation of TC is not useful as a biomarker of vitamin B_{12} status, as apoTC (normally in 5– to 10-fold excess over holoTC) responds to inflammation and infection as an acute phase protein while holoTC does not.
71. Obeid, R., Jung, J., Falk, J., et al., 2013. Biochimie 95, 1056–1061.
72. Humans typically show plasma methionine concentrations in the range of 37–136 μM.

This limits the production of thymidylate and, thus, of DNA, resulting in impaired mitosis and being manifest as macrocytosis and anemia. Similarly, deficiencies of either folate or vitamin B_{12} increase urinary FIGLU excretion, as a diminished FH_4 pool reduces the capacity to degrade that metabolite by transfer of its formimino group to produce 5′-formimino-FH_4. The only way to distinguish deficiency of vitamin B_{12} from that of folate is on the basis of the urinary excretion of MMA. Lexus intake of folate can mask the anemia or FIGLU excretion associated with vitamin B_{12} deficiency by maintaining FH_4 in spite of the methyl folate trap. However, supplemental vitamin B_{12} does not affect the anemia or other signs of folate deficiency. However, **methylmalonic aciduria** occurs *only* in vitamin B_{12} deficiency, as the adenosylcobalamin-dependent methylmalonyl CoA mutase is not affected by folate status. Therefore, patients with macrocytic anemia, increased urinary FIGLU, and low blood folate levels can be diagnosed as vitamin B_{12} deficient if their urinary MMA levels are elevated, but as folate deficient if their urinary MMA is normal.

Distinguishing Vitamin B_{12} and Folate Deficiencies

Biomarker	Vitamin B_{12}-Deficient	Folate-Deficient
Urinary FIGLU	Elevated	Elevated
Urinary MMA	Elevated	Normal
Serum Hcy	Elevated	Elevated
Serum folate	Reduced	Reduced
Serum vitamin B_{12}	Normal-reduced	Normal

9. VITAMIN B_{12} DEFICIENCY

A study of elderly Americans found >40% to show elevations in urinary MMA levels; half also showed low serum vitamin B_{12} levels.[73] This levels have been observed in 10–15% of apparently healthy, elderly Americans with adequate vitamin B_{12} intakes, and in 60–70% of those with low vitamin B_{12} intakes.[74] The prevalence of low plasma vitamin B_{12} concentrations in all Central American age groups was found to be 35–90%.[75]

Vitamin B_{12} deficiency can have primary (privational) and secondary (nonprivational) causes. The major primary cause is the consumption of strict vegetarian diets.

Vegetarian Diets

Strict vegetarian diets, i.e., those containing no meats, fish, animal products, or vitamin B_{12} supplements, contain practically no vitamin B_{12} (Tables 18.7 and 18.8). Individuals consuming such vegan diets typically show very low circulating levels of the vitamin and elevated levels of Hcy.[76] Studies have found that >50% of vegetarians in India and the United States had low serum concentrations of vitamin B_{12}, i.e., <150 pM. Yet, clinical signs among such individuals appear to be rare. Indeed, they may not be manifest for several years after starting a strict vegetarian dietary regimen. Serum vitamin B_{12} concentrations tend to vary inversely with the length of time of vegetarian practice, showing progressive declines for about 7 years—the time estimated to draw down hepatic stores of the vitamin (Fig. 18.3). Signs of vitamin B_{12} deficiency are more common among breast-fed infants of vegetarian mothers (Table 18.9). The vitamin B_{12} content of breast milk, like that of maternal serum, varies inversely with the length of maternal vegetarian practice.

TABLE 18.7 Vitamin B_{12} and Folate Status of Thai Vegetarians and Mixed Diet Eaters

Group	Vitamin B_{12} (pg/mL)	Folate (ng/mL)
Mixed Diet		
Men	490	5.7
Women	500	6.8
Vegetarian		
Men	117[a]	12.0[a]
Women	153[a]	12.6[a]

[a]$p > .05$.
Tungtrongchitr, V., Pongpaew, P., Prayurahong, B., et al., 1993. Int. J. Vit. Nutr. Res. 63, 201–207.

It should be remembered that not all vegetarians are strict vegans. Many **ovolactovegetarians** consume plant-based diets that also contain servings of dairy products, eggs, or fish to varying extents. Studies have shown that the occasional consumption of animal products (e.g., once per month) will support serum vitamin B_{12} levels comparable to those of people eating mixed diets (Table 18.10). In addition, vegetarians may include in their diets some seaweeds (*Nori* sp. and *Chlorella* sp.) and bacterially fermented foods that contain vitamin B_{12}. Some may be exposed to bacterial sources of the vitamin in contaminated foods or water. Many intentionally consume nutritional yeasts or nutritional supplements as sources of vitamin B_{12}.

The major secondary causes of vitamin B_{12} deficiency involve impaired absorption, metabolism, or metabolic function of the vitamin.

73. Norman, E.J., Morrison, J.A., 1993. Am. Med. J. 94, 589–594.
74. Carmel, R., Green, R., Jacobsen, D.W., et al., 1999, Am. J. Clin. Nutr. 70, 904–910; Carmel, R., 2000. Ann. Rev. Med. 51, 357–375.
75. Allen, L.H., 2004. Nutr. Rev. 62, S29–S33.
76. Obersby, D., Chappell, D.C., Dunnett, A., et al., 2013. Br. J. Nutr. 109, 785–794.

TABLE 18.8 Plasma Indicators of Vitamin B_{12} Status in Vegetarians and Nonvegetarians

Plasma Analyte	Omnivorous Subjects	Lacto−/−Ovo-Vegetarians			Vegans Vitamin Nonusers
		Vitamin Users	Vitamin Nonusers	Vitamin Users	
Vitamin B_{12} (pM)	287 (190–471)[a]	303 (146–771)	179 (124–330)	192 (125–299)	126 (92–267)
Transcobalamin (pM)	54 (16–122)	26 (30235)	23 (4–84)	14 (3–53)	4 (2–35)
Methylmalonic acid (nM)	161 (95–357)	230 (120–1344)	368 (141–2000)	708 (163–2651)	779 (222–3480)
Hcy (μM)	8.8 (5.5–16.1)	9.6 (5.5–19.4)	10.9 (6.4–27.7)	11.1 (5.3–25.9)	14.3 (6.5–52.1)
Folate (nM)	21.8 (14.5–51.5)	30 (14.8–119)	27.7 (16.0–76.9)	29.5 (18.8–71.8)	34.3 (20.7–72.7)

[a]*Mean, 95% confidence interval.*
Herrmann, W., Schorr, H., Obeid, R., et al., 2003. Am. J. Clin. Nutr. 78, 131–136.

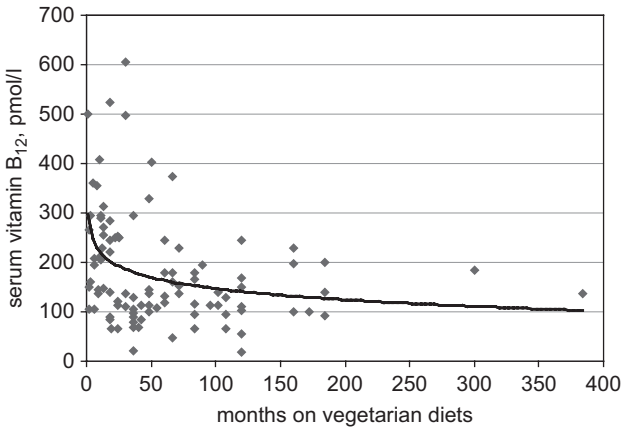


FIGURE 18.3 Inverse relationship of serum vitamin B_{12} concentrations and time following vegetarian eating practices in people in the northeastern United States. *From Miller, D.R., Specker, B.L., Ho, M.L., et al., 1991. Am. J. Clin. Nutr. 53, 524–529.*

TABLE 18.9 Ranges of Urinary Methylmalonic Acid (MMA) Excretion by Breast-Fed Infants of Vegetarian and Omnivorous Mothers

Group	MMA (μmol/mmol creatinine)
Vegetarian	2.6–791
Mixed-diet	1.7–21

Specker, B.L., Miller, D., Norman, E.J., et al., 1988. Am. J. Clin. Nutr. 47, 89–92.

TABLE 18.10 Impact of Occasional Consumption of Animal Products on Vitamin B_{12} Status in a Macrobiotic Community

Food	Consumed	Serum Vitamin B_{12} (pM)	Urine MMA (mmol/mol creatinine)
Dairy	Never	122	5.3
	≤1/week	183[a]	2.8[a]
	>1/week	179[a]	2.1[a]
Eggs	Never	139	4.8
	≤1/week	167	3.1
	>1/week	157	2.2
Sea foods[b]	Never	111	4.4
	≤1/week	145	5.3
	>1/week	161	2.6

[a]$p > .05$.
[b]*Includes various sea vegetables (e.g., wakame, kombu, hijiki, arame, nori, dulse).*
Miller, D.R., Specker, B.L., Ho, M.L., et al., 1991. Am. J. Clin. Nutr. 53, 524–529.

Malabsorption

Poor absorption of the vitamin is thought to account for at least one-third of cases of vitamin B_{12} deficiency. This can be caused by inadequate production of IF by gastric parietal cells and/or to defective functioning of ileal IF receptors.[77]

Loss of gastric parietal cell function. Vitamin B_{12} malabsorption occurs if IF production by gastric parietal cells

77. The **Schilling test** has been used to assess vitamin B_{12} absorption in clinical settings. It involves the oral administration of a tracer dose of ^{57}Co–vitamin B_{12} to a fasting subject, followed by the i.m. administration of a large dose of the vitamin to saturate plasma haptocorrin and TC. This allows the absorbed tracer to be cleared by the kidney and be quantified in the urine. Correction of low apparent absorption by orally administered IF in a stage II test indicates pernicious anemia.

is inadequate.[78] Such conditions can have causes of four general types:

- **Pernicious anemia** affects an estimated 2–3% of Americans, mostly women; although it is likely to be widely underdiagnosed. It is a disease of later life, 90% of cases are diagnosed in individuals >40 years of age. It presents as an autoimmune gastritis[79] involving destruction of the fundus and body of the stomach by antibodies to the parietal cells membrane H^+/K^+-ATPase. This causes progressive atrophy of those cells and loss of their production of acid[80] and IF, resulting in **hypochlorhydria** and vitamin B_{12} malabsorption ultimately (2–7 years) leading to macrocytic anemia.
- ***Heliobacter pylori*** infection affects an estimated 9–30% of Americans. It produces damage to the stomach referred to as type B chronic atrophic gastritis, which results in hypochlorhydria that limits the enteric absorption of vitamin B_{12}.
- **Other gastric diseases** can damage gastric parietal cells and, thus, reduce production of stomach acid and IF. Such damage can result in macrocytic anemia or, frequently, hypochromic anemia due to impaired iron absorption caused by the hypoacidic condition. These conditions can occur in patients with simple (nonautoimmune) atrophic gastritis as well as those undergoing gastrectomy. After bariatric surgery, 10–15% of patients develop vitamin B_{12} deficiency within a few years; all patients undergoing complete gastrectomy are placed in need of supplemental vitamin.
- **Chronic use of proton pump inhibitors** reduces parietal cell acid production, reducing the utilization of vitamin B_{12} from ingested food.
- **Hereditary disorders** comprise the most common vitamin B_{12} malabsorption in children. These include Imerslund–Gräsbeck syndrome and congenital IF deficiency.

Pancreatic insufficiency. The loss of pancreatic exocrine function can impair the utilization of vitamin B_{12}. For example, about one-half of all human patients with pancreatic insufficiency show abnormally low enteric absorption of the vitamin. This effect can be corrected by pancreatic enzyme replacement therapy, using oral pancreas powder or pancreatic proteases. Thus, the lesion appears to involve specifically the loss of proteolytic activity, resulting in the failure to digest intestinal R proteins, which thus retain vitamin B_{12} bound in the stomach instead of freeing it for binding by IF.

Intestinal disease. Disorders and removal of the terminal portion of the ileum, causing the loss of IF receptors, result in malabsorption of the vitamin. Such conditions include ileitis, inflammatory bowel disease (Crohn's disease) and tropical sprue.[81] In addition, intestinal parasites (e.g., the tapeworm *Diphyllobothrium latum*) and explosively growing bacterial floras can effectively compete with the host for uptake of the vitamin. Protozoal infections that cause chronic diarrhea (e.g., *Giardia lamblia*) can impair vitamin B_{12} absorption.

Chemical factors. Several factors can impair the utilization of vitamin B_{12}:

- **Xenobiotics** including biguanide antidiabetic agents, chronic alcohol consumption, and heavy smoking can damage the ileal epithelium causing loss of IF receptors.
- **Nitrous oxide (N_2O)** oxidizes cob(I)alamin to the inactive form cob(II)alamin, causing rapid inactivation of the methylcobalamin-dependent enzyme and the excretion of the vitamin. Repeated exposure to NO depletes the body of its vitamin B_{12} stores.[82]
- **Oral contraceptive steroid**[83] use has been associated with apparently asymptomatic reductions in plasma vitamin B_{12} concentrations independent of dietary intake of the vitamin.[84]

General Signs of Deficiency

Vitamin B_{12} deficiency causes **macrocytic anemia**. This type of anemia is caused by delay or failure of normal cell division in the bone marrow (it also occurs in the intestinal mucosa). The underlying biochemical lesion is arrested synthesis of DNA precursors due to diminished availability of single-C units as a result of decreased activity of the vitamin B_{12}-dependent methionine synthase.[85] This traps folate in the methyl folate trap and reduces the availability of 5,10-methylene-FH_4, which is needed for the synthesis of thymidylate and, thus, of DNA. This reduces mitotic rate results in a **megaloblastic transformation**, i.e., the formation of abnormally large, cytoplasm-rich cells. In the bone marrow, this results in a type of megaloblastic anemia referred to as macrocytic anemia.

Vitamin B_{12} deficiency also causes **neurologic abnormalities** in most species. These may also result from

78. Chronic atrophic gastritis can be a precancerous lesion, involving progressive metaplasia of the gastric mucosa leading to carcinoma.
79. It is also called type A chronic atrophic gastritis or gastric atrophy.
80. Gastric acid is needed to facilitate the dissociation of vitamin B_{12} from food proteins and to check the proliferation of enteric bacteria that compete for the vitamin. Gastric hypochlorhydria, therefore, reduces the bioavailability of vitamin B_{12} from foods.
81. Tropical sprue is endemic in south India, occurs epidemically in the Philippines and the Caribbean, and is frequently a source of vitamin B_{12} malabsorption experienced by tourists to those regions.
82. Much of the toxicity of N_2O may actually be due to impaired vitamin B_{12} function. Indeed, excessive dental use of *laughing gas* can lead to neurologic impairment.
83. i.e., Mixtures of estrogen and progestin.
84. McArthur, J.O., Tang, H.M., Petocz, P., et al., 2013. Nutrients 5, 3634–3645.
85. MET regeneration from Hcy can also be reduced by deficiencies of folate coenzymes (due to methyl folate "trapping"), which also reduce thymidylate synthesis—all leading to failed DNA replication.

TABLE 18.11 General Signs of Vitamin B_{12} Deficiency

Organ System	Signs
General	Reduced growth
Vital organs	Hepatic, cardiac, and renal steatosis
Fetus	Hemorrhage, myopathy, death
Circulatory	Anemia
Nervous	Peripheral neuropathy

TABLE 18.12 Recommended Vitamin B_{12} Intakes

US		FAO/WHO	
Age–Sex	RDA[a] (μg/day)	Age–Sex	RNI[b] (μg/day)
0–6 months	[0.4][c]	0–6 months	0.4
7–11 months	[0.5][c]	7–11 months	0.5
1–3 years	0.9	1–3 years	0.9
4–8 years	1.2	4–6 years	1.2
9–13 years	1.8	7–9 years	1.8
>13 years	2.4	>9 years	2.4
Pregnancy	2.6	Pregnancy	2.6
Lactation	2.8	Lactation	2.8

[a]*Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.*
[b]*Recommended Nutrient Intakes, Joint WHO/FAO Expert Consultation, 2001. Human Vitamin and Mineral Requirements. WHO, Rome, 286 pp.*
[c]*RDAs have not been set; AIs are given instead.*

impaired MET biosynthesis; however, some investigators have proposed that they result from altered fatty acid metabolism due to the loss of MMA mutase activity. Neurological signs typically involve diffuse and progressive nerve demyelination, manifested as progressive neuropathy, often beginning in the peripheral nerves, and proceeding to the posterior and lateral columns of the spinal cord (Table 18.11). They tend to be manifested with relatively late onset due to the effective storage and conservation of the vitamin. Because folate can correct the anemia of vitamin B_{12} deficiency, lexus intakes of folate can mask that vitamin B_{12} deficiency, such that it may not be detected until possibly irreversible neurologic damage presents. Recommended dietary intakes for vitamin B_{12} have been established (Table 18.12).

Deficiency Signs in Humans

Vitamin B_{12} deficiency in humans produces hematologic and neurologic signs and symptoms. The hematological sign is **megaloblastic anemia**. Severely deficient infants present with feeding difficulties, developmental delay, and progressive neurological symptoms. In older children and adults, chronic deficiency can also produce progressive neurologic signs that are peripheral and/or cerebral in nature.[86] The earliest peripheral nervous symptoms are usually symmetrical paresthesia of the hands and feet, loss of proprioception and vibration sense of the ankles and toes, and ataxic gait. Rarely, patients also lose manual dexterity, taste, and smell and develop poor vision and orthostatic dizziness. Cerebral and psychiatric signs include memory impairment, depression, irritability, psychosis, and dementia.

Hematologic and neurologic signs do not necessarily manifest together in vitamin B_{12}-deficient subjects.[87] In most deficient subjects, either the anemia or neurologic signs predominate.[88] The metabolic basis for this phenomenon is not clear; nor is it clear why the neurologic signs in some subjects are predominantly peripheral nerve disorders, while in others they are predominantly cerebral disorders.

Low Vitamin B_{12} Status

Marginal deficiencies of vitamin B_{12} are estimated to be at least 10 times more prevalent than clinically overt deficiencies affecting apparently healthy people. An estimated 10–15% of people over the age of 60 have low serum vitamin B_{12} levels. However, that parameter underindicates the portion of marginal deficiencies involving metabolic changes marked by elevated circulating levels of FIGLU, Hcy, and MMA. Consideration of those parameters yields estimates of 30–40%. The prevalence of low vitamin B_{12} status is greatest in the elderly. That vitamin B_{12} status declines with age is thought to be related to declining intakes of the vitamin as well as increasing prevalence of atrophic gastritis and its associated hypochlorhydria, which can affect as much as half the geriatric population.

Homocysteinemia. Vitamin B_{12} deficiency may be the primary cause of homocysteinemia in many people; almost two-thirds of elderly subjects with homocysteinemia also show methylmalonic acidemia, indicative of vitamin B_{12} deficiency (Table 18.13). Still, less than one-third of individuals with low circulating vitamin B_{12} levels also show homocysteinemia. Epidemiologic studies have indicated associations of moderately elevated plasma Hcy and risks of coronary, peripheral and carotid arterial thrombosis and atherosclerosis, venous

86. McCaddon, A., 2012. Biochimie 95, 1066–1076.
87. Healton, E.B., Savage, D.G., Brust, J.C., et al., 1991. Medicine 70, 229–245.
88. Failure to appreciate this fact can lead to the underdiagnosis of vitamin B_{12} deficiency.

TABLE 18.13 Vitamin B_{12} and Folate Status of Elderly Subjects Showing Homocysteinemia

	Serum Hcy		Serum MMA (Methylmalonic Acid)	
Parameter	>3 SD	≤3 SD	>3 SD	≤3 SD
Serum vitamin B_{12} (pM)	197 ± 77[a]	325 ± 145	217 ± 83[a]	332 ± 146
Serum folate (nM)	12.7 ± 8.2[a]	22.9 ± 19.0	18.1 ± 12.5[a]	22.7 ± 19.5

SD, standard deviation.
[a]$p > .05$.
Lindenbaum, J., Rosenberg, I.H., Wilson, P.W., et al., 1994. Am. J. Clin. Nutr. 60, 2–11.

thrombosis, retinal vascular occlusion, carotid thickening, and hypertension.[89]

Neurological Effects

Insufficient vitamin B_{12} status is thought to lead to neurodegeneration as a result of abnormal incorporation of MMA into neuronal lipids including those in myelin sheaths, stimulation of the inflammatory cytokine tumor necrosis factor-α, and/or reduced synthesis of choline, the precursor of the neurotransmitter acetylcholine. Several aspects of neurological function are affected:

- **Cognition**. Serum Hcy level has been negatively correlated with the presentation of neuropsychiatric disorders in nonanemic subjects with low serum vitamin B_{12} levels.[90] Serum vitamin B_{12} levels <257 pM predicted cognitive decline in older subjects in the Framingham Heart Study.[91] These effects have been thought to be manifestations of white matter damage in the spinal cord and brain,[92] or atrophy of the brain,[93] both of which vary inversely with serum vitamin B_{12}. Recent studies have shown that poor memory performance by low serum vitamin B_{12} subjects is associated with damage to specific microstructural regions of the hippocampus.[94]

While one trial found high doses of cyanocobalamin to improve cognitive function in subjects with only mild impairment or with symptoms of recent onset (<6 months),[95] well-controlled, randomized clinical trials have found no benefits of vitamin B_{12} administration on low vitamin B_{12} subjects with cognitive impairment/dementia.[96] One trial found vitamin B_{12} therapy without effect on patients with dementia but to improve measures of verbal fluency in cognitively impaired patients.[97] A review of clinical experience in India suggested value of the vitamin in improving language function in patients.[98]

- **Alzheimer's disease (AD)**. Several studies have noted low concentrations of vitamin B_{12} in the serum and cerebrospinal fluid of nonanemic AD patients, who also tend to have homocysteinemia.[99] AD patients have also been found to have lower plasma levels of holoTC than nondemented elderly controls, despite having similar total plasma vitamin B_{12} levels.[100] In fact, holoTC level was inversely related to the subsequent risk of elderly subjects being diagnosed with AD over a 7-year period.[101]
- **Depression**. Low plasma levels of vitamin B_{12} have been reported in nearly one-third of patients with depression, who also tend to show homocysteinemia. Patients with high vitamin B_{12} status have been reported to have better treatment outcomes.[102]
- **Parkinson's disease (PD)**. PD patients have homocysteinemia,[103] which increases their risk for cognitive impairment. Some may also have elevated circulating MMA levels. Dietary supplementation with vitamin B_{12}

89. The low prevalence of coronary heart disease among South African blacks has been associated with their typically lower plasma Hcy levels and their demonstrably more effective Hcy clearance after methionine loading.
90. Lindenbaum, J., Healton, E.B., Savage, D.G., et al., 1988. N. Engl. J. Med. 318, 1720–1728.
91. Morris, M.S., Selhub, J., Jacques, P.F., 2012. J. Am. Geriatr. Soc. 60, 1457–1464.
92. de Lau, L.M., Smith, A.D., Refsum, H., et al., 2009. J. Neurol. Neurosurg. Psychiatry 80, 149–157.
93. Vogiatzoglou, A., Refsum, H., Johnson, C., et al., 2008. Neurology 71, 826–832.
94. Köbe, T., Witte, A.V., Schnelle, A., et al., 2016. Am. J. Clin. Nutr. 103, 1045–1054.
95. Martin, D.C., Francis, J. Protetch, et al., 1992. J. Am. Geriatr. Soc. 40, 168–172.
96. Carmel, R., Gott, P.S., Waters, C.H., et al., 1995. Eur. J. Haematol. 54, 245–253; Teunisse, S., Bollen, A.E., van Gool, V.A., et al., 1996. J. Neruol. 243, 522–529.
97. Eastley, R., Wilcock, G.K., Bucks, R.S., 2000. Psychiatry 15, 226–233.
98. Moretti, R., Torre, P., Antonello, R.M., et al., 2004. Neurol. India 52, 310–318.
99. McCaddon, A., 2013. Biochimie 95, 1066–1076.
100. Refsum, H. and Smith, A.D., 2003. J. Neurol. Neurosurg. Psychiatry 74, 959–961.
101. Hooshmand, B., Solomon, A., Kareholt, I., et al., 2010. Neurology 75, 1408–1414.
102. Levitt, A.J., Wesson, V.A., Joffe, R.T., 1998. Psychiat. Res. 79,123–129.
103. PD patients are treated with L-dopa, which can increase circulating Hcy levels.

(and folate) has been shown to reduce their plasma Hcy levels.[104]

- **Multiple sclerosis**. MS patients generally have elevated circulating levels of Hcy but relatively low levels of vitamin B_{12} and folate and are rarely anemic.[105] It has been suggested that low vitamin B_{12} status may exacerbate multiple sclerosis by enhancing the processes of inflammation and demyelination and by impairing those of myelin repair.
- **Hearing loss**. Serum vitamin B_{12} levels have been reported to be lower in subjects with tinnitus compared to normal hearing controls,[106] and vitamin B_{12} supplementation has been reported to lessen tinnitus in chronically affected subjects.[107]

Response to Treatment

Subclinical vitamin B_{12} deficiency, if diagnosed, is readily addressed. Biochemical indicators of vitamin B_{12} status, plasma/serum MMA and Hcy, fall within days of treatment with the vitamin. If correctly diagnosed in infants, intramuscular treatment with vitamin B_{12} can reverse both the biochemical indicators and clinical signs (regurgitations, delayed development of motor function) within a month.[108]

The hematological signs of vitamin B_{12} deficiency also respond quickly. Morphological abnormalities in the bone marrow are corrected within 2–3 days, reticulocyte numbers increase within 3–5 days, and this is followed by increases in erythrocyte numbers with the turnover of macrocytes.

Clinically overt vitamin B_{12} deficiency is more difficult to address, as it typically involves severe malabsorption. Also, neurological signs are corrected much slower, if at all. Muscular weakness and some psychiatric signs (e.g., irritability, confusion), particularly those of relatively recent onset (e.g., <3 months), may show improvement within weeks and may be completed corrected. Sensory signs may take longer and may never be completely corrected.

Deficiency Signs in Animals

Vitamin B_{12} deficiency in nonruminant animals is characterized most frequently by reductions in rates of growth and feed intake and by impairments in the efficiency of feed utilization. In a few species (e.g., swine) a mild macrocytic anemia develops. Swine may also develop rough skin and gastrointestinal disorders. Vitamin B_{12}-deficient gilts can have delayed estrus; pregnant gilts may deliver fewer progeny, which are of low birth weight. Growing chicks and turkey poults show impairments in growth and feed utilization, macrocytic anemia, neurologic signs, and defective feathering. They can also show perosis[109] as a secondary effect of reductions in MET and choline due to the reduced availability of labile methyl groups. Limited methyl group availability (for the synthesis of phosphatidylcholine) in poultry is also manifest as increased deposition of lipids in the liver, heart, and kidneys. For this reason, vitamin B_{12} is known as a **lipotrope** for poultry. Vitamin B_{12} deficiency in the chicken also causes embryonic death, with embryos showing myopathies of the muscles of the leg, hemorrhage, myocardial hypertrophy, as well as perosis. Laboratory rodent species typically show impaired growth and feed utilization; males can show impaired spermatogenesis. Monkeys show macrocytic anemia.

Synthesis of the vitamin in the rumen is dependent on the development of a rumen microbiome, which can take several weeks. Therefore, young ruminants, i.e., those less than 6 weeks old require a dietary source of vitamin B_{12}; else they develop anorexia, poor growth, and, sometimes, macrocytic anemia. Vitamin B_{12} supplements are required for calves and lambs fed diets containing no animal protein. That need disappears with the establishment of the rumen microbiome, which depends on an adequate supply of dietary Co for the microbial synthesis of the organic portion of the corrin nucleus. Ruminal production of vitamin B_{12} can also be affected by the composition of dietary fiber, the ratio of roughage to concentrate, and the level of dry matter intake. Most microbially produced vitamin B_{12} appears to be contained within rumen microbial cells; it is released for absorption only in the small intestine. Cattle are thought to be less susceptible than sheep to Co deficiency. Sheep fed a diet containing <70 μg Co per kilogram show signs of deficiency: anorexia, wasting, diarrhea, and watery lacrimation. Co deprivation reduces hepatic Co level and increases plasma methylmalonyl CoA expression but does not produce clinical signs. Therefore, most ruminants, and species with significant cecal microbiomes (e.g., horse, rabbits, some fish), do not have dietary needs for vitamin B_{12}.

104. Lamberti, P., Zoccolella, S., Armenise, E., et al., 2005. Eur. J. Neurol. 12, 365–368.
105. Moghaddsi, M., Mamarabadi, M., Mohevi, N., et al., 2013. Clin. Neurol. Neurosurg. 115, 1802–1805; Kocer, B., Enqur, S., Ak, F. et al., 2009. J. Clin. Neurosci. 16, 399–403.
106. Houston, D.K., Johnson, M.A., Nozza, R.J., et al., 1999. Am. J. Clin. Nutr. 69, 564–571; Shemesh, Z., Attias, J., Ornan, M., et al., 1993. Am. J. Otalaryngol. 2, 94–99.
107. Shemesh, Z., Attias, J., Ornan, M., et al., 1993. Am. J. Otolaryngol. 2, 94–99.
108. Torsvik, I., Ueland, P.M., Markestad, T., et al., 2013. Am. J. Clin. Nutr. 98, 1233–1240.

109. This is the anatomical condition sometimes called "slipped tendon" in which the gastrocnemius (achilles) tendon slips from the guiding condyles of the distal end of the tibia due to the twisting of that bone and widening of the tibiometatarsal joint. This impairs locomotion including access to food, typically resulting in reduced growth. Perosis can also be caused by deficiencies of manganese, choline, or niacin.

10. VITAMIN B_{12} IN HEALTH AND DISEASE

Anticarcinogenesis

It has been suggested that subclinical deficiencies of vitamin B_{12} may enhance carcinogenesis. That hypothesis is supported by the finding that low, asymptomatic vitamin B_{12} status can alter DNA base substitution and methylation in the rat model,[110] as well as observations in a prospective study of increased breast cancer risk in women ranking in the lowest quintile of plasma vitamin B_{12} concentration.[111] In contrast, another observational study found weak positive associations of serum vitamin B_{12} level and vitamin B_{12} intake and prostate cancer risk.[112] Large, placebo-controlled, randomized intervention trials have found combined treatment of vitamin B_{12}, vitamin B_6, and folate without effect on cancer risks of breast or total cancers.[113] Therefore, it is unlikely that vitamin B_{12} has a role in reducing cancer risk.

Cyanide binding. Cobalamins can bind cyanide to produce the nontoxic cyanocobalamin. For that reason, hydroxocobalamin is a well-recognized cyanide antidote. It has been proposed that vitamin B_{12} may have a role in the inactivation of the low levels of cyanide consumed in many fruits, beans, and nuts.

Antioxidant Activity

Vitamin B_{12} has antioxidant capacity. This is indicated by the findings that high levels of the vitamin can protect cells against in vitro exposure to hydrogen peroxide (H_2O_2).[114] This protection may involve stimulation of cellular methionine synthase activity, as well as direct reaction of the vitamin with the reactive oxygen species generated by H_2O_2. Any antioxidant effects in vivo are likely to occur only at pharmacological exposures to the vitamin or other cobalamins.

Protection against mineral toxicities. Nutritional levels of vitamin B_{12} have been effective in reducing intoxicating effects of selenium in Japanese quail[115] and cadmium in the rat.[116]

11. VITAMIN B_{12} TOXICITY

Vitamin B_{12} has no appreciable toxicity. Upper tolerable intakes (ULs) for B12 have not been established. Results of studies with mice indicate that it is innocuous when administered parenterally in very high doses. Localized, injection-site, sclerodermoid reaction[117] secondary to vitamin B_{12} injection has been reported. Dietary levels of at least several hundred times the nutritional requirements are safe. High plasma levels of the vitamin are indicative of disease[118] rather than hypervitaminosis B_{12}.

12. CASE STUDY

Review the following case report, paying special attention to the diagnostic indicators on which the treatment was based. Next, answer the questions that follow.

Case

A 6-month-old boy was admitted in comatose condition. He had been born at term, weighing 3 kg, the first child of an apparently healthy 26-year-old *vegan*.[119] The mother had knowingly eaten no animal products for 8 years and took no supplemental vitamins. The infant was exclusively breast fed. He smiled at 1–2 months of age and appeared to be developing normally. At 4 months, his development began to regress; this was manifested by his loss of head control, decreased vocalization, lethargy, and increased irritability. Physical examination revealed a pale and flaccid infant who was completely unresponsive even to painful stimuli. His pulse was 136/min, respirations 22/min, and blood pressure 100 mmHg by palpation. His length was 65 cm (50th percentile for age) and his weight was 5.6 kg (<3rd percentile and at the 50th percentile for 3 months of age). His head circumference was 41 cm (3rd percentile). His optic disks[120] were pale. There were scattered ecchymoses[121] over his legs and buttocks. He had increased pigmentation over the dorsa of his hands and the feet, most prominently over the knuckles. He had no head control and a poor grasp. He showed no deep tendon reflexes. His liver edge was palpable 2 cm below the right costal margin.

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117. Such reactions are not common but have been reported for various drugs and for vitamin K.
118. Elevated cobalamin levels are typical of myelogenous leukemia and promyelocytic leukemic and are used as diagnostic criteria for polycythemia vera and hypereosinophilic syndrome. Several liver diseases (acute hepatitis, cirrhosis, hepatocellular carcinoma, and metastatic liver disease) can cause similar increases, which are due to increased levels of TC_I.
119. A strict vegetarian.
120. Circular area of thinning of the sclera (the fibrous membrane forming the outer envelope of the eye) through which the fibers of the optic nerve pass.
121. Purple patches caused by extravasation of blood into the skin, differing from petechiae only in size (the latter being very small).

Laboratory Results

Parameter	Patient	Normal Range
Hemoglobin, g/dL	5.4	10.0–15.0
Hematocrit, %	17	36
Erythrocytes, $\times 10^6/\mu L$	1.63	3.9–5.3
White blood cells, $\times 10^3/\mu L$	3.8	6–17.5
Reticulocytes, %	0.1	<1
Platelets, $\times 10^3/\mu L$	45	200–480

A peripheral blood smear revealed mild macrocytosis[122] and some hypersegmentation of the neutrophils.[123] Bone marrow aspiration showed frank megaloblastic changes in both the myeloid[124] and the erythroid[125] series. Megakaryocytes[126] were decreased in number. The sedimentation rate, urinalysis, spinal fluid analysis, blood glucose, electrolytes, and tests of renal and liver function gave normal results. An electroencephalogram was markedly abnormal, as manifested by minimal background activity and epileptiform transients in both temporal regions. Analysis of the urine obtained on admission demonstrated a markedly elevated excretion of MMA, glycine, methylcitric acid, and Hcy. Shortly after admission, respiratory distress developed, and 5 mg of folic acid was given, followed by transfusion of 10 mL of packed erythrocytes per kilogram body weight. Four days later, a repeat bone marrow examination showed partial reversal of the megaloblastic abnormalities.

Other Laboratory Results

Parameter	Patient	Normal Range
Serum vitamin B_{12} (pg/mL)	20	150–1000
Serum folates (ng/mL)	10	3–15
Serum iron (µg/dL)	165	65–175
Serum iron-binding capacity (µg/dL)	177	250–410

Cyanocobalamin (1 mg/day) was administered for 4 days. The patient began to respond to stimuli after the transfusion; however, the response to vitamin B_{12} was dramatic. Four days after the initial dose he or she was alert, smiling, responding to visual stimuli, and maintaining his or her body temperature. As he or she responded, rhythmical twitching activity in the right hand and arm developed that persisted despite anticonvulsant therapy, and despite a concomitant resolution of electroencephalographic abnormalities. The mother showed a completely normal hemogram. Her serum vitamin B_{12} concentration was 160 pg/mL (normal, 150–1000 pg/mL), but she showed moderate methylmalonic aciduria. Her breast milk contained 75 pg of vitamin B_{12}/mL (normal, 1–3 ng/mL).

With vitamin B_{12} therapy, the infant's plasma vitamin B_{12} rose to 600 pg/mL and he or she continued to improve clinically. The abnormal urinary acids and homocystine disappeared by day 10; cystathionine persisted until day 20. On day 14, Hb was 14.4 g/dL, hematocrit was 41%, and the WBC was 5700/ml. The platelet count had become normal 20 days after admission. The unusual pigment on the extremities had improved considerably 2 weeks after he or she received the parenteral vitamin B_{12} and disappeared gradually over the next month. The liver was no longer palpable. The twitching of the hands disappeared within a month of therapy. Developmental assessment at 9 months of age revealed him or her to be functioning at the 5-month age level. A month later, he or she was sitting and taking steps with support. Head circumference had exhibited catch-up growth and at 44 cm was in the normal range for the first time since admission. His length was 70 cm (10th percentile) and weight 8.4 kg (10th percentile). By this time, the mother's serum vitamin B_{12} had dropped to 100 pg/mL, and she began taking supplemental vitamin B_{12}.

Case Questions

1. Which clinical findings suggested that two important coenzyme forms of vitamin B_{12} were deficient or defective in this infant? How do the clinical findings relate specifically to each coenzyme?
2. What findings allow the distinction of vitamin B_{12} deficiency from a possible folic acid-related disorder in this patient?
3. Offer a reasonable explanation for the fact that the mother, who had avoided vitamin B_{12}-containing foods for 8 years before her pregnancy, did not show overt signs of vitamin B_{12} deficiency.

13. STUDY QUESTIONS AND EXERCISES

1. Construct a decision tree for the diagnosis of vitamin B_{12} deficiency in humans or an animal species and, in particular, the distinction of this deficiency from that of folate.
2. What key feature of the chemistry of vitamin B_{12} relates to its coenzyme functions?

122. Occurrence of unusually large numbers of *macrocytes* (large erythrocytes) in the circulating blood; also called *megalocytosis*, *megalocythemia*, and *macrocythemia*.
123. A type of mature white blood cell in the granulocyte series.
124. Related to myocytes.
125. Related to erythrocytes.
126. An unusually large cell thought to be derived from the primitive mesenchymal tissue that differentiates from hematocytoblasts.

3. What parameters might you measure to assess vitamin B_{12} status of a human or animal?
4. What is the relationship of normal function of the stomach and pancreas with the utilization of dietary vitamin B_{12}?

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Chapter 19

Vitamin-Like Factors

Chapter Outline

Anchoring Concepts

1. The designation vitamin is specific for animal species, stage of development or production, and/or particular conditions of the physical environment and diet.
2. Each of the presently recognized vitamins was initially called an accessory factor or an unidentified growth factor, and these terms continue to be used to describe biologically active substances, particularly for species of lower orders.

Have all the vitamins been discovered? From all indications in the extensive recent and current publications in the scientific literature dealing with the purification and effects of 'unidentified factors,' the answer appears to be 'no.' It is from such studies that new vitamins may be recognized and characterized

A.F. Wagner and K. Folkers[1]

LEARNING OBJECTIVES

1. To understand that the designation of a compound as a vitamin is biased in favor of avoiding deficiency in humans.
2. To understand that other substances have been proposed as vitamins.
3. To understand that choline and carnitine are vitamins for certain animal species.
4. To understand the metabolic functions of other conditionally essential nutrients: *myo*-inositol, pyrroloquinoline quinine, the ubiquinones, and orotic acid.
5. To understand why flavonoids, nonprovitamin A carotenoids, *p*-aminobenzoic acid, and lipoic acid are not called vitamins.

VOCABULARY

Acetylcholine
Acylcarnitine esters
Acylcarnitine translocase
Arachidonic acid
Beneficial dietary factor
Betaine
Betaine aldehyde dehydrogenase
Betaine:homocysteine methyltransferase
Branched-chain ketoacid dehydrogenase
γ-Butyrobetaine hydroxylase
Calcisomes
Canthaxanthin
Carnitine
Carnitine acyltransferases I and II (CATI, CATII)
Carnitine–acylcarnitine translocase (CACT)
Catechins
Ceramide
Choline
Choline acetyltransferase
Choline dehydrogenase

1. Karl Folkers (1906–97) was an American biochemist who spent most of his career at Merck Pharmaceuticals where he made many contributions isolating, identifying, and synthesizing a wide variety of bioactive natural substances including vitamin B_{12}, pyidoxaine, pyridoxal, pyrodixamine, pantothenic acid, biotin, and ubiquinone. In 1990, he received the U.S. National Medal of Science. Among his colleagues at Merck was Arthur F. Wagner (1922–2010).

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00019-8

Choline kinase
Choline oxidase
Choline phosphotransferase
Coenzyme Q10 (CoQ10)
Conditional essentiality
Cytidine diphosphatidylcholine (CDP–choline)
Dihydrolipoyl dehydrogenase
Dihydrolipoyl transacetylase
Dimethylglycine
Ethanolamine
Flavanols
Flavonols
Flavonoids
6-β-Galactinol
Glycerylphosphorylcholine
Glycerylphosphatidylcholine diesterase
Myo-inositol
Inositol 1,4,5-triphosphate (IP3)
Inositol phosphokinases
Isoflavones
α-Ketoglutarate dehydrogenase
Labile methyl groups
Lecithin
Lipoamide
Lipofuscin
Lipoic acid
Lipoic acid synthetase
Lipoyl transferase
Lycopene
Lysolecithin
Lysophosphatidylcholine
Lutein
Macula
Macular degeneration
Methionine
Mitochondrial fatty acid shuttle
Orotic acid
Phosphatidylcholine
Phosphatidylcholine–ceramide choline transferase
Phosphatidylcholine–glyceride choline transferase
Phosphatidylethanolamine
Phosphatidylethanolamine N-methyl transferase
Phosphatidylinositol (PI)
Phosphatidylinositol 4-phosphate (PIP)
Phosphatidylinositol 4,5-biphosphate (PIP2)
Phospholipases A1, A2, B, C, and D
Phosphorylcholine
Phytic acid
Phytoestrogens
Proanthocyanidins
Second messenger
Sphingomyelin
Tannic acid
Thioctic acid
Trimethylamine
ε-N-Trimethyllysine
Ubiquinones
Vitamin BT
Xanthophyll-binding protein (XBP)
Xanthophylls
Zeaxanthin

1. IS THE LIST OF VITAMINS COMPLETE?

The research that resulted in the recognition of the vitamins had both empirical and experimental phases. That is, initial associations between diet and health status generated hypotheses that could be tested experimentally. As is generally true in science, where hypotheses were clearly enunciated and adequate experimental approaches were available, insightful investigators were able to make remarkable progress in identifying these essential nutrients. Those endeavors, of course, also revealed some biologically active factors[2] to be identical or related to known nutrients,[3] some needed only by some species or under certain conditions, some to be biologically active but not essential in diets, and some not to be active at all.[4] The apparently irregular and often confusing array of informal vitamin names (Appendix A) reveals this history of discovery.

Over this history, the designation of bioactive factors as vitamins has been an informal process. It has tended to be anthropocentric in that it reflected, to a large extent, the nutritional needs of humans (Why is ascorbic acid called a vitamin, when it is synthesized by most species?). Designations have typically not been revised (Why is cholecalciferol still called a vitamin and not a hormone?). Most notably, it has been inconsistent in that several bioactives fit the traditional criteria for vitamin designation (*see* Chapter 1) under certain circumstances, e.g., for a particular species, genotype, stage of development, diet composition, nutritional status, or physical environment. Some of these have

2. In addition to the 40 or so recognized dietary essentials, foods contain an estimated 25,000+ biologically active compounds.
3. An example is vitamin T (also called "termitin," "penicin," "torutilin," "insectine," "hypomycin," "myocine," or "sesame seed factor"). This was extracted from yeast, sesame seeds, or insects and appeared to stimulate the growth of guppies, hamsters, baby pigs, chicks, mice, and insects; to promote wound healing in mice; and to improve certain human skin lesions. Ultimately, it was found to be a mixture containing folate, vitamin B_{12}, and amino acids. Similarly, vitamins M, B_c, B_{10}, T, and B_x were ultimately found to be forms of folate.
4. e.g., Early studies suggested that *p*-aminobenzoic acid, a bacterial metabolite in the biosynthesis of folate, increased growth in chicks and promoted lactation in rats, when those animals were fed marginal amounts of folate. For a time, PABA was called "vitamin B_x." Such responses were subsequently shown to be due to PABA being used by the intestinal microbiome for the synthesis of folate, which was made available to the host either directly in the gut or indirectly via the feces.

emerged as beneficial to health. Those dietary factors with vitamin-like properties fall into two categories:

- **Conditionally essential nutrients:** choline, carnitine, *myo*-inositol, ubiquinols, and lipoic acid.
- **Beneficial dietary bioactive:** nonprovitamin A carotenoids, flavonoids, lipoic acid, and orotic acid.

2. CHOLINE

Choline is a normal metabolite with essential functions in cells. Its metabolic derivatives are structural components of membranes, neurotransmitters, methyl group donors, and mediators of hepatic lipid metabolism. Choline can be biosynthesized, but there are circumstances in which that synthesis may not be sufficient for optimal physiologic functions, making it **conditionally essential**.

Recognition of a Role of Choline in Nutrition

The discovery of insulin by Banting and Best[5] in the mid-1920s led to studies with depancreatized dogs that showed dietary **lecithin** (**phosphatidylcholine, PC**) to be effective in mobilizing the excess lipids in the livers of insulin-deprived animals. Best and colleague Elinor Huntsman showed that the active component of lecithin is choline. Choline had been isolated by Strecker[6] in 1862, and its structure had been determined by Bayer[7] shortly after that. In 1940, Jukes showed that choline is required for normal growth and the prevention of the leg disorder called **perosis**[8] in turkeys; in fact, more choline was required to prevent perosis than to support normal growth. Jukes and Norris's group at Cornell then found that **betaine**, the metabolic precursor to choline, was not always effective in preventing choline-responsive perosis in turkeys and chicks. These findings made is clear that choline was more than a lipotrope.

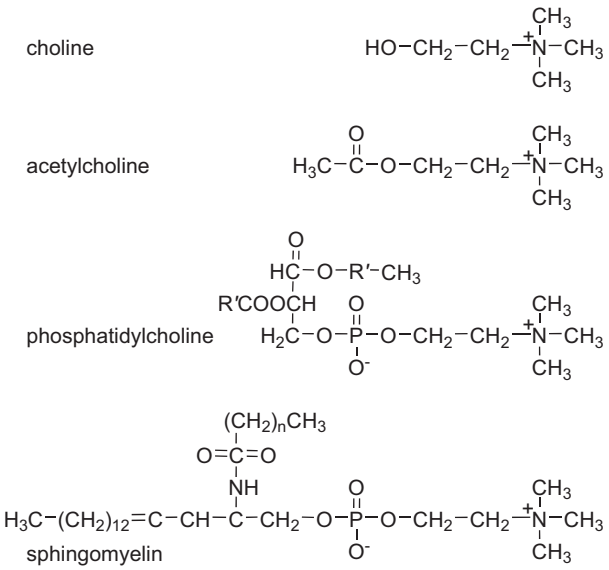


FIGURE 19.1 Choline and its functional metabolites.

Conditions of Need for Dietary Choline

For most individuals, endogenous biosynthesis of choline is sufficient to meet metabolic needs. However, that does not pertain in two situations:

- Insufficient biosynthesis of MET by 5-methyl-FH_4-dependent methylation of Hcy, e.g., due to suboptimal status with respect to folate, vitamin B_{12}, and/or preformed MET.
- Deficiencies in enzymes of choline or single-C metabolism.[9]

With low-choline intakes, these situations can, in principle, result in hepatic steatosis, hemorrhagic renal degeneration, and (in animals) depressed growth. Fatty infiltration of the liver reflects the need for PC for the synthesis of very low-density lipoprotein (VLDL), which is necessary for the export of triglycerides. Such manifestations have to do with nutritional status with respect to folate, vitamin B_{12}, and/or MET.

Conditions producing need for dietary choline are as follows:

- Deficiencies in single-C metabolism
- Deficiencies of choline-metabolizing enzymes

Chemical Properties of Choline

Choline is the trivial designation for the compound 2-hydroxy-*N*,*N*,*N*-trimethylethanaminium [also (β-hydroxyethyl)trimethylammonium] (Fig. 19.1). It is freely soluble in water

5. Frederick Banting (1891–1949), a young surgeon working in the laboratory of John James Rickard Macleod at the University of Toronto, was assisted by a medical student, Charles Best (1899–1978). Banting and Macleod (1976–35) were awarded the 1923 Nobel Prize for medicine or physiology for the discovery and isolation of insulin. Banting shared his prize money with Best, and Macleod shared his with James Collip who had worked with the team on the purification of insulin.

6. Adolph Strecker (1822–1871) was then a Professor at the University of Tübingen; he coined the name "choline" after the Greek word "*chole*" for bile.

7. Carl Josef Bayer (1847–1904) was an Austrian chemist best known for inventing the eponymous process for extracting alumina from bauxite.

8. Perosis occurs in rapidly growing heavy-bodied poultry and involves the misalignment of the tibiotarsus causing slippage of the Achilles tendon. This impairs ambulation and can reduce feeding, consequently impairing growth. Perosis can also be caused by dietary deficiencies of niacin or manganese.

9. Zeisel, S.H., 2011. J. Nutr. 141, 531–534.

and ethanol but insoluble in organic solvents. It is a strong base and decomposes in alkaline solution with the release of trimethylamine. The prominent feature of its chemical structure is its triplet of methyl groups, which enables it to serve as a methyl donor.

Distribution of Choline in Foods and Feedstuffs

Americans consume some 440–630 mg/day.[10] All natural fats contain some choline (Table 19.1). The factor occurs naturally mostly in the form of **PC**[11] (also called **lecithin**), which, because it is a good emulsifying agent, is used as an ingredient or additive in many processed foods and food supplements. Some dietary choline (<10%) is present as the free base and some as **sphingomyelin**.[12] The richest sources in human diets are egg yolk, glandular meats (e.g., liver, kidney, brain), pork (meat, bacon), soybean products, wheat germ, and peanuts. Choline is added (as choline chloride and choline bitartrate) to infant formulas as a means of fortification.

The best sources of choline for animal feeding are the germs of cereals, legumes, and oilseed meals (e.g., soybean meal). Corn is notably low in choline (half the levels found in barley, oats, and wheat). Because wheat is rich in the choline-sparing factor betaine, the choline needs of livestock fed diets based on wheat are much lower than those of animals fed diets based on corn.

Bioavailability and stability. The bioavailability of choline in foods and feedstuffs appears to be generally good, i.e., >80%. It is dependent on those factors that affect the utilization of dietary fats. Naturally occurring choline, as well as the choline salts used as supplements, have good stability. The processing of foods/feedstuffs can enhance choline bioavailability, as mechanical disruption of plant cells by chopping and grinding, etc., can activate phospholipases to release choline in free form.

Absorption and Transport of Choline

Digestion. Choline is released from **PC**[13] by hydrolysis in the intestinal lumen through the action of phospholipases produced by the pancreas (**phospholipase A_2**, which cleaves the β-ester bond) and the intestinal mucosa

TABLE 19.1 Choline Contents (mg/100 g) of Common Foods

Food	Choline Equivalents[a]
Meats	
Beef	78.2
Beef liver	418.2
Chicken	65.8
Chicken liver	290.0
Pork	102.8
Bacon	124.9
Shrimp	70.6
Cod	83.6
Salmon	65.5
Vegetables	
Beans	13.5
Broccoli	40.1
Cabbage	15.5
Carrots	8.8
Corn	22.0
Cucumber	6.0
Lettuce	6.7
Mushrooms	16.9
Onions	6.1
Peas	27.5
Potatoes	14.4
Spinach	22.1
Soybeans	115.9
Tomatoes	6.7
Peanuts	52.5
Fruits	
Apples	3.4
Avocado	14.2
Blueberry	6.0
Banana	9.8
Cantaloupe	7.6
Grapefruit	7.5
Grapes	5.6
Oranges	8.4
Peaches	6.1

Continued

10. Choline intakes of Americans have been estimated to be 443 ± 88 mg/day for women and 631 ± 157 mg/day for men (Fischer, L.M., Scearce, J.A., Mar, M.H., et al., 2005. J. Nutr. 135, 826–829).
11. Phosphatidylcholine comprises 95% of total choline in eggs and 55–70% of total choline in meats and soy products.
12. i.e., Phosphatidylcholine analogues containing, instead of a fatty acid, sphingosine (2-amino-4-octadecene-1,3-diol) at the glycerol α-carbon.
13. Also called lecithin (lécithine), the name given by the French chemist Theodore Gobley (1811–76) to the factor he isolated from egg yolk (Greek, *lékith-os*).

TABLE 19.1 Choline Contents (mg/100 g) of Common Foods—cont'd

Food	Choline Equivalents[a]
Strawberries	5.7
Cereals	
Oats	7.4
Oat bran	58.6
Rice, polished	2.1
Rice, unpolished	9.2
Wheat	26.5
Wheat bran	74.4
Other	
Milk	14.3
Eggs	251.0

[a]*Includes free choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin.*
Adapted from Zeisel, S.H., Mar, M.H., Howe, J.C., et al., 2003. J. Nutr. 133, 1302–1307.

(**phospholipases A_1** and **B**, both of which cleave the α-ester bond to yield **glycerylphosphatidylcholine, glyceryl-PC**). The mucosal enzymes are much less efficient than the pancreatic enzyme. Therefore, most of the PC that is ingested is absorbed as **lysophosphatidylcholine (lysoPC)**[14] (deacylated only in the α position), which is reacylated postabsorptively to yield PC. This reaction involves the dismutation of two molecules of lysolecithin to yield a molecule of glyceryl-PC and one of PC. Analogous reactions occur with sphingomyelin, which, unlike PC, is not degraded in the intestinal lumen but is taken up intact by the intestinal mucosa.

Absorption. LysoPC and sphingomyelin are partitioned into mixed micelles and are absorbed by micelle-dependent diffusion into enterocytes mainly in the duodenum and jejunum. Free choline is absorbed in the same region by a saturable, carrier-mediated process localized in the brush border, and efficient at low lumenal concentrations (<4 mM). It is also absorbed less efficiently by passive diffusion; at high intakes, the nonabsorbed major portion passes to the hindgut where it is catabolized by the intestinal microbiome to the end product **trimethylamine**[15] much of which is absorbed and excreted in the urine. Because PC, being better absorbed, is not subject to such extensive microbial metabolism, it produces less urinary trimethylamine. The oxidized metabolite of choline, betaine, is absorbed by way of a different carrier, the IMINO proline transporter.

TABLE 19.2 Distribution of Phospholipids in Plasma Lipoproteins

Lipoprotein Class	Phospholipid Content, %
High-density lipoproteins (HDLs)	~30
Low-density lipoproteins (LDLs)	~22
Very low-density lipoproteins (VLDLs)	10–25
Chylomicra	3–15

Transport. Within the enterocyte, most recently absorbed lysoPC is esterified to form PC, some of which is thought to be incorporated into nascent high-density lipoproteins (HDLs). Those factors are transported into the lymphatic circulation (or the portal circulation in birds, fishes, and reptiles) bound to chylomicra, which are subject to clearance to the lipoproteins that circulate to the peripheral tissues. Thus, choline is transported to the tissues predominantly as phospholipids associated with the plasma lipoproteins (Table 19.2).

Free choline, a positively charged quaternary amine, does not cross biological membranes without a carrier. Three transport systems have been identified:

- **A high-afffinity,**[16] **Na^+-dependent transporter CHT1** (SCL5A7) unique to cholinergic neurons of the brain, brain stem, and spinal column provide choline for the synthesis of acetylcholine. Genetic deletion of this transporter is lethal to mice within an hour of birth.[17]
- **Intermediate-affinity,**[18] **Na^+-dependent transporters** in the in the solute carrier family (SLC44) facilitate the transport of choline across plasma and mitochondrial membranes. Five choline transporter-like transporters have been identified. The predominant one, CTL1, is expressed in all human tissues. CTL2 is expressed mainly in lung, colon, spleen, and inner ear and to lesser extents in brain, liver, and kidney.
- **Low-affinity,**[19] **polyspecific transporters** in the solute carrier family transport choline nonspecifically. These include three organic cation transporters (OCTs) and two organic carnitine/cation transporters (OCTNs) and are expressed in many tissues.

14. Also called lysolecithin.
15. The characteristic fishy odor of this product is identifiable after consumption of a choline supplement.
16. K_m <10 μM.
17. Wurtman, R.J., Cansev, M., Ulus, I.H., 2009. Handbook of Neurochemistry and Molecular Neurobiology: Nerual Lipids. In: Lajitha, A., Tettamanti, G., Goracci, G. (Eds.). Springer, New York, p. 443–500.
18. K_m 10–30 μM.
19. K_m >30 μM.

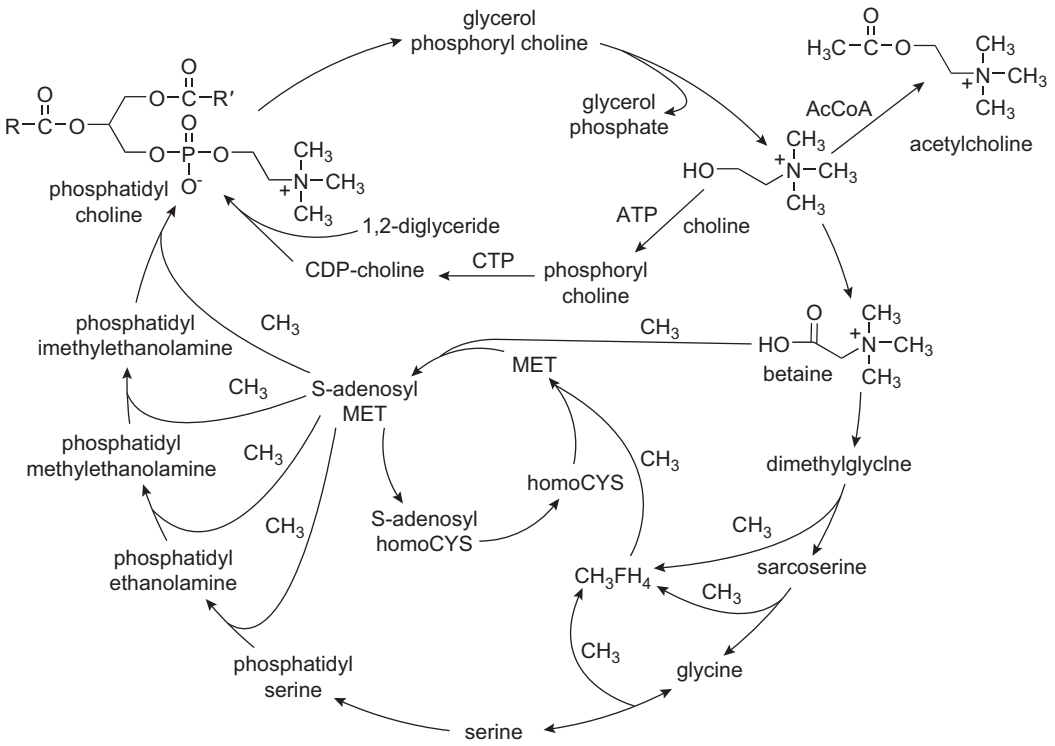


FIGURE 19.2 The biosynthesis and utilization of choline. *AcCoA*, acetyl-CoA; *CH3FH4*, 5-methyl tetrahydrofolate; *CTP*, cytidine triphosphate; *homoCys*, homocysteine; *MET*, methionine.

Tissue distribution. Choline is present in all tissues as an essential component of phospholipids in membranes of all types. Therefore, ~95% of the body pool of choline occurs as PC. It is not stored but occurs in the relatively great concentrations in the essential organs (e.g., brain, liver, kidney) mostly as PC and sphingomyelin.[20] Placental tissues are unique in that they accumulate large amounts of acetylcholine, presumably to meet fetal needs, which is otherwise present only in the parasympathetic nervous system.

Choline Metabolism

Biosynthesis. **De novo biosynthesis** of choline occurs by the sequential, *S*-adenosylmethionine (SAM)-dependent methylation of phosphatidylethanolamine by **phosphatidylethanolamine *N*-methyltransferase (PEMT)** to produce PC, which accounts for ~95% of the total choline pool (Fig. 19.2). This biosynthetic activity is due to two enzymes that use SAM as the methyl donor: (1) an inner plasma membrane enzyme adds the first methyl group and is rate limiting to choline synthesis; and (2) an outer membrane enzyme adds the second and third methyl groups. This pathway occurs in many tissues, but it greatest in liver in which it can account for as much as 40% of that organ's PC content. It is upregulated under conditions of dietary choline deficiency. PC produced by this pathway is composed mainly of long-chain polyunsaturated fatty acids.

Incorporation of choline into PC. Free choline is converted to PC in three steps. It is phosphorylated by cytosolic ATP-dependent **choline phosphotransferase** (also called **choline kinase**) in the rate-limiting step in PC synthesis. **Phosphorylcholine** is converted to **cytidine diphosphatidylcholine (CDP)** by **CDP:phosphocholine cytidyltransferase**. This step is upregulated by diacylglycerol and feedback-inhibited by PC. CDP is combined with diacylglycerol by **choline phosphotransferase** located on the endoplasmic reticulum. This pathway can account for some 70% of hepatic PC. PC produced by the CDP–choline pathway[21] is composed mainly of saturated fatty acids.

Mobilization of free choline. Cells mobilize choline by hydrolysis of PC (Fig. 19.2) and sphingomyelin. This can occur in two ways:

- The actions of **phospholipases A_2** and **B** yield lysoPC, which is converted to glyceryl-PC by **lysophospholipases**

20. Free choline comprises <1% of the total.

21. Also called the Kennedy pathway, after Eugene P. Kennedy (1919–2011) who first identified it in 1956 when he was on the faculty of the University of Chicago.

and, subsequently, to free choline by **glycerophosphodiester phosphodiesterases**. PC can also be metabolized by phospholipases C and D, which yield phosphorylcholine and phosphatidic acid, respectively. The former can be converted to free choline by **alkaline phosphatase**.

- By **phospholipid base exchange** of PC and serine, yielding choline and phosphatidylserine.

Choline oxidation. Most (60–90%) of the metabolism of free choline involves its oxidation, which frees its methyl groups to enter single-C metabolism. Choline oxidation is induced by dietary choline; it occurs in several tissues but is notably absent from brain, muscle, and blood. It is accomplished in two irreversible steps by a dual enzyme system collectively called **choline oxidase:**

- **Oxidation of choline** in the mitochondria by the FAD-linked enzyme **choline dehydrogenase** to yield betaine aldehyde and $FADH_2$.
- **Reduction of betaine aldehyde** to form **betaine** by the NAD-linked enzyme **betaine aldehyde dehydrogenase**, in both the mitochondria and cytosol, yielding NADH. This step occurs at a rate 10-fold greater than that of the incorporation of choline into phosphorylcholine.

The production of mitochondrial electron transport chain substrates ($FADH_2$ and NADH) by this process means that ~5 moles of ATP are generated for each mole of choline converted to betaine.[22]

Acetylcholine synthesis. Only a small fraction of choline is acetylated, but that amount provides the important neurotransmitter **acetylcholine**. Free choline can cross the blood–brain barrier and be taken up by presynaptic cholinergic neurons (by the low-affinity transporter) to be incorporated into PC and located in membranes. Choline can be mobilized by phospholipase D to provide free choline for the reaction with acetyl-CoA catalyzed by **choline acetyltransferase**. Because brain choline acetyltransferase is not saturated with either substrate, the availability of choline, and/or acetyl-CoA determines the rate of synthesis of acetylcholine.

Metabolic Functions of Choline

Choline and its derivative have several functions that are essential to normal metabolism and health.

- **Membrane structure**. PC is the major structural component of membranes; therefore, PC is required for membrane biogenesis in growth and cell division. With sphingomyelin it is localized in the outer leaflet of the lipid bilayer, contributing membrane asymmetry, which facilitates transmembrane signal transduction[23] and promotes lipid trafficking. In the lung, palmitate-rich PC is the active component of surfactant. PC is also a precursor to sphingomyelin,[24] the basic structure of membrane sphingolipids. Sphingomyelin comprises 10–20 mol% of the lipids in plasma membranes and is particularly abundant in the myelin sheaths of nerve cell axons. Sphingomyelin is formed by the addition of **ceramide**[25] by **PC–ceramide–phosphocholine transferase**.
- **Cell signaling**. Membrane PC also functions as a precursor of second messengers affecting cellular function. Hydrolysis by phospholipases produce diacylglycerol, an activator of **protein kinase C**, which phosphorylates a large number of target proteins in the cell; arachidonic acid (20:4), which is used in the synthesis of **eicosanoids**; phosphocholine, which is as a signaling molecule in cell division, including in tumorigenesis; free choline for incorporation into **platelet-activating factor**, which is important in clotting, inflammation,[26] uterine ovum implantation, fetal maturation, and induction of labor; free choline for incorporation into **plasmalogen**, which occurs at high levels in the sarcolemma and is important in myocardial function[27]; and free choline for the synthesis of **acetylcholine**, which is thought to act as a signaling molecule in immune cells and placenta.[28]
- **Lipoprotein synthesis**. PC is the major component of the lipid monolayer on the surface of VLDLs. The packaging of those particles occurs in the Golgi cisternae and is necessary for exporting lipids from the liver.
- **Methyl donor**. Choline is a key source of labile methyl groups via its oxidized metabolite betaine. A single methyl group is transferred from betaine to **homocysteine (Hcy)** to produce **dimethylglycine** and **methionine (MET)** by the Zn-containing enzyme **betaine:homocysteine *S*-methyltransferase (BHMT)** in the liver and kidney.[29] BHMT is downregulated by SAM and dimethylglycine. Additional methyl groups are rendered from dimethylglycine through its conversion to **sarcosine** by **dimethylg-**

22. Betaine is transported into cells by the betaine/γ-aminobutyric acid transporter.

23. Phospholipid-mediated signal transduction involves membrane phospholipases that trigger generation of inositol-1,4,5-triphosphate, which acts to release Ca^{2+} from stores in the endoplasmic reticulum.

24. Sphingomyelins can have either PC or phosphatidylethanolamine as the head group; they are found in cell membranes and myelin sheathes of nerve cell axons.

25. Ceramide is formed by adding a fatty acid to the amino group of sphingosine. Among the biological activities of ceramide are the stimulation of *apoptosis*, i.e., programmed cell death.

26. Overproduction of platelet-activating factor has been shown to produce a hyperresponsive condition, as occurs in asthma.

27. It is thought that the adverse effects of myocardial ischemia may involve the breakdown of plasmalogen.

28. Wessler, I., Kirkpatrick, C.J., 2008. Br. J. Pharmacol. 154, 1558–1571.

29. BHMT is among the more abundant proteins in liver, comprising as much as 2% of total soluble protein.

lycine dehydrogenase, and the subsequent conversion of sarcosine to **glycine** by **sarcosine dehydrogenase**. These transmethylation reactions link choline to folate metabolism. When the 5-methyl-FH_4 cannot meet intracellular demands for SAM, choline becomes an important dietary source of labile methyl groups for MET production.

- **Neurotransmission**. Acetylcholine is a key neurotransmitter in both the central and peripheral nervous systems. Through cholinergic neurons, it affects the functions of several organs (e.g., heart, lungs, gut, bladder, pancreas, and endocrine organs). Acetylcholine is also important for cognitive function and the development of the brain; choline deprivation of rat pups produce permanent memory impairment.
- **Bile**. Some 95% of biliary phosphor lipids[30] comprise a PC species that is particularly rich in palmitic (16:0) and linoleic (18:0) acids. These PCs are produced in the hepatic endoplasmic reticulum from circulating HDLs. Most (~95%) PC secreted with bile into the gut is reabsorbed; less than half of that amount returns to the liver, the major portion being used by extrahepatic tissues.
- **Osmoregulation**. The choline metabolites betaine and glyceryl-PC function as **organic osmolytes**. This function is particularly important in the renal cortex and medulla, which are exposed to high extracellular osmolarity as a consequence of concentrating urine for excretion.

These functions have several physiological effects:

- **Neurologic function**. The intake of choline can affect the concentrations of acetylcholine in the brain, suggesting that choline loading may be beneficial to patients with diseases involving deficiencies of cholinergic neurotransmission. Indeed, studies with animal models have shown choline supplementation during development to enhance cognitive performance, particularly, on more difficult tasks; to increase electrophysiological responsiveness; and to provide some protection against alcohol and other neurotoxic agents. A large cohort study found plasma choline level to be inversely associated with the incidence of symptoms of anxiety.[31] In humans, large doses (multiple gram quantities) of choline have been used to increase brain choline concentrations above normal levels, thereby stimulating the synthesis of acetylcholine in nerve terminals. Such supplementation has been found to help in the treatment of tardive dyskinesia, a movement disorder involving inadequate neurotransmission at striatal cholinergic interneurons.[32] Choline supplements have also been used with some success to improve free memory in subjects without dementia[33] and to diminish short-term memory losses associated with Alzheimer's disease,[34] a disorder involving deficiency of hippocampal cholinergic neurons. It has been suggested that autocannibalism of membrane PC may be an underlying defect in that disease; this is supported by the fact that patients treated with anticholinergic drugs develop short-term memory deficits resembling those associated with hippocampal lesions. PC has been reported to reduce manic episodes in patients, suggesting that it can be centrally active; however, such treatment has been found to exacerbate depression among tardive dyskinesia patients. A meta-analysis of 14 controlled trials found that supplementation with CDP–choline improved memory in elderly subjects with cognitive disorders.[35] Dietary choline intake was found to predict cognitive functioning in the Framingham Offspring cohort.[36]
- **Epigenetic effector**. Its contributions (via betaine) to the pool of labile methyl groups mean that the availability of choline can affect the metabolic functions of the single-C pool, particularly under circumstances that would limit that pool, e.g., when intakes of folate, vitamin B_{12}, and/or MET are limiting. Such circumstances can reduce the methylation of DNA and histones, thus modifying genetic expression. This phenomenon has been demonstrated in rodent models[37] and human volunteers.[38]

Dietary Choline in Health and Disease

Anticarcinogenesis. Choline deprivation in animal models promotes both the initiation and promotion phases of spontaneous and chemically induced

30. Phospholipids comprise ~3% of bile.

31. Bjelland, I., Tell, G.S., Vollset, S.E., 2009. Am. J. Clin. Nutr. 90, 1056–1060.

32. Tardive dyskinesia is prevalent among patients treated with neuroleptic drugs (affecting the autonomic nervous system) and is characterized by involuntary movements resembling both *chorea* (irregular and spasmodic) and *athetosis* (slow and writhing) of the face, extremities and, usually, the trunk.

33. Spiers, P., Myers, D., Hochanadel, G.S., et al., 1996. Arch. Neurol. 53, 441–448; Ladd, S.L., Sommer, S.A., LaBerge, S., et al., 1993. Clin. Neuropharmacol. 16, 540–549; Sitaram, N., Weingartner, H., Caine, E.D., et al., 1978. Life Sci. 22, 1555–1560.

34. Alvarez, X.A., Laredo, M., Corzo, D., et al., 1997. Methods Fund. Exp. Clin. Pharmacol. 19, 201–210.

35. Fioravanti, M., Yanagi, M., 2005. Cochrane Database Syst. Rev., CD000269. pub. 3.

36. Poly, C., Massar, J.M., Seshardi, S., et al., 2011. Am. J. Clin. Nutr. 94, 1584–1591.

37. Davison, J.M., Mellott, T.J., Kovacheva, V.P., et al., 2009. J. Biol. Chem. 284, 1982–1989.

38. Shin, W., Yan, J., Abratte, C.M., et al., 2010. J. Nutr. 140, 975–980; Jiang, X., Yan, J., West, A.A., et al., 2012. FASEB J. 26, 3563–3574.

hepatocarcinogenesis. These effects appear to result from decreases in tissue levels of SAM resulting in hypomethylation of DNA and, consequently, changes in gene transcription including modified expression of p53 protein. Epidemiological studies have found choline intake to be positively associated with risk to colorectal and prostate cancer[39]; although other studies have found no significant associations.[40]

Birth defects. Serum choline was inversely associated with risk of neural tube defect pregnancies.[41]

Choline Deficiency

Individuals with insufficient choline biosynthesis or deficiencies in choline or single-C metabolism can show hepatic steatosis, hemorrhagic renal degeneration, and (in animals) depressed growth.

Humans. Choline deficiency is not commonly observed in humans; this may reflect the adequate intakes of other methyl donors in the subjects most frequently studied. In a study in which healthy adults were fed a diet adequate in MET and folate, deprivation of choline produced indication of hepatic dysfunction (increased serum transaminase activities) that was corrected by feeding choline. In another study, chronic use of a choline-free parenteral feeding solution resulted in hepatic steatosis in adults.[42] Two cases have been identified in which hereditary enzyme deficiencies have produced needs for dietary choline: women with the 744C allele for PEMT, which yields that enzyme unresponsive to induction by estrogen[43]; and women with the 1958A allele for 5,10-methylenetetrahydrofolate dehydrogenase.[44] In 1998, the Food and Nutrition Board of the Institute of Medicine set recommended intakes for choline (Table 19.3).

Animals. Choline can be an indispensable dietary constituent for several species. This includes the chick, due to developmental deficiencies of PEMT, has an absolute need for dietary choline until about 13 weeks of age. Until that age, choline deprivation produces fatty liver and perosis, which signs are also see in older poultry fed diets deficient in methyl groups (e.g., MET-deficient). Clear needs for dietary choline have been demonstrated for fish,[45] signs of deficiency including impaired weight gain, reduced efficiency of feed utilization, and hepatic steatosis. It is generally assumed that most fishes cannot synthesize choline at levels sufficient to meet their physiological needs. Rodents require choline only if their capacity to methylate phosphatidylethanolamine is limited by the availability of methyl groups. In such cases, the feeding of methyl donors (MET, betaine) spares the need for choline. Rats fed a choline-deficient diet show 30–40% reductions in hepatic and brain levels of folate, resulting in a shift toward longer folylpolyglutamate metabolites and in the undermethylation of DNA. Pregnancy and lactation result in significant decreases in hepatic choline levels. Betaine can spare the need for choline to support growth and prevent fatty liver, apparently by providing single-C units.

TABLE 19.3 Recommended Choline Intakes

Age–Sex	AI[a], mg/day
0–6 months	125
7–12 months	150
1–3 years	200
4–8 years	250
9–13 years	375
>13 year males	550
14–18 year females	400
>18 year females	425
Pregnancy	450
Lactation	550

[a]*Adequate intakes are given, as RDAs have not been established.*
Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.

Biomarkers of Choline Status

There are no satisfactory biomarkers of choline status. Plasma concentrations of free choline are regulated in the range of 6–13 μM[46] and are only minimally responsive to changes in choline intake. Urinary choline represents only 2% of the amount recently consumed.

39. Johansson, M., Van Guelpen, B., Vollset, S.E., et al., 2009. Cancer Epidemiol. Biomarkers Prev. 18, 1538–1543; Lee, J.E. Giovanussi, E., Fuchs, C.S., et al., 2010. Cancer Epidemiol. Biomarkers Prev. 19, 884–887.
40. Cho, E., Willett, W.C., Colditz, G.A., et al., 2007. J. Nat. Cancer. Inst. 99, 1224–1231; Cho, E., Homes, M.D., Hankinson, et al., 2010. Br. J. Cancer 102, 489–494.
41. Shaw, G.M., Finell, R.H., Blom, H.J., et al., 2009. Epidemiol. 20, 714–719.
42. Buchman, A.L., Ament, M.E., Sohel, M., et al., 2001. J. Parenteral Enteral Nutr. 25, 260–268.
43. da Costa, K.A., Kozyreva, O.G., Song, J., 2006. FASEB J. 20, 1336–1344.
44. Kohlmeier, M., da Costa K.A., Fischer, L.M., et al., 2005. Proc. Natl. Acad. Sci. U.S.A. 102, 16025–16030.
45. For example, red drum (*Sciaenops ocellatus*), striped bass (*Morone* spp.).
46. Abratte, C.M., Wang, W., Li, R., et al., 2009. J. Nutr. Biochem. 20, 62–69.

TABLE 19.4 Recommended Tolerable Upper Limits of Choline Intake

Age–Sex	UL[a], mg/day
0–12 months	–
1–8 years	1000
9–13 years	2000
>13 year males	3000
14–18 year females	3000
>18 year females	3500
Pregnancy	
<18	3000
≥18	3500
Lactation	
<18	3000
≥18	3500

[a]*Food and Nutrition Board, 2000. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin and Choline. National Academy Press, Washington, DC, 564 pp.*

Choline Toxicity

The toxicity of choline is low. However, deleterious effects have been reported for the salt choline chloride: growth depression, impaired utilization of vitamin B_6, and increased mortality. These effects may not relate to choline but, instead, to the perturbation of acid–base balance caused by the high level of chloride administered with large doses of that salt. In humans, high doses (e.g., 20 g) have produced dizziness, nausea, and diarrhea. Upper limits of choline intake have been established (Table 19.4).

3. CARNITINE

Carnitine is a normal metabolite that functions in the transport of long-chain fatty acids from the cytosol into the mitochondria for their oxidation as sources of energy. It is biosynthesized by most species; however, some species are not capable of its biosynthesis and some circumstances limit its biosynthesis for others, making it **conditionally essential**.

Recognition of a Nutritional Role of Carnitine

In the 1950s, Fraenkel[47] and colleagues found that the successful growth of the yellow mealworm (*Tenebrio molitor*) required the feeding of a natural substance, which they found present in milk, yeast, and many animal tissues. They purified the growth factor from whey solids and named it "**vitamin B_T**." Soon vitamin B_T was found to be **carnitine**, a known metabolite that had been identified in extracts of mammalian muscle five decades earlier. The first indication of a metabolic role came in the 1960s, when carnitine was found to stimulate the in vitro oxidation of long-chain fatty acids by subcellular fractions of heart muscle.[48] Research interest in carnitine was stimulated by the finding that carnitine, it is biosynthesized by mammals from the amino acid lysine,[49] which is limiting in the diets of many of the world's poor, and by the description of clinical syndromes (of apparently genetic origin) associated with carnitine deficiency.

Conditions of Need for Dietary Carnitine

Most species can synthesize carnitine at rates sufficient for their needs. In humans, this is indicated by the fact that subjects whose cereal-based diets provide very little preformed carnitine show plasma carnitine concentrations comparable to those of whose diets provide abundant amounts of the factor.[50] However, some circumstances can limit either the biosynthesis or utilization.

Humans. **Neonates** have compromised carnitine biosynthetic capacity due to very low hepatic γ-butyrobetaine hydroxylase activities. Their carnitine status is dependent on that of the mother, on the placental transfer of carnitine in utero, and on the availability of exogenous sources after birth.[51] Appreciable amounts of carnitine are found in the milk of several species.[52] Infants fed soy-based formulas, which contain little or no carnitine, are unable to maintain normal plasma carnitine levels; intravenous administration of L-carnitine allows them to do so.

Because carnitine is a key cofactor for energy metabolism, preterm infants can be at special risk. While their plasma carnitine levels tend to be nearly normal, their stores can be depleted rapidly during the course of intravenous

47. Gottfried S. Fraenkel (1901–84) was a German-born insect physiologist who, after fleeing Germany in 1933, spent most of his career as a Professor of Entomology at the University of Illinois. In 1968, he was named to the National Academy of Sciences.

48. Fritz, I.B., Yue, K.T.N., 1963. J. Lipid Res. 4, 279–288.

49. Tanphaichtr, V., Horne, D.W., Broquist, H.P., 1971. J. Biol. Chem. 246, 6364–6366.

50. Mean ± SD: men, 59 ± 12 μM (n = 40); women, 52 ± 12 μM (n = 45).

51. Examples are human milk, prepared infant formulas and milk replacers. It has been suggested that natural selection has resulted in mother's milk containing carnitine in proportion to the needs of the infant. In fact, the greatest concentrations of carnitine in human milk occur during the first 2–3 days of suckling. During the first 3 weeks of lactation, the carnitine content of human milk varies from 50 to 70 nmol/mL; after that time, it declines to about 35 nmol/mL by 6–8 weeks. Most milk-based infant formulas contain comparable or slightly greater amounts of carnitine; however, formulas based on soybean protein or casein and casein hydrolysate contain little or no carnitine. Lipid emulsions also contain no appreciable carnitine.

52. e.g., Human milk: 28–95 nmol/mL; cow's milk: 190–270 nmol/mL.

feeding with solutions unsupplemented with carnitine. One study found the plasma carnitine concentrations of preterm infants (gestational age <36 weeks) to drop from 29 to 13 μM during total parenteral feeding. The consequences of suboptimal carnitine status are direct, as newborns change from utilizing glucose to fats as the major fuel.[53] Thus, free fatty acids are the preferred metabolic fuels for the newborn, especially for the heart and skeletal muscle, which depend on the oxidation of fatty acids for more than half of their total energy metabolism, when glucose availability is limited. Neonates fed noncarnitine-fortified soy-based formula diets have shown, with 2 weeks, reduced hepatic carnitine concentrations with associated reductions in hepatic fatty acid oxidation and ketogenesis and hypertriglyceridemia. The long-term consequences of these reductions are unknown and no clinical signs of carnitine deficiency in infants have been described.

Individuals with low muscle and/or plasma carnitine levels typically show lipid accumulation in muscle with high risk of encephalopathy, progressive muscular weakness, and cardiomyopathy. Carnitine deficiency has also been recognized as a secondary feature of various other genetic disorders, e.g., organic acidurias[54] and Fanconi syndrome,[55] in which the renal tubular loss of total carnitine and of acylcarnitine esters in particular are elevated.

Other mammals and birds. While most species studied to date appear to synthesize carnitine at rates sufficient for their needs, the biosynthetic capacity of the rat can be impaired by deprivation of its metabolic precursors, lysine and/or methionine.[56] Carnitine biosynthetic capacities may also be limited in the fetus, as carnitine supplementation of pregnant and lactating sows has been found to enhance fetal glucose oxidation and result in higher birth weights and improved growth.[57] Neonatal rabbits fed a carnitine-free colostrum replacer or a carnitine-free weaning diet showed abnormally low-tissue and urinary carnitine levels, decreased plasma total and VLDL–cholesterol levels, and increased apolipoprotein levels. Carnitine supplementation of the diets of turkey hens was found to improve egg fertility, embryonic survival, and chick growth.[58]

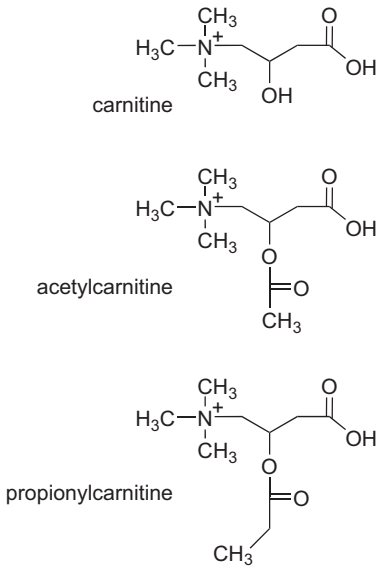


FIGURE 19.3 Carnitine and functional metabolites.

Insects. Carnitine is essential in the diets of at least some insect species. This includes beetles of the family *Tenebrionidae*,[59] the beetle *Oryzaephilus surinamensis*, and the fruit fly *Drosophila melanogaster*. It is presumed that carnitine plays the same essential role in the metabolism of fatty acids in insects that it does in mammals, and that their special dietary need is due to their inability to synthesize it from endogenous sources; however, that has not been investigated. For these species, carnitine is a vitamin.[60]

Conditions producing need for dietary carnitine are as follows:

- Limited biosynthesis in neonates
- Absent biosynthesis/transport in some insects.

Chemical Properties of Carnitine

Carnitine is the generic term for a number of compounds including L-carnitine (β[-]-β-hydroxy-γ-[*N,N,N*-trimeth ylaminobutyrate][61]) and its acetyl and propionyl esters (Fig. 19.3). Only the L-isomer is made by and is biologically active for eukaryotes. At physiological pH, carnitine

53. At birth, plasma-free fatty acids and β-hydroxybutyrate concentrations are rapidly elevated owing to the mobilization of fat from adipose tissue. These elevated levels are maintained by the utilization of high-fat diets such as human milk and many infant formulas, which typically contain more than 40% of total calories as lipid.

54. Examples include isovaleric, glutaric, propionic, and methylmalonic acidemias, which result from long- and medium-chain acyl-CoA dehydrogenase deficiencies.

55. Fanconi syndrome is a renal disease characterized by the excessive renal excretion of a number of metabolites that are normally reabsorbed (e.g., amino acids).

56. Some 0.1% of the lysine required by the rat is used to make carnitine; rats deprived of lysine develop mildly reduced tissue carnitine levels, depressed growth, and fatty liver (Eder, K., 2009. Br. J. Nutr. 102, 645–654).

57. Ramanau, A., Kluge, H., Eder, K., 2005. Br. J. Nutr. 93, 717–721; Eder, K., 2009. Br. J. Nutr. 102, 645–654.

58. Oso, A.O., Fafiolu, A.O., Adeleke, M.A., et al., 2014. J. Anim. Physiol. Anim. Nutr. 98, 766–774.

59. A family of mealworms.

60. Carnitine is an amino acid, but because it has no role in protein synthesis it meets the criteria of vitamin status (see Chapter 1).

61. Molecular weight, 161.5.

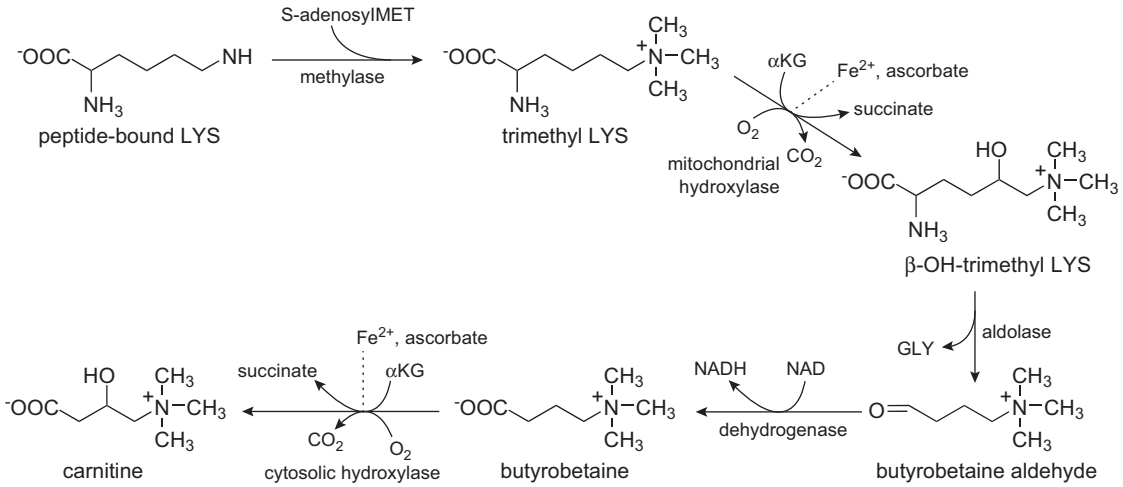


FIGURE 19.4 Biosynthesis of carnitine. *αKG*, α-ketoglutarate; *LYS*, lysine; *GLY*, glycine.

exists as a zwitterion,[62] with a positively charged quaternary amine and a negatively charged carboxyl. It forms esters with fatty acids by virtue of its hydroxyl.

Sources of Carnitine

Biosynthesis. Carnitine is synthesized in mammals. Humans are estimated to synthesize carnitine at ~1.2 μmol per kg body weight.[63] The synthesis starts with the post-translational modification of protein lysyl residues, some of which are thrice methylated to form ε-*N*-trimethyllysine using SAM as the methyl donor. This process is thought to occur in many proteins,[64] the turnover of which releases ε-*N*-trimethyllysine to be converted to L-carnitine through a sequence of reactions catalyzed by two Fe^{2+}- and ascorbate-dependent hydroxylases, a pyridoxal phosphate-dependent aldose, and an NAD^+-dependent dehydrogenase (Fig. 19.4). The first three of these enzymes occur in all tissues; but the last step, **γ-butyrobetaine hydroxylase**, occurs only in liver, kidney, and brain where it is present as multiple isoforms. It activity increases during development, peaking in the mid-teens.[65] This step does not, however, yield the quantitative conversion of γ-butyrobetaine to carnitine; therefore, the precursor is normally found in the urine. Tissue carnitine levels are depressed under conditions of vitamin B_6 deprivation and stimulated by the catabolic state (e.g., fasting), thyroid hormone, and the peroxisome proliferator clofibrate.[66]

Dietary sources. The available data concerning the carnitine contents of foods are scant and must be considered suspect, owing to the use of nonstandard analytical methods. Nevertheless, it is apparent that materials of plant origin tend to be low in carnitine, whereas those derived from animals tend to be rich in the factor (Table 19.5). Red meats and dairy products are particularly rich sources. Typical mixed diets can be expected to provide 1–16 μg carnitine per day, the actual amount depending mainly on the intake of meats.

Absorption and Transport of Carnitine

Absorption. Carnitine is absorbed across the small intestine in two ways: by an active process dependent on Na^+ cotransport; and by a passive, diffusional process that may be important for the absorption of large doses of the factor. The efficiency of absorption appears to be high, ca. 55–95%, although high doses are absorbed at lower efficiencies (≤25%), with <1% appearing in the urine and very little appearing in the feces. The uptake of carnitine from the intestinal lumen into the mucosa is rapid, and about half of that taken up is acetylated in that tissue.

Transport. Carnitine is released slowly from tissues into the plasma in both the free and acetylated forms in simple solution. Plasma total carnitine concentrations in healthy adults are 30–89 μ*M*, with men typically showing slightly greater (by ~15%) concentrations than women. Carnitine is taken up against concentration gradients by peripheral tissues, most of which can also synthesize it.

Tissue distribution. Cellular uptake of carnitine and its short-chain acyl esters is facilitated by Na^+-dependent organic cation transporters (OCTNs). A high-affinity transporter (OCTN2) is expressed in kidney, skeletal muscle, heart, pancreas, testis, and placenta but is notably low in liver, brain, lung, and colon. The transporter in the colon

62. i.e., A molecule with a positive and a negative electrical charge at different locations.
63. Rebouche, C.J., 1992. FASEB J. 6, 3379–3386.
64. e.g., Actin, myosin, ATP synthase, calmodulin, and histones.
65. For example, the hepatic γ-butyrobetaine hydroxylase activities of three infants and a 2.5-year-old boy were 12% and 30%, respectively, of the mean adult activity.
66. This effect appears to involve the peroxisome proliferator-activated nuclear receptor α (PPARα).

TABLE 19.5 Carnitine in Selected Foods and Feedstuffs

Food	Carnitine,[a] μg/100 g
Vegetables	
Avocado	1.25
Cauliflower	0.13
Alfalfa	2.00
Peanut	0.76
Cereals	
Wheat	0.35–1.22
Bread	0.24
Meats	
Beef	59.8–67.4
Beef liver	2.6
Beef kidney	1.8
Beef heart	19.3
Chicken	4.6–9.1
Lamb, muscle	78.0
Other	
Cow's milk	0.53–3.91
Casein, acid washed	0.4
Torula yeast	1.60–3.29

[a]*None detected in cabbage, spinach, orange juice, barley, corn, egg.*
Adapted from Mitchell, M., 1978. Am. J. Clin. Nutr. 31, 293–306.

may serve in the uptake of any nonabsorbed dietary carnitine as well as β-butyrobetaine produced by the hindgut microbiome. Subjects with inborn errors in OCTN2 have been identified; they readily develop signs of carnitine deficiency unless they are given supplemental carnitine. A high-affinity, OTCN-related transporter, CT-2, is expressed only in testes. Short-chain carnitine derivatives are better utilized than the parent molecule by some tissues. Acetylcarnitine, which is structurally similar to acetylcholine, crosses the blood–brain barrier more readily than carnitine whereupon it is readily converted to carnitine. Propionylcarnitine, which is lipophilic, has high affinities for skeletal and cardiac muscles. Total body carnitine occurs in nonesterified (80–90%) and esterified (10–20%) forms. The greatest concentrations of carnitine in the human body are found in epididymal tissue, seminal plasma, and sperm.

Genetic disorders of carnitine transport. An autosomal, recessive disorder involving defective transport of carnitine has been described, **primary carnitine deficiency**.[67] In involves mutations in the *SLC22A5* gene that encodes OCTN2. Defects in the transporter result in reduced cellular uptake of carnitine and its consequent wastage in the urine. This impairs fatty acid oxidation, reduces ketogenesis, and increases cytosolic accumulation of long-chain fatty acids. The clinical manifestations can include hypoglycemia, hepatomegaly, weakness, and cardiomyopathy.[68]

Carnitine Metabolism

Turnover. Total body carnitine turns over every 66 days. However, that total is comprised of three kinetically distinct tissue pools.[69] In humans, 92–97% of body carnitine occurs in muscle, where it turns over relatively slowly (~191 h), but has relatively rapid flux (~427 μmol/h) due to the size of this pool. Liver and kidney contain 2–5% of body carnitine; this pool turns over in 12 h with a flux of ~277 μmol/h. Extracellular fluids contain 0.7–1.5% of body carnitine; this pool turns over quickly, e.g., 1.1 h. Carnitine is not catabolized in the tissues, which is released in the bile, it can be degraded by hindgut microbiome to γ-butyrobetaine, trimethylamine, and malic semialdehyde which appear in the feces. Trimethylamine can be absorbed across the colon to be oxidized to trimethylamine oxide by the liver.

Excretion. Carnitine is highly conserved by the human kidney, which reabsorbs >90% of filtered carnitine, being the dominant means of regulating plasma carnitine concentration. Renal reabsorption is facilitated by a brush border transporter, OTCN2, which recovers carnitine as well as its short-chain acyl esters. Renal tubular excretion of carnitine, its short-chain acyl esters, and γ-butyrobetaine adapt to circulating carnitine concentrations; some of this may come from the renal secretion of carnitine either in free form or as short-chain acylcarnitine esters. That urinary carnitine is typically comprised of a higher proportion of acylcarnitine esters than the general circulation suggests selective secretion of carnitine esters by renal tubular cells. Trimethylamine can be metabolized in the liver to yield trimethylamine oxide, which is also excreted in the urine.

Metabolic Functions of Carnitine

Mitochondrial fatty acid shuttle. Carnitine functions in the transport of long-chain fatty acids (fatty acyl-CoA) from the cytosol into the mitochondrial matrix for oxidation as sources of energy (Fig. 19.5). The mitochondrial inner membrane is impermeable to long-chain fatty acids and their CoA esters, which are therefore dependent on activation as carnitine esters for entry into that organelle. This transport process, referred to as the **carnitine transport**

67. Also called "carnitine uptake defect," the incidence is ~1:40,000 with a carrier rate of ~1%.

68. Fu, L., Huang, M., Chen, S., 2013. Korean Circ. 43, 785–792.
69. Rebouche, C.J., Engle, A.G., 1984. J. Clin. Invest. 73, 857–867.

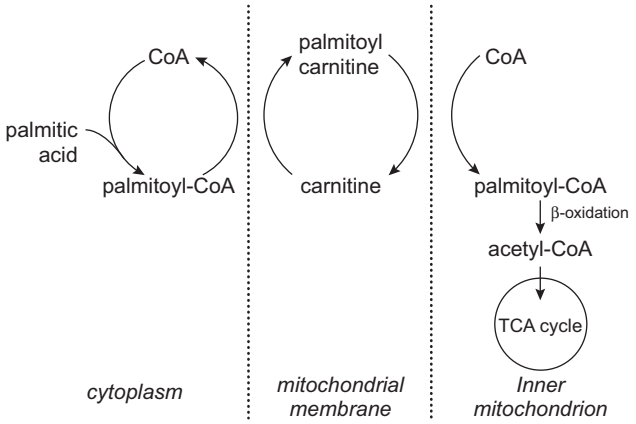


FIGURE 19.5 The mitochondrial fatty acid shuttle.

shuttle, is effected by two transesterifications involving fatty acyl-CoA esters and carnitine, and the action of three mitochondrial enzymes: **carnitine–acyltransferases I** and **II (CATI** and **CATII)** and **carnitine–acylcarnitine translocase (CACT)**.

CATI resides on the outer side of the inner mitochondrial membrane, while CATII is located on the matrix side and acylcarnitine translocase spans the inner membrane. These enzymes catalyze the formation and hydrolysis of fatty acylcarnitine esters.[70] The CATs provide an outlet for acetyl-CoA when the production of short-chain acyl-CoAs exceeds their rate of use by the TCA cycle, as in the transitions from fasting to feeding or from rest to vigorous physical activity, as well as in the overfed state.[71] Their activities in tissues are inhibited by accumulation of lipids, including palmitoyl-CoA, as in obesity.[72]

CACT catalyzes the exchange of carnitine and acylcarnitines produced by CATs across the membrane. The result of the concerted action of these enzymes is that long-chain fatty acids are brought into the mitochondrion by esterification to carnitine and transported as fatty acylcarnitine esters, after which carnitine is released and returned to the outer side of the membrane, thus, rendering the free fatty acid available for β-oxidation within the mitochondrion. It has been suggested that the carnitine transport shuttle may also function in the reverse direction by transporting acetyl groups back to the cytoplasm for fatty acid synthesis.[73]

Under normal metabolic conditions, it appears that short-chain acyl-CoAs are generated at rates comparable to the rates of their use, such that acylcarnitine does not accumulate. However, under conditions of propionic acidemia or methylmalonic academia/aciduria, which occur in vitamin B_{12} deficiency, the urinary excretion of acylcarnitine is enhanced owing to the increased formation of short-chain acylcarnitines.

The activity of the carnitine transport shuttle is typically low at birth but increases dramatically after birth. For example, the CATI activity in rat liver increases nearly fivefold within 24 h of birth, peaking within 2–3 days. Similar increases in the hepatic activities of the CATI and CATII have been observed in human infants. That these increases correspond to the development of fatty acid oxidation in the heart, liver, and adipose tissue suggests that the carnitine transport shuttle is rate limiting to that process.

Genetic disorders of carnitine metabolism. Genetic deficiencies have been described in both CATI and CATII. These deficiencies are rare; each involves impaired oxidation of long-chain fatty acids. CATI[74] deficiency is due to a single nucleotide variation in the enzyme. Clinical signs are typically seen in children, presenting as hepatic encephalopathy under conditions of increased energy demands are increased, e.g., in febrile illness.[75] At least 90 nucleotide changes have been identified in CATII; one variant accounts for some 60% of all mutant alleles. Those of CATII deficiency[76] present from infancy to adulthood in three multisystem diseases: a lethal neonatal form, a severe infantile hepatocardiomuscular form, and a mild myopathic form.[77]

Other functions. Carnitine and its esters have other biological actions:

- **Peroxisomal fatty acid shuttle**—Chain-shortened fatty acyl-CoAs are transesterified to carnitine within peroxisomes from which they are transported by OCTN3 into the cytoplasm for mitochondrial uptake.
- **Glucocorticoid-like actions**—The ability of carnitine to bind the glucocorticoid receptor-α has been related to its glucocorticoid-like effects in causing receptor-mediated release of cytokines and maintaining urea cycle activity to attenuate hyperammonemia.[78]
- **Phospholipid remodeling**—Carnitine-bound fatty acids are incorporated into erythrocyte membrane phospholipids during repair after oxidative attack, as well as into the lung surfactant dipalmitoylphosphatidylcholine.

70. The carnitine acyltransferases are actually a family of six related enzymes with different, but overlapping, chain length specificities that have been isolated from mitochondria (three each from the inner and outer sides of the inner membrane).
71. Muoio, D.M., Noland, R.C., Kovalik, J.P., et al., 2012. Cell Metab. 15, 764–777.
72. Seiler, S.E., Martin, O.J., Noland, R.C., et al., 2014. J. Lipid Res. 55, 635–644.
73. Even if such a reverse shuttle were to function, its contribution to fatty acid synthesis would be insignificant in comparison with that of the citrate shuttle, which transports acetyl-CoA to the cytoplasm by the action of a citrate cleavage enzyme.
74. Fewer than 60 cases are known, mostly in the native Alaskan population where the frequency of the variant is estimated to be 1.3/1000 live births.
75. Bennett, M.J., Santini, A.B., 2013. GeneReview NCI Bookshelf.
76. Wieser, T., 2014. GeneReview NCI Bookshelf.
77. These phenotypes have been described in 28, 28, and 300 families, respectively.
78. Manoli, I., De Martino, M.U., Kino, T., et al., 2004. Ann. N.Y. Acad. Sci. 1033, 147–157.

- **Insulin sensitivity**—Tissue carnitine levels, regulated through local expression of CATI and CACT, affect mitochondrial levels of acyl-CoA substrates control of which has been linked to insulin sensitivity.[79]
- **Cardiac function**—Carnitine stabilizes the cardiac mitochondrial membrane, protecting it from permeability transition in the presence of high-fatty acid β-oxidation.[80]
- **Thyroid function**—Carnitine appears to be a peripheral agonist of thyroid hormone. A randomized, controlled trial found administration of carnitine effective in reversing symptoms of hyperthyroidism.[81]

Dietary Carnitine in Health and Disease

Hepatic function. Hypocarnitinemia (plasma concentrations <55 μM) and tissue carnitine depletion appear to be common in patients with advanced cirrhosis, who not only tend to have marginal intakes of carnitine and its precursors but also have diminished capacity to synthesize carnitine. Carnitine supplementation has been found to protect against ammonia-induced encephalopathy in cirrhotics.[82]

Renal function. Patients with renal disease managed with chronic hemodialysis can be depleted of carnitine[83] due to losses in the dialysate that greatly exceed the amounts normally lost in the urine.[84] Tissue depletion of carnitine has been related to the complications of hemodialysis: hyperlipidemia, cardiomyopathy, skeletal muscle asthenia, and cramps. A meta-analysis of 49 randomized clinical trials found that carnitine treatment of dialysis patients with end-stage renal disease reduced serum concentrations of LDL and C-reactive protein (CRP)[85].

Cardiovascular health. Studies with animal models have shown that carnitine supplementation can benefit cardiac function.[86] Administration of propionylcarnitine improved cardiac function, as well as the functional recovery of the myocardium after ischemia.[87] Treatment with propionylcarnitine had a prostacyclin-like affect in countering vasoconstrictor activity, promoted endothelium-dependent arterial dilation in hypertensive individuals, and had antihyperlipidemic and antiatherosclerotic effects. Rats supplemented with carnitine showed prostaglandin responses associated with cardioprotection (i.e., reduced ratios of 6-keto-prostaglandin $F_{1\alpha}$ to thromboxane B_2 and leukotriene B_4) and reduced myocardial injury after ischemia/reperfusion.

Randomized, controlled trials with cardiac patients have found carnitine treatment to reduce hypertension, enhance vascular function, reduce left ventrical dilatation, and prevent ventricular remodeling. A multicenter, randomized trial found propionylcarnitine to enhance the exercise capacity of chronic heart failure patients with relatively intact myocardial function.[88]

Neurologic health. Studies have found that supplementation with carnitine can restore mitochondrial function in aging animals, which typically declines due to changes that include diminished expression of CACT.[89] Treatment with acetylcarnitine has been shown to reduce memory loss in old rats, the effect was associated with the release of acetylcholine in the striatum and hippocampus.

A multicenter, randomized, clinical intervention trial with Alzheimer's disease patients found attenuated progression for several parameters of behavior, disability, and cognitive performance.[90] Trials with Alzheimer's disease patients have found acetylcarnitine treatment to reduce deterioration in reaction time, reduce depressive symptoms, and improve cognitive performance.[91] A meta-analysis of 21 studies with patients with mild cognitive impairment or mild Alzheimer's disease concluded that treatment with acylcarnitine (1.5–2 g/day) improved both clinical and psychomotor assessments.[92] A randomized clinical trial found acylcarnitine to be as effective as, but better tolerated than, the antidepressive drug amisulpride in reducing depressive symptoms in dysthymia patients.[93] Studies have found carnitine supplementation effective in improving adaptive behavior and socialization skills, and reducing attention problems in subjects with attention deficient/hyperactivity

79. Noland, R.C., Koves, T.R., Seiler, S.E., et al., 2009. J. Biol. Chem. 284, 22840–22852.
80. Oyangi, E., Yano, H., Uchida, M., et al., 2011. Biochem. Biophys. Res. Commun. 412, 61–67.
81. Benvenga, S., Amato, A., Calvani, M., et al., 2004. Ann. N.Y. Acad. Sci. 1033, 158–167.
82. Malaguarnera, M., Pistone, G., Elvira, R., et al., 2005. World J. Gastroenterol. 11, 7197–7202.
83. In one study, the muscle carnitine concentrations of eight patients after hemodialysis was only 10% of that of healthy controls. It is of interest, however, that not all hemodialysis patients experience carnitine depletion. Some show chronic hypocarnitinemia, whereas others show a return of plasma carnitine concentrations to normal or higher than normal within about 6 h after dialysis. The recovery of the latter group is hastened (to about 2 h) if each patient is given 3 g of D,L-carnitine orally at the end of the dialysis period.
84. This can be prevented by adding carnitine to the dialysate, e.g., 65 μM.
85. Chen, Y., Abbate, M., Tang, L., et al., 2014. Am. J. Clin. Nutr. 99, 408–422.
86. Ferrari, R., Merli, E., Cicchitelli, G., et al., 2004. Ann. N.Y. Acad. Sci. 1033, 79–91.
87. Lango, R., Smoleński, R.T., Rogowski, J., et al., 2005. Cardiovasc. Drug Ther. 19, 267–275.
88. i.e., Ejection fractions of 30–40%; anonymous, 1999. Eur. Heart J. 20, 70–77.
89. Ames, B.N., Liu, J., 2004. Ann. N.Y. Acad. Sci. 1033, 108–116.
90. Spagnoli A., Lucca, U., Menasce, G., et al., 1991. Neurology 41, 1726–1732.
91. Rai, G., Wright, G. and Scott, L., et al., 1990. Curr. Med. Res. Opin. 11, 638–647.
92. Montgomery, S.A., Thal, L.J., Amrein, R., 2003. Int. Clin. Psychopharmacol. 18, 61–71.
93. Zanardi, R., Smeraldi, E., 2006. Eur. Neuropsychopharmacol. 16, 281–287.

disorder and fragile X syndrome.[94] A randomized trial found supplemental carnitine to reduce excessive daytime sleepiness in patients with narcolepsy.[95]

Exercise performance. It has been suggested that carnitine supplementation may improve exercise performance (i.e., acting as an ergogenic aid) and attenuate the deleterious effects of hypoxic training to hasten recovery from strenuous exercise. Implicit in such suggestions is the notion that carnitine supply may be rate limiting to fatty acid oxidation in muscle, a phenomenon demonstrated only when muscle carnitine drops to less than half normal levels due to the low K_m of CATI. A review of the published literature found no evidence that carnitine supplements can improve athletic performance.[96] A few studies, however, have reported modest improvements in exercise-induced muscle lactate accumulation in resistance-trained athletes,[97] and muscle soreness in nontrained subjects.[98]

Weight management. Some, but not all, studies with animals have found supplementation with carnitine or propionlycarnitine to enhance the loss of body fat under conditions of negative energy balance.[99] Controlled trials with humans, however, have not found such effects.

Diabetes. Reduction in the carnitine-dependent transport of fatty acids into the mitochondria results in cytosolic triglyceride accumulation and has been implicated in the development of insulin resistance. Prediabetic subjects and those with T2D show increased circulating levels of several acylcarnitines.[100] Studies with animal models have suggested that carnitine status can affect the control of glycolysis and/or gluconeogenesis; carnitine supplements have been found to stimulate the insulin-mediated disposal of glucose and confer protection of vascular function in insulin resistance.[101] Studies in the rat have shown experimentally induced diabetes to result in depletion of lens carnitine and acetylcarnitine, and carnitine supplementation to reduce the development of cataracts.

A clinical trial found carnitine to reduce fasting plasma glucose levels and increase fasting triglycerides in patients with T2D.[102] Intramuscular acetylcarnitine reduced pain in patients with diabetic neuropathy and in T2D patients with poorly controlled blood glucose.[103] Intravenous L-carnitine has been shown to improve insulin sensitivity,[104] and oral L-carnitine was found to reduce glycation end products in the skin of hemodialysis patients.[105]

Male reproductive health. Epididymal tissue and spermatozoa typically contain high amounts of carnitine. Studies in both rodent models and humans have related carnitine levels to sperm count, motility, and maturation, and have suggested that carnitine supplementation can improve sperm quality.[106] Two randomized trials found treatment with carnitine and/or acylcarnitine to improve sperm motility in low-fertility males. Propionylcarnitine was found to enhance the efficacy of the drug sildenafil in treating erectile dysfunction in diabetic patients and in postprostatectomy patients.[107]

Biomarkers of Carnitine Status

Carnitine status can be assessed on the basis of the concentration of carnitine in muscle or plasma/serum. Plasma carnitine levels are subject to several effects. They are reduced in individuals with a single-nucleotide polymorphism in medium-chain acyl-CoA dehydrogenase, a mitochondrial enzyme that catalyzes fatty acid β-oxidation,[108] protein malnutrition (Table 19.6), and have been noted in children with schistosomiasis and associated anemia. As Fe^{2+} is required in two steps in carnitine biosynthesis (ε-*N*-trimethyllysine hydroxylase, γ-butyrobetaine hydroxylase), it is possible that deficiencies of iron may reduce carnitine biosynthesis.

Carnitine Safety

A systematic review of published literature concluded that evidence of safety is strong for carnitine intakes as great

94. Torrioli, M.G., Vernacotola, S., Mariotti, P., et al., 1999. Am. J. Med. Genet. 87, 366–368; Van Oudheusden, L.J., Scholte, H.R., 2002. Prostaglandins Leukotrienes Essent. Fatty Acids 67, 33–38; Torrioli, M.G., Vernacotola, S., Peruzzi, L., et al., 2008. Am. J. Med. Genet. 146, 803–812.
95. Miyagawa, T., Kawamura, H., Obuchi, M., et al., 2013. PLOS One 8, e53707.
96. Brass, E.P., 2004. Ann. N.Y. Acad. Sci. 1033, 67–78.
97. Jacobs, P.L., Goldstein, E.R., Blackburn, W., et al., 2009. J. Int. Soc. Sports Nutr. 6, 9.
98. Spierling, B.A., Kraemer, W.J., Vingren, J.L., et al., 2007. J. Strength Cond. Res. 21, 259–264.; Ho, J.Y., Kraemer, W.J., Volek, J.S., et al., 2010. Metabolism 886, 223–230.
99. Heo, K., Odle, J., Han, I.K., et al., 2000. J. Nutr. 130, 1809–1814; Mingorance, C., del Pozo, M.G., Herra, M.D., et al., 2009. Br. J. Nutr. 102, 1145–1153; Schmengler, U., Ungru, J., Boston, R., et al., 2013. Livestock Sci. 155, 301–307.
100. Particularly, tetradecenoylcarnitine (C14:1), tetradecadienylcarnitine (C14:2), octadecenoylcarnitine (C18:1), and malonylcarnitine/hydroxylbutyrylcarnitine (C3DC+C4OH). Mai, M., Tönjes, A., Kovacs, P., et al., 2013. PLoS One 12, e382459.
101. Mingorance, C., del Pozo, M.G., Herra, M.D., et al., 2009. Br. J. Nutr. 102, 1145–1153.
102. Rahbar, A.R., Shakerhosseini, R., Saadat, N., et al., 2005. Eur. J. Clin. Nutr. 59, 592–596.
103. DeGrandis, D., Minardi, C., 2002. Drugs R. D. 3, 223; Sima, A.A., Calvani, M., Mehra, M., et al., 2005. Diabetes Care 28, 89–94.
104. Giancaterini, A., De Gaetano, A., Mingrone, G., et al., 2000. Metabolism 49, 704–708.
105. Fukami, K., Yamagishi, S.I., Sakai, K., et al., 2013. Rejuvenation Res. 16, 460–466.
106. Ng, C.M., Blackman, M.R., Wang, C., et al., 2004. Ann. N.Y. Acad. Sci. 1033, 177–188.
107. Gentile, V., Vicini, P., Prigiotti, G., et al., 2004. Curr. Med. Res. Opin. 20, 1377–1384; Cavalini, G., Modenini, F., Vitali, G., et al., 2005. Urology 66, 1080–1085.
108. Couce, M.L., Sánchez-Pintos, P., Diogo, L., et al., 2013. Orphanet J. Rare Dis. 8, 102–108.

TABLE 19.6 Apparent Carnitine Deficiency in Protein-Malnourished Children

Group	Plasma Carnitine, µmol/dL	Plasma Albumin, g/dL
Healthy controls	9.0 ± 0.6 (8)[a]	3.5 ± 0.1 (8)
Undernourished patients	6.4 ± 0.9 (10)	2.7 ± 0.2 (5)
Marasmus patients	3.7 ± 0.5 (12)	2.7 ± 0.2 (8)
Kwashiorkor patients	2.6 ± 0.5 (13)	1.7 ± 0.1 (9)

[a]*Mean ± SD for (n) children.*
Adapted from Khan, L., Bamji, M.S., 1977. Clin. Chim. Acta 75, 163–166.

as 2000 mg/day on a chronic basis, although studies using higher levels have not observed adverse effects.[109]

4. *MYO*-INOSITOL

Myo-inositol is a normal metabolite that, as **phosphatidylinositol (PI)**, affects membrane structure and function, supporting the production of eicosanoids and cell signaling in the regulation of metabolism. *Myo*-inositol is biosynthesized by most species; however, some species are not capable of its biosynthesis, and dietary shortages of other vitamins may also limit its biosynthesis, making it **conditionally essential**.

Recognition of a Nutritional Role of *Myo*-Inositol

Although *myo*-inositol had been discovered in extracts of animal tissues almost 100 years earlier, interest in its potential nutritional role first occurred in 1940 when it was reported to be a new vitamin required for normal growth, hair and skin of the mouse, i.e., the "mouse antialopecia factor."[110] That original report was later questioned regarding the adequacy of the diet with respect to other known vitamins. Nevertheless, several groups found dietary supplements of *myo*-inositol to stimulate the growth of several species (chicks, turkeys, rats, mice) in ways dependent on shortages of other factors including pantothenic acid, biotin, and folate. Whether the observed effects were actually responses to a missing nutrient was debated. *Myo*-inositol was found to be essential for the growth of most cells in culture. However, it had been found earlier that the daily urinary excretion of *myo*-inositol by the rat exceeded the amount ingested, suggesting the factor was biosynthesized.[111] More recently, deprivation of *myo*-inositol has been shown to render hepatic triglyceride accumulation by the rat susceptible to the effects of the fatty acid composition of the diet, indicating a function resembling that of an essential nutrient. Further, Hegsted and colleagues found that the female Mongolian gerbil[112] develops intestinal lipodystrophy when deprived of the factor.[113] In fact, that group showed that the gerbil required a source of *myo*-inositol to prevent the disorder when fed a diet-containing adequate levels of all other known nutrients.

Conditions of Need for Dietary *Myo*-Inositol

A few species have overt dietary needs for *myo*-inositol. These include several fishes and the gerbil. Studies with fishes have shown dietary deprivation of *myo*-inositol to result in anorexia, fin degeneration, edema, anemia, decreased gastric emptying rate, reduced growth, and impaired efficiency of feed utilization. Studies with gerbils have shown *myo*-inositol deprivation to result in intestinal lipodystrophy, with associated hypocholesterolemia and reduced survival. These effects are observed only in females; males appear to have a sufficient testicular synthesis of *myo*-inositol. For these species, *myo*-inositol is a dietary essential.

Studies with nutritionally complete diets have failed to confirm early reports that suggested dietary needs for *myo*-inositol to prevent alopecia in rodents; fatty liver in rats and growth retardation in chicks, guinea pigs, and hamsters. It is possible that those earlier findings may have indicated beneficial effects of dietary sources of *myo*-inositol on the hindgut microbiome of those animals, i.e., that dietary *myo*-inositol might be beneficial in diets containing marginal amounts of such factors as choline and biotin, which are known to be synthesized by the microbiome. Support for this hypothesis comes from findings that supplemental *myo*-inositol reduced hepatic lipid accumulation in rats fed a choline-deficient diet, improved growth in rats fed a diet deficient in several vitamins, and reduced the incidence of fatty liver in and improved the growth of chicks fed a biotin-deficient diet containing an antibiotic.[114] Thus, it is plausible that a dietary source of *myo*-inositol could be useful under conditions that increase the need for myo-inositol for lipid transport (e.g., high-fat diets[115]) or disturb the

109. Hathcock, J.N., Shao, A., 2006. Regul. Toxicol. Pharmacol. 46, 23–28.
110. Woolley, D.W., 1940. Science 92, 384–385.
111. Needham, J., 1924. Biochem. J. 18, 891–904.
112. *Meriones unguiculatus*.
113. Hegsted, M, Hayes, K.C., Gallagher, A., et al., 1973. J. Nutr. 103, 302–307.
114. Antibiotics can reduce the numbers of microorganisms that normally produce *myo*-inositol and other nutrients.
115. High-fat diets may increase the needs for *myo*-inositol for lipid transport.

hindgut microbiome (e.g., antibiotic use, stressful physical environments).

Conditions producing need for dietary inositol are as follows:

- Limited biosynthesis in fish and gerbils
- Limited synthesis by the gut microbiome?

Chemical Nature

Myo-inositol is a water-soluble, hydroxylated, cyclic six-carbon compound (*cis*-1,2,3,5-*trans*-4,6-cyclohexanehexol) (Fig. 19.6). It is the only one of the nine possible stereoisomeric forms of cyclohexitol with biological activity.

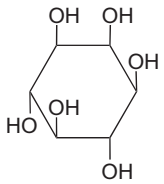


FIGURE 19.6 *Myo*-inositol.

Sources of *Myo*-Inositol

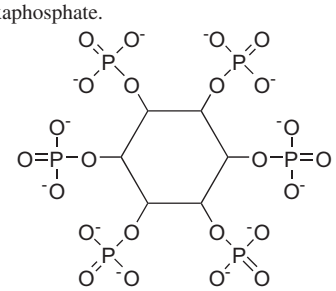
Biosynthesis. Most, if not all, mammals are capable of synthesizing *myo*-inositol de novo ultimately from glucose. Biosynthetic capacity has been found in the liver, kidney, brain, and testis of rats and rabbits, and in the kidney and other tissues in humans. Biosynthesis involves the cyclization of glucose-6-phosphate to inositol-1-phosphate by inositol-1-phosphate synthase, followed by a dephosphorylation by inositol-1-phosphatase. The human kidney produces several grams of *myo*-inositol daily. Renal synthesis of *myo*-inositol has been found to be about 4 g/day (~2 g/kidney/day).[116]

Dietary sources. *Myo*-inositol occurs in foods and feedstuffs in three forms: free *myo*-inositol, **phytic acid**,[117] and inositol-containing phospholipids. The richest sources of *myo*-inositol are the seeds of plants (e.g., beans, grains, and nuts) (Table 19.7). However, the predominant form occurring in plant materials is phytic acid (which can comprise most of the total phosphorus present in materials such as cereal grains[118]). Because most mammals have little or no intestinal phytase activity, phytic acid is poorly utilized as a source of either *myo*-inositol or phosphorus.[119,120] In animal products, *myo*-inositol occurs in free form as well as in inositol-containing phospholipids (primarily PI); free *myo*-inositol predominates in brain and kidney, whereas phospholipid inositol predominates in skeletal muscle, heart, liver, and pancreas. The richest animal sources of inositol are organ meats. Human milk is relatively rich in *myo*-inositol (colostrum, 200–500 mg/liter; mature milk, 100–200 mg/liter) in comparison with cow's milk (30–80 mg/liter). A disaccharide form of *myo*-inositol, 6-β-galactinol (6-*O*-β-D-galactopyranosyl-*myo*-inositol), comprises about one-sixth of the nonlipid *myo*-inositol in that material.

Myo-inositol is classified by the US FDA among the substances generally recognized as safe and, therefore, can be used in the formulation of foods without the demonstrations of safety and efficacy required by the Food, Drug and

116. Troyer, D.A., Schwertz, D.W., Kreisberg, J.I., et al., 1986. Ann. Rev. Physiol. 48, 51–71.

117. Inositol hexaphosphate.



118. Of the total P present, phytic acid P comprises 48–73% for cereal grains (corn, barley, rye, wheat, rice, sorghum), 48–79% for brans (rice, wheat), 27–41% for legume seeds (soybeans, peas, broad beans), and 40–65% for oilseed meals (soybean meal, cottonseed meal, rapeseed meal).

119. The bioavailability of P from most plant sources is >50% for ruminants, which benefit from the phytase activities of their rumen microflora. Nonruminants, lacking intestinal phytase, derive much less P from plant phytic acid, depending on the phytase contributions of their intestinal microflora. For pigs and rats, such contributions appear to be significant, giving them moderate abilities (~40%) to utilize phytic acid P. In contrast, the chick, which, has only a sparse intestinal microflora, can use only ~8% of phytic acid P.

120. Phytic acid can also form a very stable chelation complex with zinc (Zn^{2+} is held by the negative charges on adjacent pyrophosphate groups), thus reducing its nutritional availability. For this reason, the bioavailability of zinc in such plant-derived foods as soybean is very low.

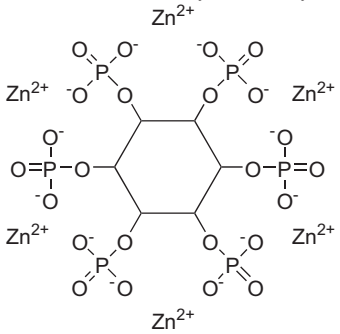


TABLE 19.7 Total *Myo*-Inositol Contents of Selected Foods

Food	Myo-inositol, mg/g
Vegetables	
Asparagus	0.29–0.68
Beans	
Green	0.55–1.93
White	2.83–4.40
Red	2.49
Broccoli	0.11–0.30
Cabbage	0.18–0.70
Carrot	0.52
Cauliflower	0.15–0.18
Celery	0.05
Okra	0.28–1.17
Pea	1.16–2.35
Potato	0.97
Spinach	0.06–0.25
Squash, yellow	0.25–0.32
Tomato	0.34–0.41
Fruits	
Apple	0.10–0.24
Cantaloupe	3.55
Grape	0.07–0.16
Grapefruit	1.17–1.99
Orange	3.07
Peach	0.19–0.58
Pear	0.46–0.73
Strawberry	0.13
Watermelon	0.48
Cereals	
Rice	0.15–0.30
Wheat	1.42–11.5
Meats	
Beef	0.09–0.37
Beef liver	0.64
Chicken	0.30–0.39
Chicken liver	1.31
Lamb	0.37
Pork	0.14–0.42
Pork liver	0.17
Turkey	0.08–0.23
Trout	0.11
Tuna	0.11–0.15
Dairy Products and Eggs	
Milk	0.04
Ice cream	0.09
Cheese	0.01–0.09
Egg, whole	0.09
Egg yolk	0.34
Nuts	
Almond	2.78
Peanut	1.33–3.04

Adapted from Clements, Jr., S.R., Darnell, B., 1980. Am. J. Clin. Nutr. 3, 1954–1967.

Cosmetic Act. It is added to many prepared infant formulas (at about 0.1%). It is estimated that typical American diets provide adults with about 900 mg of *myo*-inositol per day, slightly over half of which is in phospholipid form.

Absorption and Transport of *Myo*-Inositol

Absorption. The enteric absorption of free *myo*-inositol occurs by active transport; uptake from the small intestine is virtually complete. The enteric absorption of phytic acid, however, depends on its digestion and the amounts of divalent cations in the diet/meal. Most animal species lack intestinal phytase activities and are, therefore, dependent on the presence of a gut microbiome with those enzymes. For species with foregut microbiomes (e.g., ruminants and long-gutted nonruminants), phytate is digestible, making it a useful source of *myo*-inositol. Dietary cations (particularly Ca^{2+}) can reduce the utilization of phytate by forming insoluble (and, thus, nondigestible and nonabsorbable) phytate **chelates**. Because a large portion of the total *myo*-inositol in mixed diets is typically in the form of phytic acid, its utilization from high-calcium diets can be <50% of that from diets containing low to moderate amounts of the mineral.[121]

121. For the same reason, the bioavailability of calcium is also low for high-phytate diets. This same effect occurs for the nutritionally important divalent cations Mn^{2+} and Zn^{2+}; the bioavailability of each is reduced by the presence of phytic acid in the diet.

Absorption of phospholipid *myo*-inositol is thought to be analogous to that of PC.[122]

Transport. The normal circulating concentration of *myo*-inositol in humans is about 30 μM. It circulates predominantly in the free form; a small amount occurs as PI associated with lipoproteins. Free *myo*-inositol is taken up by an active transport process in some tissues (kidney, brain) and by carrier-mediated diffusion in others (liver). The active process requires Na^+ and energy and is inhibited by high levels of glucose. Untreated diabetics show impaired tissue uptake and impaired urinary excretion of *myo*-inositol.

Tissue distribution. In tissues, *myo*-inositol is found as the free form and as the mono-, di-, and triphosphorylated metabolites. These PIs differ from other phospholipids by being enriched in **stearic acid** (predominantly at the 1-position) and **arachidonic acid** (predominantly at the 2-position). For example, the *myo*-inositol-containing phospholipids on the plasma membrane from human platelets contain about 42 mol% stearic acid and about 44 mol% arachidonic acid. The greatest concentrations of *myo*-inositol are found in neural and renal tissues.

Metabolism of *Myo*-Inositol

Conversion to PIs and soluble inositides. Free *myo*-inositol is converted to PI within cells by PI synthetase, which catalyzes its reacting with the liponucleotide cytidine diphosphate-diacylglycerol.[123] In turn, PI can be sequentially phosphorylated to the monophosphate (phosphatidylinositol 4-phosphate, PI_2) and diphosphate (phosphatidylinositol 4,5-diphosphate, PIP_2) forms by membrane **inositolphosphate kinases (IPKs)**.[124] Higher phosphorylated forms (PIP_4, PIP_5, PIP_6, and PIP_7) are formed by the action of **inositolpolyphosphate multikinase (IPMK)**. These PIs can be dephosphorylated by three phosphatases (PTEN, SHIP1, SHIP2[125]) that produce various soluble inositol phosphatides (e.g., IP, IP_2, IP_3, etc.) depending on the local expression of IPKs.

Turnover. In the presence of cytidine monophosphate, PI synthetase functions (in the reverse direction) to break down that form to yield CDP-diacylglycerol and *myo*-inositol. The kidney performs most of the further catabolism, clearing *myo*-inositol from the plasma, converting it to glucose, and then oxidizing it to CO_2 via the pentose phosphate shunt. The metabolism of *myo*-inositol appears to be relatively rapid; the rat can oxidize half of an ingested dose within 48 h.

Metabolic Functions of *Myo*-Inositol

The metabolically active forms of *myo*-inositol are PIs and IPs, which have several physiologically important roles [126,127]:

- **PIs affect membrane structure and function**. The PIs are membrane active due to their unique fatty acid compositions. For example, their polar head group and highly nonpolar fatty acyl chains facilitate specific electrostatic interactions while providing a hydrophobic microdomains in membranes that facilitate the recruitment of proteins and, ultimately, control the cytoskeleton and membrane dynamics during mitosis. This role also allows PIs to function as an activator of microsomal Na^+, K^+-ATPase, an essential constituent of acetyl-CoA carboxylase, a stimulator of tyrosine hydroxylase, cofactors of alkaline phosphatase and 5´-nucleotidase, a membrane anchor for acetylcholinesterase, a necessary factor for insulin sensitivity, and a regulator of endosome–lysosome trafficking and induction of pathogen killing and antigen-processing pathways in lymphoid cells.
- **PIs are ready sources of arachidonic acid**. PI serves as a source of releasable arachidonic acid for the formation of the **eicosanoids**[128] by cyclooxygenase and/or lipoxygenase. Although PI is less abundant in cells than other phospholipids (PC, phosphatidylethanolamine,

122. This would involve hydrolysis by pancreatic phospholipase A in the intestinal lumen to produce a lysophosphatidylinositol, which, on uptake by the enterocyte, would be reacylated by an acyltransferase or hydrolyzed further to yield glycerylphosphorylinositol.

123. This step is catalyzed by the microsomal enzyme CDP-diacylglycerol–inositol 3-phosphatidyltransferase (also called PI synthetase).

124. These are ATP:PI-4-phosphotransferase and ATP:PI-4-phosphate 5-phosphotransferase, respectively. They are located on the cytosolic surface of the erythrocyte membrane. There is no definitive evidence that *myo*-inositol can be isomerized or phosphorylated to the hexaphosphate level; however, such prospects would be of interest, as the isomer D-*chiro*-inositol has been shown to promote insulin function, and inositol hexaphosphate (phytic acid) has been found to be anticarcinogenic in different animal models.

125. Phosphatase and tensin homologue; PI-3,4,5-triphosphate 5-phosphatases 1 and 2.

126. Additional roles have been identified in lower organisms: transcriptional regulation in yeasts; stress tolerance in plants.

127. In addition, IP kinases have metabolic roles that appear unrelated to their catalytic activities. IPMK has been shown to mediate the effects of growth factors by stimulating the protein kinase Akt, which activates the central signaling regulator mTOR complex 1. This involves IPMK binding to mTORC1, promoting its stability and, thus, mediating the ability of amino acids to activate mTOR. IPMK similarly affects AMPK signaling, and a similar mechanism may be involved in the effect attributed to IP7 in maintaining exocytosis of insulin-containing secretory granules from mouse pancreatic β-cells.

128. The eicosanoids include prostaglandins, thromboxanes, and leukotrienes. The prostaglandins are hormone-like substances secreted for short-range action on neighboring tissues; they are involved in inflammation, in the regulation of blood pressure, in headaches, and in the induction of labor. The functions of the leukotrienes and thromboxanes are less well understood; they are thought to be involved in regulation of blood pressure and in the pathogenesis of some types of disease.

and phosphatidylserine), its enrichment in arachidonic acid renders it an effective source of that eicosanoid precursor.

- **IPs are second messengers of Ca^{2+} signaling**. IPs mediate the responses of target tissues to the signaling by cholinergic or β-adrenergic agonists (producing rapid responses) and mitogens (producing medium-term responses). This mediation involves its activation to the less abundant species, IP_2 to IP_3, which serves as a **second messenger** to activate the release of Ca^{2+} from intracellular stores (in discrete organelles called **calcisomes**) and stimulate the entry of Ca^{2+} into the cell across the plasma membrane.[129] Cell surface IP_3 receptors have been shown to effect primary control over the hydrolysis of IP_2 by regulating the activity of phospholipase C (phosphodiesterase) on the plasma membrane. Receptor occupancy, thus, activates the hydrolysis of PIP_2, which is favored at low intracellular concentrations of Ca^{2+} to produce IP_3 and, perhaps, other inositol polyphosphates. The former process involves a specific IP_3 receptor on the calcisomal membrane; IP_3 binding to this receptor opens an associated **Ca^{2+} channel**. IP_3-stimulated entry of Ca^{2+} into the cell requires an increase in the permeability of the plasma membrane signaled by the emptying of the IP_3-sensitive intracellular pool. Evidence suggests that 1,2-diacylglycerol, formed from receptor-stimulated metabolism of the *myo*-inositol-containing phospholipids, may signal the activation of protein kinase C for the phosphorylation of various proteins important to cell function. According to this hypothesis, 1,2-diacylglycerol functions with Ca^{2+} and phosphatidylserine, both of which are known to be involved in the activation of protein kinase C. The maintenance of balanced Ca^{2+} flux has also been shown to be important in left–right symmetrical development in the zebrafish; this is disrupted by genetic deletion of IPKs.[130]

Dietary *Myo*-Inositol in Health and Disease

Positive clinical findings have been reported for myo-inositol supplements in human patients:

- **Preterm infants**. Three randomized, clinical trials found *myo*-inositol supplementation to improve survival and reduce retinopathy of prematurity, bronchial dysplasia, and intraventricular hemorrhage.[131]
- **Obesity**. *Myo*-inositol supplementation has been found to reduce insulin resistance and gestational diabetes in two cohorts of obese pregnant women[132] and improve ovarian function in three cohorts of overweight/obese women with polycystic ovary syndrome.[133]
- **Psoriasis**. A small, randomized clinical trial found supplementation with *myo*-inositol to reduce the severity of symptoms of psoriasis patients.[134]
- **Neuropsychological responses**. Because mood stabilizers such as lithium, valproate, and carbamazepine function by stabilizing inositol signaling, it has been suggested that *myo*-inositol may have value in the treatment of depression and other psychiatric disorders. While *myo*-inositol supplementation has been reported to be helpful in treating bipolar depression and bulimia nervosa with binge eating, the limited findings in this area to date do not indicate clear benefits. A small randomized trial found *myo*-inositol to increase the efficacy of high-dose n-3 fatty acid therapy in reducing symptoms of mania and depression in children with mild to moderate bipolar spectrum disorders.[135] Another study found *myo*-inositol supplementation to improve symptoms in women with premenstrual dysphoric disorder.[136]

Biomarkers of *Myo*-Inositol Status

Plasma concentration of *myo*-inositol in humans are typically ~30 μ*M* most of which is the free form. This parameter is not informative regarding the physiologically active PIs in cells.

Safety of Myo-Inositol

There are no reports of adverse effects of oral intakes of myo-inositol. It is presumed safe.

129. The Ca^{2+}-mobilizing activity of IP_3 is terminated by its dephosphorylation (via a 5-phosphatase) to the inactive inositol-1,4-bisphosphate, or by its phosphorylation (via a 3-kinase) to a product of uncertain activity, IP_4. There is some controversy concerning whether other inositol phosphates [e.g., inositol 1,3,4,5-tetraphosphate (IP_4), which is a product of a 3-kinase acting on IP_3] can also signal Ca^{2+} mobilization. Evidence suggests that, in at least some cells, IP_3 and IP_4 may have cooperative roles in Ca^{2+} signaling.

130. Tsui, M.M., York, J.D., 2010. Adv. Enzyme Regul. 50, 324–337.

131. Howlett, A., Ohlsson, A., 2003. Cochrane Database Syst. Rev. CD000366.

132. D'Anna, R., Di Benedetto, A., Scilipoti, A., et al., 2015. Obstet. Gyncecol. 126, 310–315; Matarelli, B., Vitacolonna, E., D'Angelo, M., et al., 2015. J. Matern. Fetal Neonatal. Med. 26, 967–972.

133. Kamenov, Z., Kolarov, G., Gatvba, A., et al., 2015. Gynecol. Endocrinol. 31, 131–135; Pizzo, A., Laganà, A.S., Barbaro, L., 2015. Gynecol. Endocrinol. 30, 205–208; Artini, P.G., Di Berardino, O.M., Papini, F., et al., 2015. Gynecol. Endocrinol. 29, 375–379.

134. Allan, S.J.R., Kavanagh, G.M., Herd, R.M., et al., 2004. Br. J. Dermatol. 150, 966–969.

135. Wozniak, J., Faraone, S.V., Chan, J., et al., 2015. J. Clin. Psychiatry 76, 1548–1555.

136. Gianfranco, C., Vittorio, U., Silvia, B., et al., 2011. Hum. Psychopharmacol. 26, 526–530.

5. UBIQUINONES

Ubiquinones are normal metabolites that are essential components of the mitochondrial electron transport chains of all cells. They are biosynthesized by most species; however, several conditions can limit their biosynthesis, making them **conditionally essential**.

Recognition of Nutritional Roles of Ubiquinones

Isolated from the unsaponifiable fractions of the hepatic lipids from vitamin A-deficient rats, the principal species of the group, ubiquinone(50) was subsequently identified as an essential component of the mitochondrial electron transport chains of most prokaryotic and all eukaryotic cells. Folkers called this factor "vitamin Q." In subsequent decades, the term "coenzyme Q" came to be used as the general descriptor of this family of compounds.

Conditions of Need for Dietary Ubiquinones

Dietary sources of ubiquinones have been found to be beneficial under specific conditions: limited biosynthesis and increased need for mitochondrial antioxidant protection.

- Conditions limiting ubiquinone biosynthesis:
  Genetic disorders. Several genetic variants in enzymes in the ubiquinone biosynthetic pathway or mitochondrial electron transport chain have been found to reduce tissue CoQ_{10} losses markedly (33–97%).[137] Subject with these rare deficiencies that reduce plasma CoQ_{10} concentrations to more than two SD below the mean normal level show five major phenotypes: encephalopathy (4 cases reported), cerebellar ataxia (94 cases reported), an infantile multisystemic disease (17 cases reported), nephropathy and isolated myopathy (10 cases reported).[138]
  Aging. Deficient tissue levels of CoQ occur in older animals, suggesting that ubiquinone biosynthesis may decline with age. Tissue-specific deficiencies of CoQ have been identified in association with genetic deficiencies of enzymes involved in that ubiquinone biosynthesis. These deficiencies can be corrected with dietary supplements of CoQ_{10}.
  Statin therapy. Tissue CoQ levels can be reduced as a result of statin therapy, which impairs the synthesis of CoQ_{10} by inhibiting HMG-Co reductase to reduce farnesyl pyrophosphate, an intermediate in the pathway to CoQ. Statin treatment has been associated with myopathic conditions ranging from mild myalgia to fatal rhabdomyolysis as well as with subclinical cardiomyopathy. These conditions have been found to respond to CoQ_{10} supplementation or its synthetic analogue idebenone.[139]
- Conditions increasing antioxidative need are as follows:
  Vitamin E deficiency. Metabolic needs for CoQ are linked to vitamin E status, as the oxidized form of CoQ_{10}, can support antioxidant functions *in lieu* of that vitamin. This has been demonstrated in the reduction of clinical signs of vitamin E deficiency in both rats[140] and rhesus monkeys.[141] In fact, responses to CoQ_{10} have been found to have more rapid onset than those to α-tocopherol.
  Disease-producing oxidative stress. Increased oxidative stress occurs in **hyperthyroidism**, which is associated with low circulating levels of CoQ_{10} and α-tocopherol.[142] **Parkinson's disease** patients face a higher than normal risk of CoQ deficiency,[143] which may be related to their excessive production of reactive oxygen species (ROS).

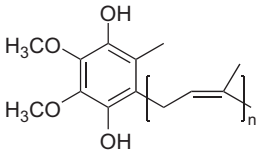


FIGURE 19.7 Structure ubiquinones (reduced form shown).

Conditions producing need for dietary ubiquinones are as follows:
- Limited biosynthesis: genetic disorders, aging, statin therapy
- Increased oxidative stress.

Chemical Nature of the Ubiquinones

The **ubiquinones** are a group of tetrasubstituted 1,4-benzoquinone derivatives with isoprenoid side chains of variable length (Fig. 19.7). The structure of the 6-chromanol moiety is remarkably similar to the oxidized form of vitamin E, tocopherylquinone, the difference being that ubiquinones have two methoxyl groups in ring positions where

137. Bentinger, M., Tekle, M., Dallner, G., 2010. Biochem. Biophys. Res. Commun. 396, 74–79.
138. Emmanuele, V., López, L.C., Berardo, A., et al., 2012. Arch. Neurol. 69, 978–983.
139. Littarru, G.P., Langsjoen, P., 2007. Mitochondrion 7S, S168–S174; Mancuso, M., Orsuci, D., Vopli, L., et al., 2010. Curr. Drug Targets 11, 111–121.
140. i.e., "Resorption–gestation" syndrome, *see* Chapter 8.
141. i.e., Anemia and the myopathy.
142. Mancini, A., Festa, R., Raimondo, S., et al., 2011. Int. J. Molec. Sci. 12, 9216–9225.
143. Mischley, L.K., Allen, J., Bradley, R., 2012. J. Neurol. Sci. 318, 72–75.

tocopherylquinone has methyl groups. The conventions of nomenclature for this group are similar to those for vitamin K. The terms ubiquinone and coenzyme Q (or CoQ) are synonymous, but their actual species, which are defined by the nature of their respective side chain, is indicated differently: the number of side chain carbons is indicated parenthetically for the ubiquinone designation and the number of side chain isoprenyl units is indicated in a subscript for the CoQ designation.[144]

Sources of Ubiquinones

Biosynthesis. CoQ_{10} is synthesized in all tissues from precursors available in inner mitochondrial membranes. The biosynthetic process derives the isoprenyl side chain is derived from mevalonate, the ring system from tyrosine, the hydroxyl groups from molecular oxygen, and the methyl groups from *S*-adenosylmethionine to produce a 50-C polyisoprene chain comprised of 10 isoprenoid units. A key enzyme in this pathway is hydroxymethylglutaryl-CoA reductase, which is feedback-inhibited by CoQ_{10}.[145] Endogenous biosynthesis appears sufficient to support membrane saturation levels.

Dietary sources. CoQ_{10}, localized in the mitochondria and other cellular membranes, is found in plant and animal tissues of high cellularity, particularly, those rich in mitochondria such as heart and muscle (Table 19.8).

Absorption and Transport of Ubiquinones

Absorption. Ubiquinones appear to be absorbed, transported, and taken up into cells by mechanisms analogous to those of the tocopherols. In the rat, the greatest absorption of CoQ_{10} has been found to occur in the duodenum, with demonstrable absorption also in the colon, ileum, and jejunum, suggesting the possibility of enterohepatic circulation.

Transport. Because all cell synthesizes apparently adequate amounts of ubiquinones, there is little redistribution via the circulation. The small amounts in plasma reflect CoQ recently absorbed from the diet and released by the liver in association with VLDL. Evidence indicates that the lipoprotein pool does not exchange with the tissues.

Tissue distribution. CoQ_{10} is normally present in membranes of all cells in the body as a result of ubiquitous biosynthesis. The total CoQ_{10} pool size in the human adult is estimated to be 0.5–1.5 g. Relatively great concentrations of CoQ_{10} are found in the liver, heart, spleen, kidney, pancreas, and adrenals.[146] Tissue ubiquinone levels increase under the influence of oxidative stress, cold acclimation, and thyroid hormone treatment and appear to decrease with cardiomyopathy, other muscle diseases, and aging. Dietary supplementation increases CoQ levels only in the liver and spleen.

Metabolism of Ubiquinones

The ubiquinones undergo reversible, two-electron redox reactions (Fig. 19.8).

Metabolic Functions of Ubiquinones

CoQ has several essential metabolic functions.

- **Mitochondrial respiratory chain**. CoQ is an electron acceptor for complexes I and II, passing electrons from flavoproteins (e.g., NADH or succinic dehydrogenases) to the cytochromes via cytochrome b_5. In this process, CoQ undergoes reversible reduction/oxidation to cycle between the 1,4-quinone (oxidized) and 1,4-dihydroxybenzene (reduced) species (Fig. 19.9).
- **Antioxidant**. CoQ_0 is a lipid-soluble antioxidant that protects α-tocopherol in membranes; protects LDL from oxidation; and generally participates in the prevention of the oxidation of lipids, proteins, and DNA. In LDLs, which are particularly susceptible to oxidation, CoQ_{10} is the primary antioxidant, being oxidized before α-tocopherol.[147]
- **Regulation of intracellular NAD^+/NADH balance**. CoQ is an essential cofactor for NADH oxidase in plasma membranes. This enzyme regulates intracellular NAD^+/NADH balance, which is important in cell growth and development.
- **Mitochondrial membrane pore regulation**. CoQ is a factor preventing the opening of mitochondrial membrane transition pores that otherwise admit large molecules capable of antagonizing the function of that organelle and promoting apoptosis.
- **Uncoupling of oxidative phosphorylation**. CoQ is required for the delivery of protons from fatty acids to uncoupling proteins. The uncoupling of the proton gradient from oxidative phosphorylation is used to produce heat.
- **Antiinflammatory effects**. CoQ stimulates lymphocytes to release factors signaling the expression of NF-κB-1-dependent genes to produce antiinflammatory cytokines.
- **Endothelial function**. CoQ stimulates endothelial release of nitric oxide.

144. e.g., Ubiquinone(50) is equivalent to CoQ10.

145. HMG-CoA reductase is upstream of the branch point in the mevalonate pathway whereby farnesyl pyrophosphate can be used for the synthesis of either CoQ or cholesterol. This fact has been exploited to reduced the cholesterol contents of eggs by supplementing the diets of laying hens with CoQ_{10} (Honda, K., Saneyasu, T., Motoki, T., et al., 2013. Biosci. Biotechnol. Biochem. 77, 1572–1574).

146. The contributions of foods and feedstuffs, many of which are now known to contain appreciable concentrations of ubiquinones, to these high tissue levels are unknown.

147. Stocker, R., Bowry, V.W., Frei, B., 1991. Proc. Natl. Acad. Sci. 88, 1646–1650.

TABLE 19.8 CoQ_{10} Contents of Foods

Food	CoQ_{10}, μg/g
Vegetables	
Asparagus	2
Avocado	10
Bean	2
Broccoli	6–9
Cabbage	1–5
Cauliflower	1–7
Eggplant	1–2
Onion	1
Parsley	8–26
Pepper, sweet	3
Potato	1
Soybean	7–19
Spinach	1–10
Sweet potato	3–4
Fruits	
Apple	1
Banana	1
Orange	1–2
Strawberry	1
Tomato	<1
Cereals	
Corn germ	7
Rice bran	5
Wheat germ	4–7
Meats	
Beef, muscle	16–37
Beef, liver	39–51
Beef, heart	113
Pork, muscle	14–45
Pork, liver	23–54
Pork, heart	118–282
Chicken, muscle	11–25
Chicken, liver	1116–132
Chicken, heart	92–192
Fish	3–130
Dairy products and eggs	
Milk	2
Butter	7
Eggs	1–4
Other	
Corn oil	13–139
Olive oil	4–160
Soybean oil	54–279

Adapted from Pravst, I., Zmitek, K., Zmitek, J., 2010. Crit. Rev. Food Sci. Nutr. 50, 269–280.

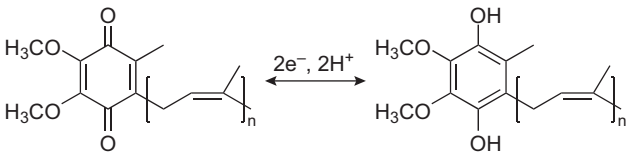


FIGURE 19.8 Redox function of ubiquinones.

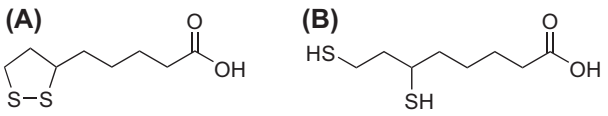


FIGURE 19.9 Lipoic acid (A) and dihydrolipoic acid (B).

Dietary Ubiquinones in Health and Disease

Cardiovascular health. Studies with animal models have shown that supplemental CoQ_{10} can help maintain the integrity of cardiac muscle under cardiomyopathic conditions.[148] Administration of CoQ_{10} has been found to protect against myocardial damage mediated by free-radical mechanisms (ischemia, drug toxicities) in animal models. Clinical trials with humans have indicated benefits of supplemental CoQ_{10} of several types:

- **Migraine**—A small, open-label trial found CoQ_{10} to reduce headache frequency.[149]

148. e.g., Cardiomyopathy induced in the rat by feeding a fructose-based, copper-deficient diet.
149. Sándor, P.S., Di Clemente, L., Coppola, G., et al., 2005. Neurol. 64, 713–715.

- **Congestive heart failure**—A meta-analysis of randomized controlled trials showed CoQ_{10} to reduce dyspnea, edema, and the frequency of hospitalization.[150]
- **Coronary artery disease**—Its antioxidant activity may be the basis of CoQ_{10} supplementation reducing inflammatory markers in patients with coronary artery disease.[151]
- **Hypertension**—A systematic review of eight randomized trials found that CoQ_{10} supplementation can decrease blood pressure (systolic: −16 mm Hg; diastolic: −10 mm Hg).[152]
- **Atherosclerosis**—A randomized trial found CoQ_{10} after myocardial infarction to reduce subsequent myocardial events and cardiac deaths.[153]
- **Endothelial dysfunction**—A randomized trial found CoQ_{10} to improve endothelial function of peripheral arteries of dyslipidemic patients with type 2 diabetes.[154]
- **Recovery from coronary artery bypass surgery**—In a randomized trial, patients preoperatively supplemented with CoQ_{10} had significantly fewer reperfusion arrhythmias and shorter hospitalizations than controls.[155]
- **Friedrich's ataxia**—High doses of CoQ_{10} administered with vitamin E improved cardiac and muscular function in patients with Friedrich's ataxia.[156,157]

Neurologic health. A clinical trial found CoQ_{10} supplementation to improve symptoms in children with autism.[158] Modest improvements in symptoms were reported from a small, randomized trial with Parkinson's disease patients.[159]

Other effects. CoQ_{10} supplementation has been reported to improve semen quality in men with idiopathic infertility, to reduce the risk of preeclampsia, to reduce ultraviolet light-induced skin wrinkling, to confer protection against cardiac or hepatic toxicity associated with cancer chemotherapy, and to improve muscular strength in patients with Duchenne muscular dystrophy. Studies in animal models have suggested that CoQ_{10} supplementation may improve insulin sensitivity.

Biomarkers of Ubiquinone Status

Assessments of CoQ in the plasma/serum reflect mostly the small amounts recently absorbed from the diet as well as that released by the liver in association with lipoproteins. In healthy individuals, plasma CoQ concentrations are typically ~1 μg/mL. While not informative regarding tissue CoQ status, plasma CoQ levels can be useful in evaluating the status of LDLs, which are particularly susceptible to oxidation. Tissue ubiquinone status is best assessed by direct measurement of CoQ in mitochondrial lipids.

Safety of Ubiquinones

Human studies have found CoQ to be safe and well tolerated as a dietary supplement. A chronic, high-level treatment (0.26% of diet) has been shown to exacerbate some cognitive and sensory impairments in older mice.[160]

6. LIPOIC ACID

Lipoic acid, also known as **thioctic acid**, is a normal metabolite first isolated from bovine liver in 1951.[161] It has subsequently been shown to function both as a coenzyme for mitochondrial enzymes and as an amphipathic antioxidant capable of quenching free radicals, regenerating other cellular antioxidants, and chelating prooxidative metal ions. While lipoic acid is biosynthesized in the mitochondria of apparently all animals, evidence suggests that its biosynthesis is likely to decline with age-related losses of mitochondrial mass, making lipoic acid **conditionally essential**. Its antioxidant properties appear to be the basis of beneficial health effects of lipoic acid supplements, making lipoic acid *also* a **beneficial dietary bioactive** for animals capable of its biosynthesis.

Benefits of dietary lipoic acid are as follows:
- Improved mitochondrial function in aging
- Increased antioxidant protection.

Chemical Nature of Lipoic Acid

Lipoic acid (1,2-dithiolane-3-pentanoic acid) contains two vicinal sulfur atoms that are subject to oxidation/reduction;

150. Soja, A.M., Mortensen, S.A., 1997. Mol. Aspects Med. 18, S159–S168.
151. Lee, B.J., Huang, Y.C., Chen, S.J., et al., 2012. Nutrition 28, 767–772.
152. Rosenfeldt, F., Hilton, D., Pepe, S., et al., 2003. Biofactors 18, 91–100.
153. Singh, R.B., Neki, N.S., Kartikey, K., et al., 2003. Mol. Cell. Biochem. 246, 75–82.
154. Watts, G.F., Playford, D.A., Croft, K.D., et al., 2002. Diabetolgia 45, 420–426.
155. Makhija, N., Sendasgupta, C. Kiron, U., et al., 2008. J. Cardiothorac. Vasc. Anesth. 22, 832–839.
156. Cooper, J.M., Schapira, A.H.V., 2007. Mitochondrion 7S, S127–S135.
157. Friedreich's ataxia is an autosomal recessive condition involving deficient production of a mitochondrial protein, frataxin, thought to function in antioxidant regulation and/or iron metabolism. The condition manifests in adolescence as progressive limb and gait ataxias, and losses of deep tendon reflexes and position and vibration senses due to the loss of large sensory neurons in the dorsal root ganglia and deterioration of other cerebellar-spinal tracts.
158. Gvozdjáková, A., Kucharská, J., Ostatníková, D., et al., 2014. Oxid. Med. Cell. Long. Article ID:798957, 6 pp.
159. Muller, T., Büttner, T., Gholipour, A.F., et al., 2003. Neurosci. Lett. 341, 201–204.
160. Sumien, N., Heinrich, K.R., Shetty, R.A., et al., 2009. J. Nutr. 139, 1926–1932.
161. Reed, L.J., DeBusk, B.G., Gansalus, I.C., et al., 1951. Ciences 114, 93–94.

the reduced form, dihydrolipoic acid, has two thiols. Lipoic acid contains one chiral center[162]; thus, the molecule has two possible optical isomers (*R*- and *S*-) (Fig. 19.9).

Sources of Lipoic Acid

Biosynthesis. Lipoic acid (the *R*-enantiomer) is synthesized in the mitochondria from unsaturated fatty acids and cysteine catalyzed by a **lipoyltransferase** and **lipoic acid synthetase**.[163] It is quickly reduced by **dihydrolipoyl dehydrogenase**. The amounts synthesized are limited,[164] but appear adequate for all species studied, e.g., no adverse effects have been observed in chicks, rats, or turkey poults fed lipoic acid-free diets.[165] However, because mitochondrial mass declines with age, lipoic acid biosynthesis may also decline in older individuals. This hypothesis is supported by findings that lipoic acid supplementation increased mitochondrial enzyme activities in older rats but not younger ones.[166] Genetic deficiencies have been identified in lipoic acid synthetase and lipoyltransferase I; these result in nonketotic hyperglycemia, defective energy metabolism, encephalopathy, and cardiomyopathy.

Dietary sources. Lipoic acid is present in a wide variety of foods, generally at low levels. The best sources are tissues rich in mitochondria (e.g., red meats, liver, heart, kidney) or chloroplasts (i.e., spinach, broccoli, Brussel sprouts, peas, tomatoes, potatoes, rice bran). In these foods, most lipoic acid is covalently bound to ε-amino groups of lysyl residues in proteins. Foods contain only the *R*-enantiomer; synthetic sources consist of equimolar mixtures of both the *R*- and *S*-enantiomers.

Absorption and Transport of Lipoic Acid

The enteric absorption of lipoic acid is thought to be facilitated by the monocarboxylate transporter and/or Na^+-dependent multivitamin transporter. *R*-lipoic acid is absorbed with moderate efficiency (~40%); *S*-lipoic acid is absorbed less well (~30%).[167] The bioavailability of food forms (lipoyllysine) remain unexamined, but are likely to be no more than 30–35%, depending on protein digestibility. Absorbed lipoic acid appears quickly in the plasma, apparently in free solution. It is cleared quickly, indicating ready tissue uptake. The same transporters are thought to facilitate the tissue uptake of lipoic acid. In tissues, lipoic acid is found in mitochondria.

Metabolism of Lipoic Acid

Lipoic acid is reduced to dihydrolipoic acid by the pyridine nucleotides, NADH or NADPH. Substrate stereospecificity varies; mitochondrial **NADH-dependent dihydrolipoamide dehydrogenase** shows a preference for *R*-lipoic acid; while cytosolic **NAPH-dependent glutathione reductase** shows a preference for *S*-lipoic acid. Lipoic acid is added to lipoic acid-dependent enzymes by covalently linking it to the ε-amino group of a lysyl residue through that action of **lipoyltransferase I**. Lipoic acid is catabolized in mitochondria by extensive β-oxidation of the carbon backbone, resulting in at least a dozen apparently inactive metabolites, including 3-ketolipoic acid; 2,4-bismethylmercaptobutanoic acid; and 4,6-bismethylmercaptohexanoic acid.[168]

Metabolic Functions of Lipoic Acid

Lipoic acid has two metabolic functions:

- **Coenzyme** for five multisubunit redox enzymes: two involved in energy metabolism (**α-ketoglutarate dehydrogenase, pyruvate dehydrogenase complex** [specifically, one of its three components, **dihydrolipoyl transacetylase**, also referred to E2[169]]); and three involved in amino acid metabolism (**branched-chain ketoacid dehydrogenase**, **2-oxoadipate dehydrogenase**) and the **glycine cleavage system**. In all cases, catalysis involves the amide form, **lipoamide**, undergoing reversible acylation/deacylation to transfer acyl groups to CoA as well as reversible redox ring opening/closing, coupled with the oxidation of an α-keto acid.
- **Antioxidant** involved in cellular antioxidant protection through both direct and indirect functions. This function is based on the redox cycling between the oxidized disulfide (lipoic acid) and the reduced sulfhydryl (dihydrolipoic acid) forms. This is a potent redox couple (0.32 V reduction potential) capable of reducing oxidized forms of other cellular antioxidants, including glutathione, α-tocopherol, ascorbic acid, and CoQ_{10}, as well as quenching ROS and reactive nitrogen species. Lipoic acid can also chelate metal ions (iron, copper,

162. i.e., The lipoic acid molecule has a carbon atom to which four different moieties are bound, for which reason the molecule lacks an internal plane of symmetry.
163. Mayr, J.A., Feichtinger, R.G., Tort, F., et al., 2014. J. Inherit. Metab. Dis. 37, 553–563.
164. Carreau, J.P., 1979. Meth. Enzymol. 62, 152–158.
165. Exogenous sources of lipoic acid are, however, required by some species of bacteria (*Streptococcus faecalis*, *Lactobacillus casei*) and protozoa (*Tetrahymena geleii*).
166. Arivazhagan, P., Ramanathan, K., Panneerselavam, C., et al., 2001. Chem. Biol. Interact. 138, 189–198.
167. Hermann, R., Niebach, G., Borbe, H.O., et al., 1996. Eur. J. Pharm. Sci. 4, 167–174.
168. The dominant metabolites include bisnorlipoate, tetranorlipoate, β-hydroxy-bisnorlipoate, and the corresponding bis-methylated mercapto derivatives.
169. PDC also includes pyruvate dehydrogenase (E1) and dihydrolipoyl dehydrogenase (E3).

zinc, magnesium), which otherwise catalyze ROS-generating reactions. Its antioxidant properties underlie several metabolic functions of lipoic acid: increasing Nrf2, which induces expression of catalytic and regulatory subunits of γ-glutamylcysteine ligase, the rate-limiting enzyme in glutathione synthesis[170]; stimulating the insulin receptor and inducing phosphorylation of Akt to activate phosphoinositide-3 kinase; participating in the recruitment of the glucose transporter GLUT4 from its Golgi storage site in muscle; protection against bone loss by inhibiting osteoclastogenic ROS[171]; and preventing cysteine oxidation, which otherwise stimulates protein–tyrosine phosphatases.

Dietary Lipoic Acid in Health and Disease

The metabolic effects of dietary lipoic acid are related to age-related deficiencies in its biosynthesis and/or to its antioxidant properties, which over a wide range of exposure can affect cellular "redox tone."

Aging. Studies with animal models have shown lipoic acid supplementation to protect cardiac mitochondria from age-related dysfunction[172] and correct age-related deficits in the activities of mitochondrial enzymes of the TCA cycle and electron-transport chain affecting energy metabolism.[173] Other studies have found lipoic acid supplements and lipoic acid-rich foods to improve cognitive function and motor skills in aged animals,[174] deficits of which are thought to be related to mitochondrial dysfunction.

Antioxidant effects. Dietary lipoic acid has been shown to have value in preventing and/or treating conditions related to oxidative stress:

- **Diabetes**. Lipoic acid supplementation has been shown to enhance glucose utilization and improve glycemic control. Studies with animal models of diabetes have shown that supplemental lipoic acid can reduce glucose uptake by muscle, reduce exercise-induced lipid peroxidation, increase glucose disposal, reduce cataract formation, and improve motor neuron conductivity. Clinical trials with type 2 diabetic patients have found lipoic acid treatment to increase glucose clearance,[175] improve endothelium-dependent vasodilation,[176] and reduce blood glucose levels and lipid peroxidation products.[177] Meta-analyses of clinical trials concluded that lipoic acid treatment (300–600 mg/day) significantly improved diabetic neuropathies of the feet and lower limbs.[178] A clinical study found lipoic acid supplementation to improve visual contrast sensitivity caused by retinal microvascular damage in patients with either type 1 or type 2 diabetes.[179]
- **Cardiovascular health**. **A** small study found oral lipoic acid (300 mg/day for 4 weeks) to improve endothelial-dependent flow-mediated vasodilation in subjects with metabolic syndrome.[180]
- **Multiple sclerosis**—A clinical trial indicated that lipoic acid may be useful in treating patients by reducing serum matrix metalloproteinase-9 and reducing T cell migration into the central nervous system.[181]
- **Alzheimer's disease**—An uncontrolled clinical experiment with a small number of patients with probable Alzheimer's disease reported lipoic acid treatment to stabilize declining cognitive function.[182]
- **High-fat feeding**. Dietary supplementation with lipoic acid has been found to attenuate the oxidative stress, dyslipidemic and immunosuppressive effects of a high-fat diet for mice.[183]

Safety of Lipoic Acid

Lipoic acid is considered safe, having been widely used as in clinical therapy for several decades. Studies in dogs have indicated an LD_{50} of 400–500 mg/kg of body weight; however studies with rats have suggested LD_{50} values four to five times that range.[184] At very high doses, gastrointestinal signs (nausea, abdominal pain, vomiting, diarrhea), allergic skin reactions, and malodorous urine have been reported.

170. Suh, J.H., Shenvi, S.V., Dixon, B.M., et al., 2004. Proc. Natl. Acad. Sci. U.S.A. 101, 3381–3386.
171. Roberts, J.L., Moreau, R., 2015. Nutr. Rev. 73, 116–125.
172. Janson, M., 2006. Clin. Interv. Aging 13, 261–265.
173. Arivazhagan, P., Ramanathan, K., Panneerselavam, C., et al., 2001. Chem. Biol. Interact. 138, 189–198.
174. Stoll, S., Hartmann, H., Cohen, S.A., et al., 1993. Pharmacol. Biochem. Behav. 46, 799–805; Bickford, P.C., Gould, T., Briederick, L., et al., 2000. Brain Res. 866, 211–217; Roudebush, P., Zicker, S.C., Cotman, C.W., et al., 2005. J. Am. Vet. Med. Assoc. 227, 722–728; Head, E., Nukala, V.N., Fenoglio, K.A., et al., 2009. Exp. Neurol. 220, 171–176.
175. Jacob, S., Henricksen, E.J., Tritschler, H.J., et al., 1996. Exp. Clin. Endcrinol. Diabetes 104, 284–288.
176. Heinsch, B.B., Francessconi, M., Mittermayer, F., et al., 2020. Eur. J. Clin. Invest. 40, 148–154.
177. Ziegler, D., Hanefeld, M., Ruhnau, K.J., et al., 1995. Diabetology 38, 1425–1433.
178. Ziegler, D., Nowack, H., Kempler, P., et al., 2004. Diabet. Med. 21, 114–1121; Han, T., Bai, J., Liu, W., et al., 2012. Eur. J. Enodccrinol. 167, 465–471.
179. Gębka, A., Serkies-Minuth, E., Raczyńska, D., 2014. Mediators Inflam. Article ID:131538, 7 pp.
180. Sola, S., Mir, M.Q., Cheema, F.A., et al., 2005. Circulation 111, 343–346.
181. Yadav, V., Marracci, G., Lovera, J., et al., 2005. Mult. Scler. 11, 159–165.
182. Hager, K., Marahens, A., Kenklies, M., et al., 2001. Arch. Gerotol. Geriatr. 32, 275–282.
183. Yang, R.L., Li, W., Shi, Y.H., et al., 2008. Nutrition 24, 582–588; Cui, J., Le, G., Yang, R., et al., 2009. Cell. Immunol. 260, 44–50.
184. Cremer, D.R., Rabeler, R., Roberts, A., 2006. Regul. Toxicol. Pharmacol. 46, 29–42.

7. NONPROVITAMIN A CAROTENOIDS

Of the hundreds of carotenoids that give orange and red colors to foods, a small number are absorbed, and an even smaller number are sequestered in certain tissues. These carotenoids lack β-ionone rings; hence, they cannot be metabolized to retinol. Nevertheless, a few are sequestered in the tissues where they exert important physiological effects, making them **beneficial dietary bioactives**.

Benefits of Nonprovitamin A Carotenoids

The significant fact of biology is that these pigments are accumulated in visual and nervous tissues where they appear to function as screening pigments and antioxidants. These functions would appear to underlie at least some of the demonstrated benefits of diets rich in fruits and vegetables. For some, this is grounds for establishing recommended intakes, although that has not been done.

Benefits of dietary nonprovitamin A carotenoids are as follows:

- Antioxidant protection
- Support of visual function
- Support of neurological function.

Chemical Properties of Nonprovitamin A Carotenoids

The carotenoids are polyisoprenoid compounds produced by plants for the purposes of harvesting light for photosynthesis and quenching free radicals, thereby, protecting plant tissues against oxidative stress. Both functions are due to the capabilities of the conjugated double bond systems of these compounds, which enable them to accept unpaired electrons by delocalizing that electronegativity across multiple carbons. Accordingly, carotenoids have potent antioxidant capabilities. This property allows some carotenoids to function in vision. However, most, i.e., those without the β-ionone head group necessary for that and other vitamin A functions (*see* Chapter 6), lack provitamin A activity. These include some carotenes and oxygenated analogues called **xanthophylls**. The most common nonprovitamin A carotenoids in human diets are **lycopene**, **lutein**, **zeaxanthin**, and **canthaxanthin** (Fig. 19.10).

The most common nonprovitamin A carotenoids in human diets are as follows:
lycopene, lutein, zeaxanthin, and canthoxanthin.

Sources of Nonprovitamin A Carotenoids

Most nonprovitamin A carotenoids are pigments, occurring in red-, yellow-, and orange-colored plant tissues (Table 19.9). The dominant form in US diets is lycopene, the nonaromatic, polyisoprenoid precursor to the biosynthesis of β-carotene in plants.[185] It is found in significant amounts, mainly as the all-*trans*-isomer, in such red-colored foods as tomatoes, watermelon, pink grapefruit, and guava. It is estimated that Americans consume an average of 3–11 mg lycopene daily; estimates from European studies have been similar. The xanthophyll lutein is present in significant concentrations in spinach, kale, corn, broccoli, collards, and eggs. Lutein and zeaxanthin in corn represent major sources of pigmentation for poultry diets.[186] The xanthophyll **astaxanthin** is the source of pink coloration of salmon.

Absorption and Transport of Nonprovitamin A Carotenoids

Nonprovitamin A carotenoids in foods are utilized in the same ways as their provitamin A counterparts (*see* Chapter 6). Their utilization as carotenoids depends on their not being cleaved by the carotene oxygenases.[187] The enteric absorption of nonprovitamin A carotenoids occurs by micelle-dependent diffusion, which depends on the presence of luminal fat and is subject to the antagonistic effects of binding to heat-labile food proteins. Hence, cooking or heat processing improves the bioavailability of lycopene from tomato products. Carotenoids enter the circulation as components of chylomicra from which they are moved to lipoproteins. Most are transported by HDL; however, lutein and zeaxanthin are equally distributed among HDL and LDL. Because the transfer of lipoprotein lipid contents depends on interactions with cell surface receptors, it is thought that the distribution of lutein and zeaxanthin to peripheral tissues, and the retinal capture of lutein and zeaxanthin in particular, depends on an individual's particular lipoprotein (particularly, apoE) profile. Serum levels of lycopene appear to decline with age, but that affect appears to be related to the lower consumption of fat and lycopene-rich, tomato-based foods by older adults (Fig. 19.11).[188]

Tissue distribution. Of the some three dozen carotenoids that have been identified in human serum, lycopene is preferentially accumulated by the prostate, and lutein and zeaxanthin are preferentially captured by the retinal pigment epithelium, the frontal lobe, and visual processing regions of the brain (Fig. 19.12). The human retina contains 25–200 ng of these pigments, zeaxanthin being concentrated in the center and lutein being distributed about the periphery. Greatest concentrations

185. Plants convert lycopene to β-carotene by forming to β-rings at its ends through the action of lycopene cyclase.
186. i.e., To promote coloration of egg yolks and broiler skin.
187. Carotenoid cleavage activity is highly variable between individuals; this is explained in part by single nucleotide polymorphisms in the carotene oxygenase-1 (BCO1) (Leitz, G., Lange, J., Rimbach, G., 2010. Arch. Biochem. Biophys. 502, 8–16.
188. Ganji, V., Kafai, M.R., 2005. J. Nutr. 135, 567–572.

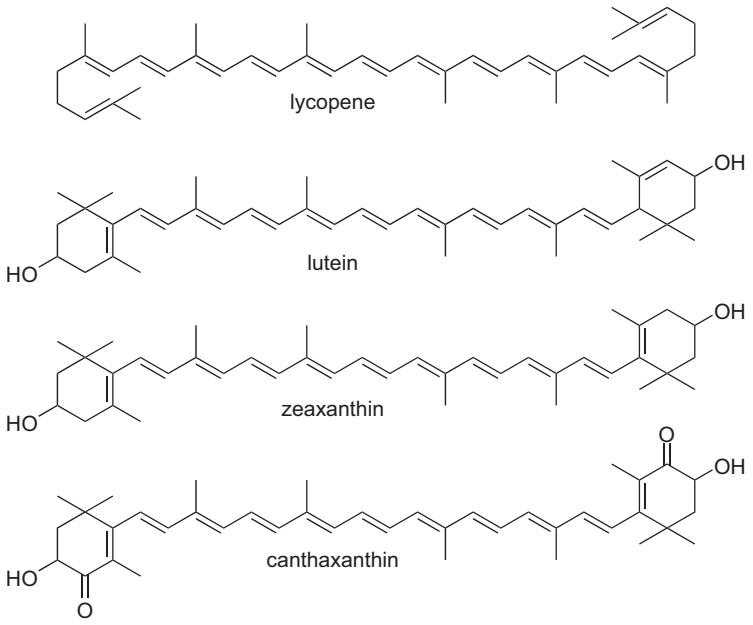


FIGURE 19.10 Structures of major nonprovitamin A carotenoids.

accumulate in the central region of the retina, known as the macula, giving that region, its characteristic yellow color.[189] Lutein and zeaxanthin are typically present in greatest amounts in the fovea (in a 2:1 molar ratio) but their concentrations decrease 100-fold in the periphery. The capture of the macular pigments is believed to be facilitated by a **xanthophyll-binding protein (XBP)**. Adipose tissue may also serve as a storage site for xanthophylls, as serum levels of lutein and zeaxanthin increase in response to weight loss, while macular pigment density is unaffected.[190] Retention of other flavonoids, notably quercetin in liver and naringenin in muscle, is effected by prenylation.[191]

Metabolism of Nonprovitamin A Carotenoids

Lycopene. The dominant forms of lycopene in the serum are its *cis*-isomers, which comprise 50–90% of the circulating carotenoid. This appears to be due to both the better absorption of that isomer than the dominant all-*trans*-isomer found in plant tissues, as well as the continuous *cis*-isomerization of the latter in the body.[192] In humans, lycopene has a half-life of 5 days; it is turned over by conversion to more polar metabolites. The enzyme **carotene oxygenase II** is thought to be involved in this metabolism, producing acylo-retinoids and carbonyl compounds, which are subject to autoxidation or radical-mediated oxidation. A large number of lycopene degradation products are possible.[193]

Lutein and zeaxanthin. While the macular pigments are exclusively of dietary origin, they include a zeaxanthin isomer, 3R,3′S-*meso*-zeaxanthin, that is *not* found in the diet. This isomer appears to be formed by the oxidation–reduction and double-bond isomerization of lutein (i.e., 3R,3′R,6′R-lutein). It is not found in other tissues, suggesting that it may be catalyzed photochemically in the retina or by enzymes expressed only in that tissue. Studies have shown that dietary supplementation with lutein and zeaxanthin increases the macular pigments in two ways: by direct capture and by stimulating the migration of retinal pigment epithelial cells to the macula.[194]

Metabolic Functions of Nonprovitamin A Carotenoids

Antioxidant. **Lycopene** is the most potent carotenoid antioxidant in vitro, being twice as effective (due to its extended conjugated diene system) than β-carotene in quenching singlet oxygen ($^1O_2$) and 10 times as effective as α-tocopherol. Whether this activity is the basis of its beneficial health effects is not clear, as tissue levels of lycopene tend to be much lower than those other antioxidants; such

189. Macular pigments occur in greater amounts in the central area, i.e., the **fovea**. Other mammals lack maculae; however, carotenoid-rich oil droplets have been found in the retinas of birds, reptiles, amphibians, and fish.

190. Kirby, M.L., Beatty, S., Stack, J., et al., 2011. Br. J. Nutr. 105, 1036–1046.

191. Terao, J., Mukai, R., 2014. Arch. Biochem. Biophys. 559, 12–16.

192. Unlu, N.Z., Bohn, T., Francis, D.M., 2007. Br. J. Nutr. 98, 140–146; Ross, A.B., Vuong, L.T., Ruckle, J., 2011. J. Nutr. 93, 1263–1273.

193. Several have been identified in humans or animals: 5,6-dihydroxy-5,6-dihydrolycopene; 2,6-cyclolycopene-1,5-diols; 5,6-dihydrolycopene; 5,6-dihydro-5-*cis*-lycopene; apo-8′-lycopenal; apo-10′-lycopenal; apo-10′-lycopenoic acid.

194. Leung, I.Y., Sandstrom, M.M., Zucker, C.L., et al., 2004. Invest. Ophthalmol. Vis. Sci. 45, 3244–3256.

TABLE 19.9 Food Contents of Nonprovitamin A Carotenoid Contents (μg/100 g or μg/100 mL)

Food	Lutein	Zeaxanthin	Lycopene
Fruits			
Apricot	123–188	0–39	54
Banana	86–192		0–254
Fig	80		320
Grapefruit, red			750
Guava			769–1816
Kiwi			<10
Mango			10–724
Nectarine, flesh			2–131
Papaya	93–318		0–7564
Pineapple			265–605
Watermelon, red			4,770–13,523
Watermelon, Yellow			56–287
Vegetables			
Avocado	213–361	8–18	
Basil	7050		
Bean, green	883		
Cabbage, white	450		
Carrot	254–510		
Cress	6510–7540		
Cucumber	459–840		
Dill weed	13,820		
Egg plant	170		
Endive	2060–6150		
Kale	4,800–11,470		
Leek	3680		
Lettuce	1000–4780		
Parsley	6,400–10,650		
Peas, green	1910		
Pepper, green	92–911	0–42	
Pepper, red	248–8506	593–1350	
Pepper, yellow	419–638		
Potato, sweet	50		
Pumpkin	630		500
Rhubarb			120
Sage	6350		
Spinach	5930–7900		

Continued

TABLE 19.9 Food Contents of Nonprovitamin A Carotenoid Contents (μg/100 g or μg/100 mL)—cont'd

Food	Lutein	Zeaxanthin	Lycopene
Tomato	46–213		850–12,700
Tomato ketchup			4,710–23,400
Tomato sauce			5,600–39,400
Grains			
Corn, flakes	0–52	102–297	
Durum lour	164		
Wheat flour	76–116		
Other			
Olive oil	350		
Butter	15–25	0–2	
Egg yolk	384–1320		
Milk, 4% fat	0.8–1.4	0–0.1	

Adapted from Maiani, G., Castón, M.J.P., Catasta, G., et al., 2009. Mol. Nutr. Food Res. 53, S194–S218.

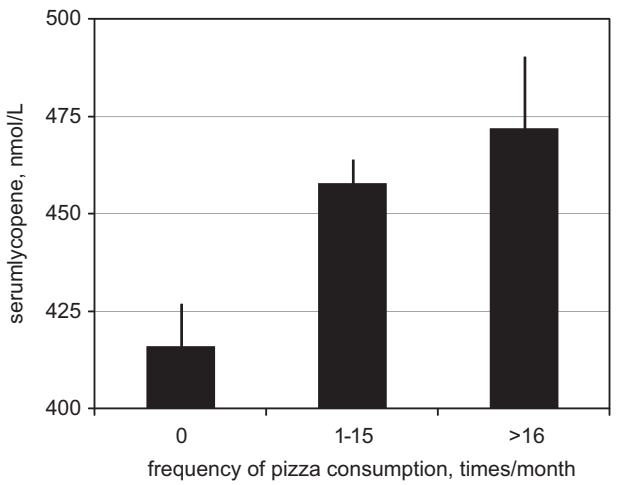


FIGURE 19.11 Relationship of serum lycopene concentration to pizza consumption in the NHANES 1988–94. *Ganji, V., Kafai, M.R., 2005. J. Nutr. 135, 567–572.*

effects may be mediated by lycopene metabolites (e.g., apo-10′-lycopenoid acid). These, and other carotinoids, have been shown to induce superoxide dismutase, glutathione-S-transferase, quinone reductase, and several phase I and II enzymes. Therefore, these responses appear to involve upregulation of **antioxidant response elements (ARE)**. Studies have found lycopene to have a very low affinity for the retinoid X receptor α (*see* Chapter 6) and, thus, to be a very weak transactivator of genes using the retinoic acid response element (RARE). The metabolite apo-10′-lycopenoid acid, however, has been shown to induce RARβ mRNA expression in some cell lines.[195] A meta-analysis of published epidemiological data found evidence that higher intakes of lutein being associated with lower risks of coronary heart disease and stroke.[196]

Vision. Lutein and zeaxanthin comprise the pigment of the posterior central region of the retina, i.e., the macula, where they are typically present in higher concentrations than in any other tissue. These carotenoids protect the macula from the damaging effects of blue-wavelength photons, as they absorb in the range of 420–480 nm, reducing by as much as 90% the incoming energy in this range from reaching macular photoreceptors. They accumulate selectively in the most vulnerable domains of membranes—those containing unsaturated phospholipids. Lutein and zeaxanthin can also scavenge ROS formed in photoreceptors, likely as a results of extramitochondrial oxidative phosphorylation in the rod outer segment. Light-catalyzed oxidative reactions also occur in the retina, as indicated by the accumulation of the autofluorescent pigment **lipofuscin**.[197] These xanthophylls support visual

195. Lian, F., Smith, D.E., Ernst, H., et al., 2007. Carcinogenesis 28, 1567–1574.

196. Leermakers, E.T.M., Darweesh, S.K.L., Baena, C.P., et al., 2016. Am. J. Clin. Nutr. 103, 481–494.

197. This appears to result from the condensation of two molecules of all-*trans*-retinal with one of phosphatidylethanolamine, which complex is taken up by the retinal pigment epithelium and converted to a stable pyridnium bis-retinoid that is cytotoxic and causes apoptosis and, hence, macular degeneration.

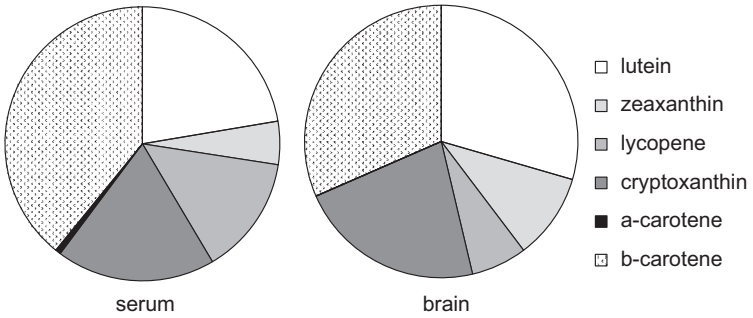


FIGURE 19.12 Distribution of carotenoids in serum and brain (average of cerebellum and frontal, occipital, and temporal cortices) of centenarian decedents. *After Johnson, E.J., Vishwanathan, R., Johnson, M.A., et al., 2013. J. Aging Res. Article ID:951786, 13 pp.*

development and protect against cataracts and age-related macular degeneration (AMD)[198]:

- **Visual function**. Evidence indicates that macular pigment carotenoids support visual acuity by reducing the effects of chromatic aberration or by preferentially absorbing short-wavelength dominant air light that produces veiling luminance in distance viewing (i.e., blue haze). Two well-controlled, randomized trials found lutein supplementation to improve central visual field and reduce loss of central field sensitivity.[199]
- **Cataracts**.[200] The human lens contains significant amounts of **lutein** and **zeaxanthin**, most of which is located in the epithelium and cortex. These antioxidant carotenoids are thought to be important in reducing the photoperoxidation of lens lipids both by quenching free radicals and by maintaining lens glutathione in the reduced state. Several cohort studies have produced strong evidence for relatively high intakes of lutein, zeaxanthin, and/or **lycopene** being associated with reduced risk for nuclear cataracts. One study found lutein supplementation (7 mg/day, i.e., the equivalent of ~100 g spinach) to improve visual acuity and reduce glare sensitivity in subjects with age-related cataracts.[201]
- **AMD**.[202] Evidence suggests that the macular pigments lutein and zeaxanthin may protect against AMD. A case–control study found that high consumption of carotenoid-rich foods was associated with a 43% reduction in risk for neovascular AMD.[203] The Age-Related Eye Disease Study (AREDS) found that individuals in the highest quintile of lutein and zeaxanthin consumption had risk reductions of 27–55% for various signs of AMD.[204] A randomized trial using lutein plus other antioxidants as the intervention agent in patients with progressive atrophic macular degeneration reported improvements in several aspects of visual acuity[205]; other trials found no protective effect of similar treatments.[206] These apparently discrepant findings may be due to different genetic susceptibilities to AMD in those cohorts, as intervention with lutein, zeaxanthin and other antioxidants was found effective in reducing AMD risk only in subjects with the greatest genetic risk.[207] AMD patients are advised to consume low-glycemic diets rich in green, leafy vegetables and to consume at least two

198. Vishwanathan, R., Johnson, E.J., 2012. In: Erdman, J.W., Macdonald, I.A., Zeisel, S.H. Eds.), Present Knowledge in Nutrition, tenth ed. Wiley-Blackwell, Ames, IA, pp. 939–981.

199. Bahrami, H., Melia, M., Dagnelie, G., 2006. BMC Ophthalmol. 6, 23; Berson, E.L., Rosner, B., Sanmdberg, M.A., et al., 2010. Arch. Ophthalmol. 122, 403–411.

200. Age-related cataract is a major cause of visual impairment and blindness, affecting as many as 50 M people worldwide and present in clinically significant ways in almost half of Americans 75–85 yrs, increasing from ~8% in those 52–64 yrs. Cataract can occur in different parts of the lens: center (nuclear, the most common), outer rim (cortical), or central posterior cortex (posterior subcapsular).

201. Olmedilla, B., Granado, F., Blanco, I., et al., 2003. Nutrition 19, 21–24.

202. AMD is a leading cause of severe vision loss in industrialized countries. It affects ~1.75 million Americans, including ~8% of adults 43–54 years. and ~30% of those >75 yrs with early signs (7% of the latter group with advanced disease). It affects the macula, which is responsible for high-acuity vision by virtue of its high density of concentration of cones. The fist sign of AMD is the accumulation of extracellular debris ("drusen") between the retinal pigment epithelium (RPE) and its basement membrane due to the phagocytic failure of RPE cells.

203. Seddon, J.M., Ajani, U.A., Sperduto, R.D., et al., 1994. JAMA 272, 1413–1420.

204. AREDS, 2007. Arch. Ophthamol. 125, 1225–1232.

205. Richer, S., Stiles, W., Statkute, L., et al., 2004. Optometry 75, 216–230.

206. Van den Langenberg, G., Mares-Perlman, J., Klein, R., et al., 1998. Am. J. Epidemiol. 148, 204–214; Taylor, H., Tikellis, G., Robman, L., et al., 2002. Br. Med. J. 325, 11–14; Moeller, M.S., Parekh, N., Tinker, L.L., et al., 2006. Arch. Ophthalmol. 124, 1151–1162; Bartlett, H.E., Eperjesi, F., 2007. Eur. J. Clin. Nutr. 61, 1121–1127.

207. Wang, J.J., Buitendijk, G.H.S., Rochtchina, E., et al., 2014. Ophthalmology 121, 667–675.

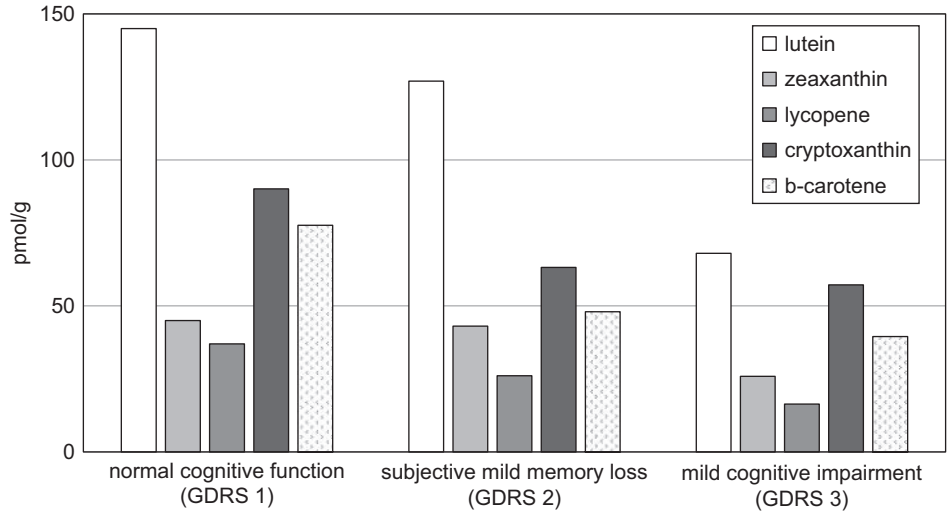


FIGURE 19.13 Carotenoids in brains (average of cerebellum and frontal, occipital and temporal cortices) of centenarian decedents according to premortem Global Deterioration Scores. *After Johnson, E.J., Vishwanathan, R., Johnson, M.A., et al., 2013. J. Aging Res. Article ID:951786, 13 pp.*

servings of fish weekly.[208] The use of an AREDS-based supplement (400 IU vitamin E, 500 mg vitamin C, 10 mg lutein, 2 mg zeaxanthin, 80 mg zinc, and 2 mg copper) is the most effective means of slowing the progression of atrophic AMD.[209]

Neurologic function. Lutein and zeaxanthin comprise more than two-thirds of the carotenoids in the frontal lobe and visual processing regions of the brain, with concentrations highly correlated with those of the retina. In vitro studies have shown that lutein can have a structural role in PUFA-rich neural membranes while also influencing interneuronal and neural–glial cell communications.[210] That such functions occur in vivo and affect visual processing is indicated by findings that the proxy variable macular pigment density was directly related to visuomotor ability in older subjects.[211] A study of octagenarians and centigenarians found that the serum lutein, zeaxanthin, and β-carotene were associated with better cognition, and that brain lutein and β-carotene concentrations determined postmortem were positively related to cognition (Fig. 19.13).[212] A double-blinded, randomized intervention trial found supplementation with lutein (12 mg/day) to improve verbal fluency, and supplementation with both lutein and docosahexaenoic acid (800 mg/day) to improve memory and learning rate.[213]

Dietary Nonprovitamin A Carotenoids in Health and Disease

Anticarcinogenesis. Epidemiological investigations have shown consumption of tomato-rich diets to be associated with reduced risk to cancers of the prostate and lung.[214] A meta-analysis of 31 prospective studies of breast cancer found β-carotene to be the only carotenoid intake of which was related (negatively) to breast cancer risk. A case–control analysis of 3004 women in the EPIC[215] cohort found significant inverse relationships of plasma concentrations of β- and α-carotenes and risk for estrogen receptor-negative (ER^-) breast cancer risk, but no relationship with ER^+ breast cancers.[216] One study found serum lutein concentration to be inversely related to breast cancer risk.[217] A meta-analysis of 11 case–control studies and 10 cohort or nested case–control studies found prostate cancer risk to be inversely related

208. Consumption of n-3 PUFAs has been shown to prevent and reduce progression of retinal lesions in animal models (Pinazo-Durán, M.D., Gómez-Ulla, F., Arias, L., et al., 2014. J. Ophthalmol. Article ID:901686).
209. Broadhead, G.K., Grigg, J.R., Chang, A.A., et al., 2015. Nutr. Rev. 73, 488–462.
210. Stahl, W., Sies, H., 2001. Biofactors 15, 95–98; Johnson, E.J., McDonald, K., Caldarella, S.M., et al., 2008. Nutr. Neurosci. 11, 75–98.
211. Hammond, B.R., Fletcher, L.M., 2012. Am. J. Clin. Nutr. 96, 1207S–1213S.
212. Johnson, E.J., Vishwanathan, R., Johnson, M.A., et al., 2013. J. Aging Res. Article ID:951786, 13 pp.
213. Johnson, E.J., McDonald, K., Caldarella, S,M., et al., 2008. Nutr. Neurosci. 11, 75–83.
214. Giovannucci, E., 2002. Exp. Biol. Med. 227, 852–859; Giovannucci, E., 2005. J. Nutr. 135, 2030S–2031S; Arab, L., Steck-Scott, S., Fleishaur, A.T., 2002. Exp. Biol. Med. 227, 894–899.
215. European Prospective Investigation into Cancer and Nutrition; this ongoing study includes 521,468 subjects managed in 23 centers in 10 countries.
216. Bakker, M.F., Peeters, P.H.M., Klaasen, V.M., et al., 2016. Am. J. Clin. Nutr. 103, 454–464.
217. A 25 μg/dL increment in serum lutein was associated with a 32% reduction in breast cancer risk. Aune, D., Chan, D.S.M., Vieira, A.R. et al., 2012. Am. J. Clin. Nutr. 96, 356–373.

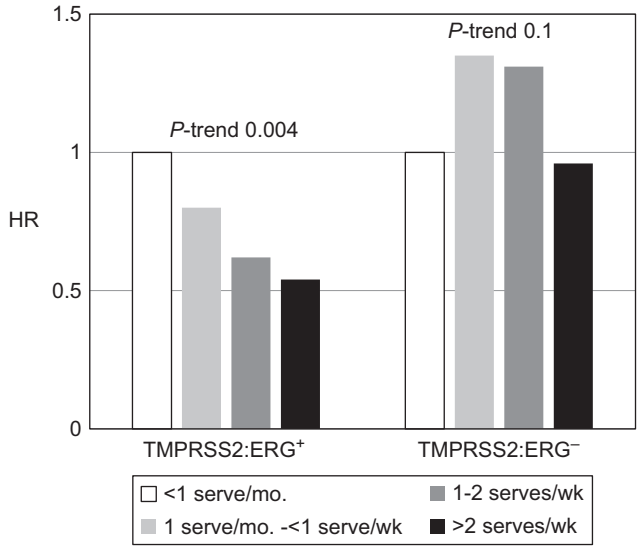


FIGURE 19.14 Relationship of tomato sauce intake and prostate cancer risk by TMPRSS2:ERG status. *Data are expressed as hazards ratios (HRs) comparing each intake group to the lowest one. After Graff, R.E., Pettersson, A., Lis, R.T., et al., 2016. Am. J. Clin. Nutr. 103, 851–860.*

to serum concentration of **lycopene**,[218] suggesting risk reduction of 25–30%. A more recent meta-analysis of 24 case–control and nested case–control studies found both dietary intake and plasma/serum levels of lycopene plus β-carotene (but not β-carotene alone) to be associated with reduced risk of prostate cancer.[219] A recent evaluation of the 23 years of follow-up of 884 man subset of the Health Professionals Follow-up Study found that the intake of tomato sauce was inversely related to risk of developing prostate cancer.[220] This effect was found to be driven by a strong protective effect for men expressing the transmembrane protease, TMPRSS2:ERG (Fig. 19.14).[221]

Studies with animal tumor models have shown lycopene treatment to affect molecular mechanisms associated with antitumorigenesis, e.g., reduced cell proliferation, increased apoptosis, reduced markers of oxidative stress. Two studies have found lycopene to reduce the incidence of spontaneous or chemically induced mammary cancers in the rat and of chemically induced lung cancers in male (but not female) mice. Lycopene has been found to cause cell cycle arrest and apoptosis in cultured prostate cancer cells, which is associated with upregulation of the expression of intercellular gap junction communication associated with decreased cell proliferation.[222] Only a few intervention studies have been conducted. One found lycopene supplementation to reduce cancer biomarkers and disease progression in patients with benign prostatic hyperplasia[223]; another found no effects on progression of high-grade prostatic intraepithelial neoplasias.[224]

Skin health. Epidemiological studies have pointed to a protective effect of tomato-rich diets against UV damage to the skin. A randomized trial found that consumption of lycopene-rich tomato paste conferred significant protection against UV damage in a small cohort of healthy women, increasing the median erythemal dose by 16%.[225]

Biomarkers of Nonprovitamin A Carotenoid Status

Carotenoid status can be assessed in two ways:

- **Plasma carotenoids** can be determined in plasma by visual absorbance after chromatographic separation. The responses of plasma lutein, lycopene, and β-carotene to meals containing those carotenoids are highly variable between individuals. The postprandial plasma lutein response has been found to be affected by at least 15 genes and single-nucleotide polymorphisms related to lutein metabolism and the chylomicron triacylglycerol response.[226] Serum lycopene levels have been similarly found to be affected by polymorphisms in at least three genes.[227]
- **Skin carotenoids** can be assessed in the skin optically by resonance Raman spectroscopy palmer scanning.[228] This method yields results that are correlated with plasma total carotenoid analyses (Fig. 19.15) and off the advances of a rapidly, noninvasive procedure.

Safety of Nonprovitamin A Carotenoids

Both **lycopene** and **lutein** are generally regarded as safe. A systematic risk review found no reports of adverse effects for either lutein (highest chronic exposures noted for

218. Emtinan, M., Takkouche, B., Caamano-Isoma, F., 2004. Cancer Epidemiol. Biomarkers Prev. 13, 340–345.
219. Wang, Y., Cui, R., Xiao, Y., et al., 2015. PLoS One 10, e0137427.
220. Graff, R.E., Pettersson, A., Lis, R.T., et al., 2016. Am. J. Clin. Nutr. 103, 851–860.
221. Serine 2:v-ets avian erythrocyte blastosis virus E26 oncogene homologue; this protein is expressed by ~50% of patients with prostate cancer.
222. i.e., Connexin 43; Heber, D., Lu, Q.Y., 2002. Exp. Biol Med. 227, 920–923.
223. Schwarz, S., Obermüller-Jevec, U.C., Hellmis, E., et al., 2008. J. Nutr. 138, 49–53.
224. Mariani, S., Lionetto, L., Cavallari, M., et al., 2014. Int. J. Mol. Sci. 15, 1433–1440.
225. Rizwan, M., Rodriguez-Blanco, I., Harbottle. A., et al., 2010. Br. J. Dermatol. 154, 154–162.
226. Borel, P., Desmarchelier, C., Nowicki, M., et al., 2014. Am. J. Clin. Nutr. 100, 168–175.
227. Zubair, N., Kooperberg, C., Liu, J., et al., 2015. J. Nutr. 145, 187–192.
228. Mayne, S.T., Cartmel, B., Scarmo, S., et al., 2013. Arch. Biochem. Biophys. 539, 163–170.

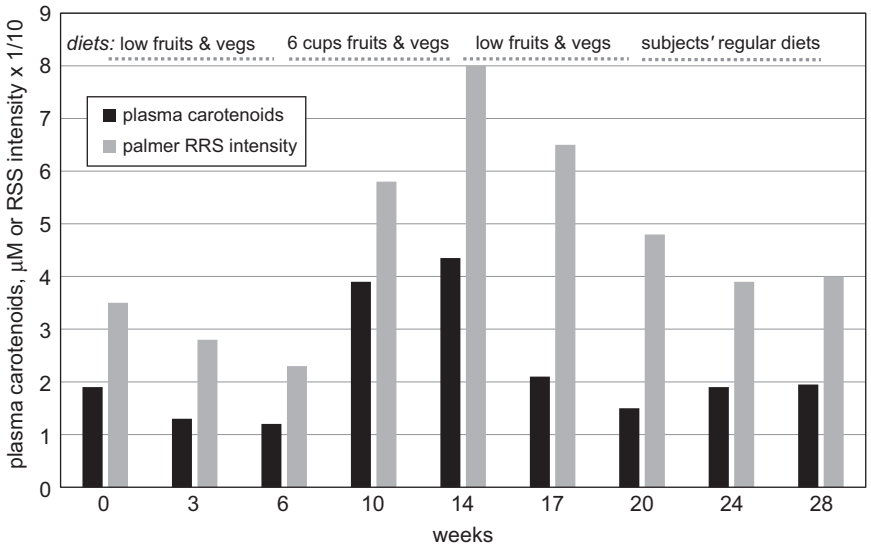


FIGURE 19.15 Responses of plasma carotenoids and palmar scanning by Raman resonance spectrometry (RRS) to changes in intakes of carotenoid-containing fruits and vegetables. In phases I (0–6 wks) and III (14–20 wks), subjects were asked to avoid carotenoid-containing fruits and vegetables. In phase II (6–14 wks), they were fed a diet that provided 1–2.5 cups of fruits and 3–4.5 cups of vegetables daily. In phase IV (20–28 wks) they returned to their regular self-selected diet. *After Jahns, L., Johnson, L.K., Mayne, S.T., et al., 2014. Am. J. Clin. Nutr. 100, 930–937.*

humans were 40 mg/day[229]) or lycopene (highest chronic exposures for humans were 150 mg/day).[230] Therefore, the observed safe levels (OSLs) for these carotenoids were set at ≤20 mg/day for lutein, ≤75 mg/day for lycopene.[231] However, because these OSLs were calculated without the benefit of no- or low-observed effect levels (NOAELs or LOAELs), they are necessarily cautious underestimates. Actual safe upper limits of exposure are likely to be at least several times greater.

8. FLAVONOIDS

Flavonoids are ubiquitous plant metabolites. More than 6000 different flavonoids have been identified, representing the major sources of red, blue, and yellow plant pigments other than the carotenoids. They have a wide variety of functions in plants: natural antibiotics,[232] predator feeding deterrents, photosensitizers, UV-screening agents, metabolic modulators. Several flavonoids and flavonoid-containing food have been found to have health benefits, making them **beneficial dietary factors**.

Recognition of Nutritional Roles of Flavonoids

The flavonoids were discovered by Szent–Györgyi as the component of lemon juice or red pepper that potentiated the antiscorbutic activities of those foods for the guinea pig. That factor was called by various groups "citrin," "vitamin P,"[233] and "vitamin C_2," but it was ultimately found to be a mixture of phenolic derivatives of 2-phenyl-1,4-benzopyrane, the flavane nucleus.

Benefits of Dietary Flavonoids

None of the flavonoids meet the criteria of essential nutrients. Still, it is clear that at least some of this diverse group of natural components of foods are beneficial to health. So, while there presently appears to be no situations of conditional need for flavonoids, there is clear wisdom in including sources of flavonoids in healthy diets. Diets containing such sources, particularly those that provide significant amounts of flavonols, flavones, flavanones, and anthocyanins, have been associated with greater likelihood of health and well-being in older age (Figs. 19.16 and 19.17).[234]

Benefits of dietary flavonoids are as follows:
- Protection against inflammation and related disorders
- Promotion of immune function
- Reduced risks for chronic disease

Chemical Properties of Flavonoids

Flavonoids are secondary metabolites of shikimic acid.[235] They are polyphenolic compounds containing two aromatic

229. Dagnelie, G., Zorge, I.S., McDonald, T.M., 2000. Optometry 71, 147–164.
230. Rao, A.V., Agarwal, S., 1998. Nutr. Cancer 31, 199–203.
231. Shao, A., Hathcock, J.N., 2006. Reg. Toxicol. Pharmacol. 45, 289–298.
232. e.g., Phytoalexins, antimicrobial plant metabolites which accumulate in infected tissues.
233. *P* indicated the *permeability* vitamin, because it improved capillary permeability.
234. Samieri, C., Sun, Q., Townsenmd, M.K., et al., 2014. Am. J. Clin. Nutr. 100, 1489–1497.
235. (3*R*,4*S*,5*R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid.

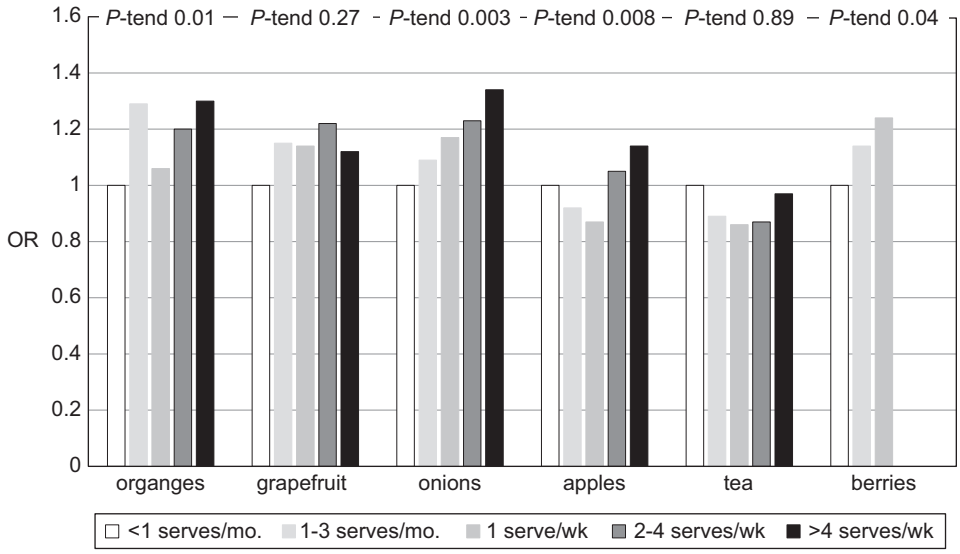


FIGURE 19.16 Relationship of intake of flavonoid-rich foods and healthy aging. Data from the Nurses' Health Study presented by quintiles of each class of flavonoid intake; relative healthy aging scores are presented as odds ratios (ORs) for each of the higher quintiles (Q2-Q5) compared to lowest quintile (Q1), n's 242–373. *After Samieri, C., Sun, Q., Townsend, M.K., et al., 2014. Am. J. Clin. Nutr. 100, 1489–1497.*

rings linked by an oxygen-containing, heterocyclic ring (Fig. 19.18). The hydroxyl groups of these polyphenols enable them to form glycosidic linkages with sugars, and most flavonoids occur naturally as glycosides.

There are six general classes of flavonoids, classified by their common ring substituents:

- **Flavonol**s (R_3 hydroxy-, R_4 keto-derivatives) include quercetin, kaempferol, isorhamnetic and myricetin, the most abundant flavonoids in human diets. Flavonols are found in different fruits and vegetables, often as glycosides. Relatively high amounts (15–40 mg/100 g) are found in broccoli, kale, leeks, and onions.
- **Flavanols** (R_3 hydroxy derivatives), also called **catechins**, do not exist as glycosides (unlike other flavonoids). They include catechin, epicatechin, epigallocatechin, and their gallate derivatives found in apples, apricots, and red grapes (2–20 mg/100 g); green tea and dark chocolate are rich in catechins (40–65 mg/100 g).
- **Flavones** are a group of some 300 compounds that retain the basic flavane nucleus structure. It includes apigenin and luteolin, which occur in high concentrations (>600 mg/100 g) in parsley, and in lower but significant amounts in cereal grains, celery, and citrus rinds (as polymethoxylated forms).
- **Anthocyanins** (R_3 and R_4 reduced derivatives) exist as glycosides; their aglycones are called anthocyanidins of which there are several hundred, the most common being cyanidin, delphinidin, malvinidin, pelargonidin, peonidin, petunidin, and malvidin. Most are red or blue pigments. The richest sources (up to 600 mg/100 g) are raspberries, black berries, and blue berries; cherries, radishes, red cabbage, red skinned potato, red onions, and red wine are also good sources (50–150 mg/100 g). Anthocyanins have antioxidant properties. Unlike other flavonoids, anthocyanins are relatively unstable to cooking and high-temperature food processing.
- **Flavanones** (R_4 keto, "C" ring reduced derivatives) are found primarily in citrus pulp (15–50 mg/100 g) where they are also present as *O*- and *C*-glycosides and methoxylated derivatives. They include eriocitrin, neoericitrin, hesperetin, neohespiridin, naringin, narirutin, didymin, and poncirin.
- **Isoflavones** ("B" aromatic ring derivatives linked at R_3) are contained only in legumes, mostly as glycosides. They include daidzein, genistein, and glycitein, which are also referred to as **phytoestrogens** due to the affinities of their 7- and 4'-hydroxyl groups to binding mammalian estrogen receptors. Soy products (soy flour, tofu, tempeh) can contain 25–200 mg/100 g.
- **Tannins** are polymeric flavonoids present in all plants. Those conjoined by covalent, nonhydrolyzable C–C bonds are called **condensed tannins** or **proanthocyanidins**. Others containing hydrolysable nonaromatic polyol carbohydrate moieties include gallic and ellagic acids and have strong antioxidant properties in vitro.

Dietary Sources

Dietary intake of flavonoids varies widely according to dietary habits and preferences. Americans are estimated to consume ~190 mg/person/day, mostly as flavonols.[236] Similar estimates have been made for northern Europeans.

236. Chun, O.K., Chung, S.J., Song, W.O., 2007. J. Nutr. 137, 1244–1252.

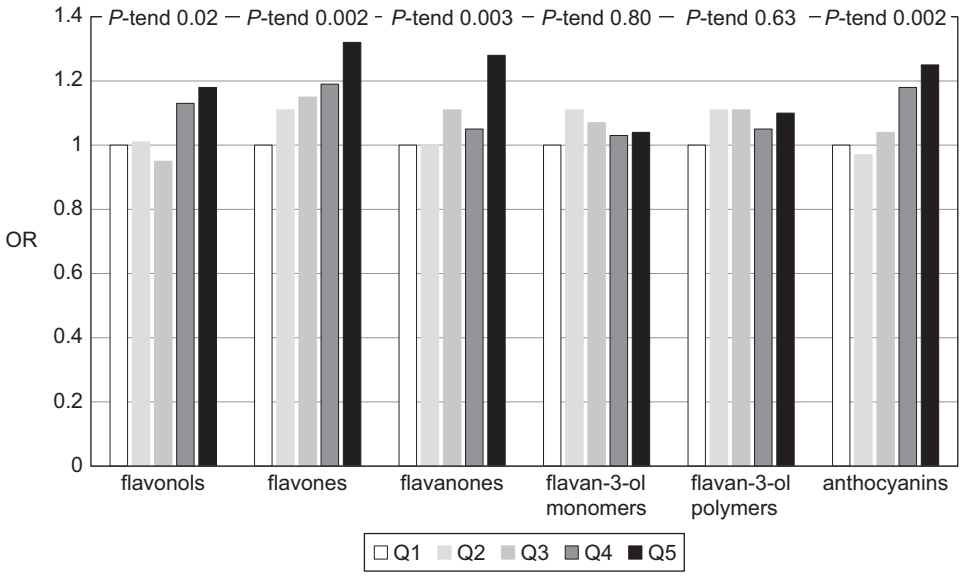


FIGURE 19.17 Relationship of flavonoid intake and healthy aging. Data from the Nurses' Health Study presented by quintiles of each class of flavonoids imputed from food intake data; relative healthy aging scores are presented as odds ratios (ORs) for each of the higher quintiles (Q2-Q5) compared to lowest quintile (Q1), n's 242–373. *After Samieri, C., Sun, Q., Townsend, M.K. et al., 2014. Am. J. Clin. Nutr. 100, 1489–1497.*

The greatest contributors of flavonoids in human diets are fruits and vegetables, fruit juices, green tea, and dark chocolate (Table 19.10). Most flavonoids tend to be concentrated in the outer layers of fruit and vegetable tissues (e.g., skin, peel). In general, the flavonoid contents of leafy vegetables are high; whereas, those of root vegetables are low, with the notable exception of red-skinned onions. The flavonoid contents of vegetables and fruits can vary between cultivars; for example, the quercetin contents of six commercial onion varieties were found to vary by 18-fold.[237] The greatest contributors to total flavonoid intakes tend to be tea, citrus fruits and juices, and wine. Flavonoid aglycones are stable during food processing and cooking; however, anthocyanidins are unstable to such conditions.

Absorption and Transport of Flavonoids

Absorption. Most flavonoids in foods occur as glycosides, which must be hydrolyzed by glycosidases in saliva and brush border of the intestine to be absorbed. The efficiency of these processes appears to be low, e.g., <10%, as many dietary flavonoids are esterified in nonhydrolyzable ways. Glucosylated flavonoids (e.g., quercetin, hesperetin) are hydrolyzed; their aglycone moieties are absorbed by the Na^+-dependent glucose transporter-1 (SGLT-1), as are nonglycosylated flavonoids (e.g., epicatechin, epigallocatechin).

The large portions of flavonoids that are not absorbed are metabolized by the hindgut microbiome. This includes anthocyanins, procyanidins, and flavonoids linked to glucose and rhamnose, e.g., hesperidin, naringin, and rutin.

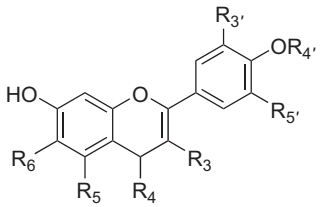


FIGURE 19.18 General structure of flavonoids.

Microbial metabolites of these flavonoids appear to be absorbed across the colon and are likely to be biologically active. Indeed, it has been suggested that the demonstrated benefits of black tea (and, perhaps, other) flavonoids in reducing inflammation, lowering blood pressure, and improving platelet and endothelial cell functions may be mediated by specific microbial metabolites.[238] The hindgut microbiome responds to the host's consumption of dietary flavonoids. This was demonstrated by the finding that pigs fed cocoa powder (a source of flavan-3-ols, epicatechin and catechin) showed increased abundance of *Lactobacillus* and *Bifidobacterium* species in their colonic microbiome.[239]

Transport. Most absorbed flavonoids are conjugated in the liver such that glucuronides, sulfates, and methylated derivatives comprise the dominant forms in the circulation. The notable exception is epigallocatechin gallate (EGCG), which circulates predominantly in unconjugated form.

237. Lee, J., Mitchell, A.E., 2011. J. Agr. Food Chem. 59, 857–863.

238. Van Duynhoven, J., Vaughan, E.E., van Dorsten, F., et al., 2013. Am. J. Clin. Nutr. 98, 1631S–1641S.

239. Jang, S., Sun, J., Chen, P., et al., 2016. J. Nutr. 146, 673–680.

TABLE 19.10 Dietary Sources of Flavonoids

Type	Flavonoid	Food sources
Flavonols	Quercetin, kaempferol, myricetin, isorhamnetin	*Rich sources:*[a] capers, dock, lovage leaves Apple, beans, black grapes, broccoli, buckwheat, carob, chokecherry, coriander, cranberry, elderberry, fennel leaves, ginger, Goji berry (wolfberry), juniper berries, kale, cress, mustard greens, New Zealand spinach, okra, onions, peppers (hot, sweet), plums, radicchio, radish leaves, sweet potato leaves
Flavanols	Epigallocatechin, epicatechin gallate, epigallocatechin gallate, isorhamnetin	Apples, arugula, asparagus, Chinese cabbage, chocolate, dill weed, grapes, parsley, sea buckthorn berries; service (Saskatoon) berries, watercress
Flavan-3-ols	Catechin, epicatechin, epicatechin 3-gallate	Bilberry, broad beans, chocolate (dark, milk), peaches, plums, soybeans, soy products, tea (brewed, black and green)
Flavones	Apigenin, luteolin	*Rich sources:*[a] celery seed, juniper berries, oregano, parsleyCelery, celery hearts, Chinese celery, kumquat, radicchio, thyme, vine spinach
Anthocyanidins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, petunidin	*Rich sources:*[a] acai berries, apple skin, blackberries, blueberries, cabbage (red), Cedar bay cherries, chokecherries, currants, elderberries, grapes (red), Illawarra plums, pecans, radicchio, radishes, raspberries, service (Saskatoon) berries, Tasmanian peppers Apple skin, arctic bramble berry, bilberry, cherry (sweet, tart), black beans, cranberry, eggplant, lingonberry, strawberries, wines (red)
Flavanones	Hesperetin, naringenin, eriodictyol	*Rich sources:*[a] oregano Artichokes, lemons, limes, grapefruit, kumquat, oranges, pummelo, rosemary
Isoflavones	Daidzein, genistein, glycitein	Soybeans, soy products

[a]*Foods containing ≥100 mg of respective flavonoids per 100 g.*
Adapted from Foods containing ≥10 mg of respective flavonoids per 100 g.
From Bhagwat, S., Haytowitz, D.B., 2015. USDA Database for the Flavonoid Content of Selected Foods, Release 3.2
http://www.ars.usda.gov/nutrientdata/flav.

Metabolism of Flavonoids

Absorbed flavonoids are conjugated in the intestinal mucosa and are degraded to different phenolic compounds that are rapidly excreted in the bile and urine. Urinary flavonoids show highly variability, suggesting interindividual variation in flavonoid metabolism.

Significant flavonoid catabolism occurs in the hindgut **microbiome**. This includes cleavage of the heterocyclic ring, deesterification, and hydrolysis of sugars, resulting in the formation of various phenolic acids and their lactones. Some constituents of that microbiome, e.g., *Bacteroides* spp., have glycases and are, thus, able to metabolize polyphenylglycones. Their numbers are increased by consuming flavonoid-rich foods. The result is that ~80% of flavonoid metabolites are ultimately absorbed from the colon.[240]

Metabolic Effects of Flavonoids

Enzyme modulation. Flavonoids can interact with many enzymes, selectively affecting the activities of some. This includes induction of some (e.g., phase II enzymes) by binding to promoter regions of their respective genes, and inhibition of others (e.g., aldose reductase, phosphodiesterase, *O*-methyltransferase, and several serine and threonine kinases) by direct binding to the respective protein. For example, tea flavanols inhibit redox-sensitive transcription factors (NF-κB, AP-1) and prooxidative enzymes (lipoxygenases, cyclooxygenases, nitric oxide synthase, xanthine oxidase) but induce phase II and antioxidant enzymes (glutathione *S*-transferases, superoxide dismutases).[241] Flavanones can induce phase II enzymes and exert antiinflammatory effects; naringin, in particular, has been implicated in the effect of grapefruit juice in inhibiting cytochrome *P*450-dependent drug metabolism.[242] Flavonoids with B-ring catechols can

240. Stoupi, S., Williamson, G., Viton, F., et al., 2010. Drug Metab. Dispos. 38, 287–291.

241. Such effects have been cited as the basis of prospective antiinflammatory roles of flavonoids (Middleton, Jr., E., Kandaswami, D, Theoharides, T.C., 2000. Pharmacol. Rev. 52, 673–751).

242. This effect, which involves inhibition of the CYP3A4 isoform, may also involve other flavanones in grapefruit juice. The potency is evidenced by the fact that a single glass of grapefruit juice can affect the biological activity of drugs metabolized by this enzyme system, increasing the activities of some and decreasing the activities of others.

promote mitochondrial production of ROS by inhibiting succinoxidase. Others can cause uncoupling of mitochondrial oxidative phosphorylation and Ca^{+2} release by reducing membrane fluidity. Isoflavones can affect estrogen synthesis and the transactivation of estrogen receptors α and β to affect signal transduction pathways.

Antiestrogenic effects. That soy isoflavones can be ant-estrogenic was demonstrated by a study of Asian women whose intake of soy products was inversely associated with their circulating estrogen level.[243] This effect involves the binding of those isoflavones, genistein and daidzein to intranuclear type II estrogen receptors α and β, thus, affecting the estrogen-synthetic activity of 17β-steroid oxidoreductase and estrogen-dependent signal transduction pathways. This is believed to underlie epidemiological observations of inverse associations of soy products and loss of bone mineral density and symptoms of menopause or premenstrual syndrome. Some, but not all, clinical trials have found the consumption of soy products to reduce menopausal symptoms by as much as 50–60%.[244] One study found soy consumption effective in reducing premenstrual syndrome symptoms.[245] The flavonol quercetin is also a phytoestrogen, interacting with type II estrogen receptors.

As their estrogenic character might suggest, the consumption of soy isoflavones has been associated with higher bone mineral density in a limited number of epidemiological studies. Studies in animal models have shown maternal consumption of soy isoflavones to increase offspring bone mineral density. Such studies have shown that flavonoids modulate the expression of transcription factors that affect osteoblast function by affecting cellular signaling via mitogen-activated protein kinase (MAPK), bone morphogenic protein, estrogen receptor, and osteoprotegerin/receptor activator of NF-κB ligand.[246] Clinical trials conducted to test the hypothesis that soy isoflavones may be useful in improving bone mineralization for the prevention of osteoporosis have yielded inconsistent results.[247]

Putative antioxidant effects. It has been suggested that flavonoids must have metabolic value as antioxidants because of their ability to chelate divalent metal cations (e.g., Cu^{2+}, Fe^{2+}), which removes those catalysts of lipid peroxidation reactions and by scavenging radical

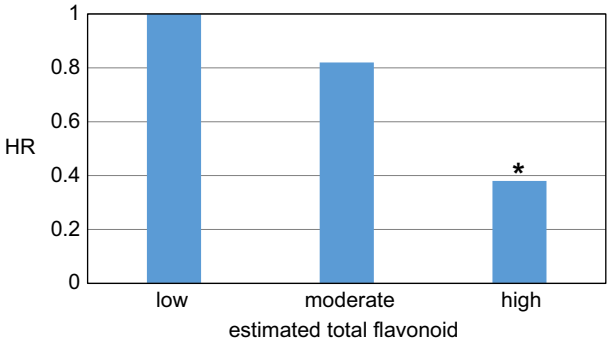


FIGURE 19.19 Risk of all-cause mortality by level of flavonoid intake among women. Data expressed as hazard ratio (HR). *After Ivey, K.L., Hodgson, J.M., Croft, K.D., et al., 2015. J. Nutr. 101, 1012–1020.*

intermediates.[248] Flavonols and some proanthocyanins have been shown to inhibit macrophage-mediated LDL oxidation in vitro, protecting LDL–α-tocopherol from oxidation. Quercetin, which has multiple phenolic hydroxyl groups (a carbonyl at C-4, and free hydroxyl groups at C-3 and C-5), can scavenge superoxide radical ions, hydroxyl radicals, and fatty acyl peroxyl radicals. This chemical property is the basis for in vitro chemical measurements of "total antioxidant capacity" in foods.[249] Such methods may be good indicators of total phenolic content; however, it is highly questionable whether they yield information of any physiologic relevance, as flavonoids are extensively metabolized after ingestion, so their circulating levels are low.[250] There is no direct evidence that health effects of flavonoids involve antioxidant functions in vivo.

Dietary Flavonoids in Health and Disease

Epidemiologic studies have demonstrated associations of diets high in flavonoids with increased longevity and reduced risks of cardiovascular diseases and cancer (Fig. 19.19). However, because such diets are typically rich in fruits and vegetables, it can be difficult to determine from these results whether the protective factor(s) are flavonoids or some other phytochemicals, vitamins, or minerals (e.g., β-carotene, ascorbic acid, fiber) also provided by those foods.

Antiinflammatory effects. Epidemiologic observations have found diets rich in fruits and vegetables to be associated with relatively low levels of inflammatory markers in the people consuming them. That flavonoids contribute to such antiinflammatory effects was suggested by an analysis of the NHANES 1999–2002 data,

243. Nagata, C., Takatsuka, N., Inaba, S., et al., 1998. J. Nat. Cancer Inst. 90, 1830–1835.
244. Albertazzi, P., Pansini, F., Bonaccorsi, G., et al., 1998. Obstet. Gynecol. 91, 6–11; Upmalis, D.H., Lobo, R., Bradley, L., et al., 2000. Menopause 7, 236–242.
245. Bryant, M., Cassidy, A., Hill, C., et al., 2005. Br. J. Nutr. 93, 731–739.
246. Trzeciakiewicz, A., Habauzit, V., Horcajada, M.N., 2009. Nutr. Res. Rev. 22, 68–81.
247. Messina, M., Ho, S., Alekel, D.L., 2004. Curr. Opin. Clin. Nutr. Metab. Care 7, 649–658.
248. Galleano, M., Verstraeten, S.V., Oteiza, P.I., et al., 2010. Arch. Biochem. Biophys. 510, 23–30.
249. Several systems have been used for this in vitro assessment: TRAP (total reactive antioxidant potential), TEAC (Trolox equivalent antioxidant capacity), ORAC (oxygen radical absorbance capacity).
250. Hollman, P.C.H., Cassidy, A., Comte, B., et al., 2011. J. Nutr. 141, 989S–1009S.

which showed that intakes of the flavonols, quercetin and kaempferol; the anthocyanins, malvidin and peonidin; and the isoflavone, genistein were each inversely related to serum concentrations of the inflammatory marker CRP.[251] Similarly, the Nurses' Health Study found that flavonol intake to be inversely related to circulating levels of soluble vascular adhesion molecule-1, and that intakes of flavonoid-rich foods to be associated with lower circulating levels of CRP and soluble tumor necrosis factor receptor-2 (sTNF-R2).[252] Studies in animal and cell models have pointed to several mechanisms underlying these antiinflammatory effects, including modulation of proinflammatory gene expression, inhibition of NF-κB activation, and inhibition of nuclear poly(ADP-ribose) polymerase-1 to reduce macrophage cytokine release.

Immunity. Quercetin has been found to affect aspects of immune cell function. This includes inhibition of induction and function of cytotoxic T lymphocytes, liposaccharide-induced production of NO and TNF-α by macrophages, and Ca^{+2} uptake and consequent histamine release by mast cells. Based on the latter effect, quercetin was administered in nasal spray containing other flavonols to small cohort of patients with allergic rhinitis and nasal congestion; results showed symptom relief within minutes and suppression of symptoms for several hours in most subjects.[253]

Cardiovascular health. Epidemiologic evidence points to cardiovascular disease mortality being inversely related to flavonoid intake, and particular flavanoids being protective against cardiovascular disease.[254] Diets high in the flavonol quercetin were associated with 21–53% reduced risks of cardiovascular disease prevalence.[255] An analysis of data from the Nurses' Health Study found that consumption of anthocyanins, some flavones, and some flavanols was associated with prevention of hypertension.[256] An analysis of a cohort of ~41,000 French women found individuals with high intakes of flavonols, anthocyanin and proanthocyanidins to be less likely to have hypertension.[257]

Several clinical intervention trials have found dark chocolate, green tea, or blueberry flavonols to improve vascular endothelial function,[258] coco and red grape flavanols to reduce platelet reactivity.[259] Intervention with quercetin was found to reduce blood pressure, particularly in hypertensive subjects.[260] Clinical trials with resveratrol and cardiovascular end points have yielded mixed results.[261]

Meta-analyses of clinical trials have found evidence for chocolate consumption increasing flow-mediated dilation and reducing blood pressure, for soy protein isolate consumption being associated with reductions in diastolic blood pressure and LDL cholesterol, and for green tea consumption being associated with reduced LDL cholesterol and stroke risk.[262]

Metabolic studies indicate several mechanisms for beneficial effects of flavonoids on vascular function. Quercetin has been found to inhibit the activity of angiotensin-converting enzyme and the activation of *c*-Jun N-terminal kinase in the modulation of angiotensin-induced hypertrophy of vascular smooth muscle cells. Various proanthocyanins have been shown to inhibit platelet activation; to inhibit the expression of interleukin-2; and to lower serum levels of glucose, triglyceride, and cholesterol. Flavonoid-rich foods (cocoa, grape juice, red wine) have been found to be antithrombotic by inhibiting platelet aggregation and promote vascular endothelial function by stimulating nitric oxide production. Both the flavanone hesperetin and the flavanol epigallocatechin have been found to block oxidized LDL-induced endothelial apoptosis,[263] a key process in atherosclerosis. Epicatechin and its metabolite methylepicatechin have been shown to inhibit NADPH oxidase and arginase, to modulate concentrations of nitric oxide in endothelial cells.[264] Flavonones, flavanols, and flavonols have been found to inhibit NF-κB activity.

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252. Landberg, R., Sun, Q., Rimm, E.B., et al., 2011. J. Nutr. 141, 618–625.
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254. Knekt, P., Jarvinen, R., Renuanen, A., et al., 1996. Br. Med. J. 312, 478–481; Huxley, R.R., Neil, A.A., 2003. Eur. J. Clin. Nutr. 57, 904–980; Mink, P.J., Scraffod, C.G., Barraj, L.M., et al., 2007. Am. J. Clin. Nutr. 85, 895–909.
255. Arts, I.C.W., Hollman, P.C.H., 2005. Am. J. Clin. Nutr. 81, 317S–325S.
256. Cassidy, A., O'Reilly, E.J., Kay, C., et al., 2011. Am. J. Clin. Nutr. 93, 338–347.
257. Lajou, M., Rossignol, E., Fagherazzi, G., et al., 2016. Am. J. Clin. Nutr. 103, 1091–1098.
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259. Keevil, J.G., Osman, H.E., Reed, J.D., et al., 2000. J. Nutr. 130, 53–56; Rein, et al., 2000; Hubbard, G.P., Wolfram, S., Lovegrve, J.A., et al., 2004. J. Thromb. Haemost. 2, 2138–2145.
260. Edwards, R.L., Lyon, T., Litwan, S.E., et al., 2007. J. Nutr. 137, 2405–2411; Edwards, R.L., Lyon, S.E., Rabovsky, A., et al., 2007. J. Nutr. 137, 2405–2411; Egert, S., Bosy-Westphal, A., Seiberl, J., et al., 2009. Br. J. Nutr. 102, 1065–1074.
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263. Choi, J.S., Choi, Y.J., Shin, S.Y., et al., 2008. J. Nutr. 138, 983–990.
264. Steffen, Y., Schewe, T., Sies, H., 2007. Biochem. Biophys. Res. Commun. 359, 828–833; Schnorr, O., Brosssett, T., Momman, T.Y., et al., 2008. Arch. Biochem. Biophys. 476, 211–215.

Neurologic health. Clinical trials have found fruit flavonoids, flavanoid-rich foods (wine, tea, chocolate, grape juice, orange juice), and soy isoflavones to enhance cognitive function.[265] Studies in animal models have found extracts of flavonoid-rich foods (blueberry, spinach, strawberry) to reduce age-related declines in neuronal signal transduction and cognitive function.[266] Several mechanisms have been indicated in studies with animal models: increased expression of estrogen receptor-β, enhanced protein kinase, and lipid kinase signaling of transcription of factors involved in synaptic plasticity and cerebrovascular blood flow.

Obesity and diabetes. Flavonoids have been suggested as having antiobesity effects. A 14-year cohort study with 4280 men and women in the Netherlands, which found diets rich in flavonoids to be associated with lower increases in body mass index (BMI); the effect was significant only for women.[267] A study of ~1500 European adolescents found regular consumption of chocolate to be associated with lower adiposity.[268] Other trials have shown regular intake of flavonoid-rich fruits or green tea to reduce body weight. The green tea flavonol EGCG has been shown to inhibit obesity in the mouse model, but a meta-analysis of 15 studies found that green tea catechins were effective in producing modest reductions in BMI and body weight only in the presence of caffeine.[269] Such effects are thought to be due to increased thermogenesis and appetite suppression. The consumption of flavonoids has been shown to favor *Bacteroides* spp. in the hindgut. This may be a mechanism whereby flavonoids exert weight-lowering effects, as a *Bacteroides*-dominant hindgut microbiome has been associated with a relatively low yield of absorbable energy from fermentation.[270]

Flavonoids may be protective against the development of type 2 diabetes (T2D). Studies in three different cohorts found T2D risk to be inversely related to intakes of flavonoids (inputed from food frequency questionnaire data). In the ~340,000 subject EPIC[271] cohort, T2D risk was inversely related to intakes of flavan-3-ol and proanthocyanidin intakes.[272] In the 17-year follow-up of the Framingham Offspring cohort, a difference of 2.5-fold in flavonol intakes were associated with a 26% reduction in T2D risk.[273] In a 9-year follow-up of the ~18,200 subject Physicians Health Study cohort, T2D risk was inversely related to chocolate consumption.[274] A meta-analysis of clinical trials found interventions with cocoa/chocolate found those foods to reduce insulin resistance.[275] Another meta-analysis found resveratrol to improve glycemic control and insulin sensitivity in individuals with diabetes but not on nondiabetics.[276] A small, randomized clinical trial found intervention with anthocyanins to improve dyslipidemias and prevent insulin resistance in T2D patients.[277] Studies with rodent models have shown that flavonoids can improve glycemic control by enhancing insulin secretion and sensitivity.[278]

Anticarcinogenesis. Some (but not all) epidemiologic studies have found consumption of diets high in flavonoids to be associated with reduced risks of cancers of the lung and rectum (fruit catechins), and lung and prostate (soy isoflavones).[279] An analysis of the food intake data from the Nurses' Health Study cohorts (~172,000 women) found risk of developing ovarian cancer to be lowest among individuals in the highest quintile of tea consumption; the flavonoid intakes (imputed from food frequency data) suggested modest protective effects of flavonols and flavanones.[280]

Various flavonoids have been found to inhibit cell proliferation and angiogenesis in vitro and to inhibit phorbol ester-induced skin cancer in the mouse model. Several studies have shown that in vitro exposure to quercetin can synergize the effects of chemotherapeutic drugs on both resistant and nonresistant tumor cells. Underlying these effects may

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268. Cuena-García, M., Ruiz, J.R., Ortega, F.B., et al., 2014. Nutrition 30, 236–239.

269. Phung, O.J., Baker, W.L., Matthews, L.J., et al., 2010. Am. J. Clin. Nutr. 91, 73–81.

270. Ley, R.E., Turnbargh, P.J., Klein, S., et al., 2006. Nature 444, 1022–1023.

271. European Prospective Investigation into Cancer and Nutrition, an 8-country, 26-center study.

272. Zamora-Ross, R., Forouhi, N., Sharp, S.J., et al., 2014. J. Nutr. 144, 335–343.

273. Jacques, P.F., Cassidy, A., Rogers, G., et al., 2013. J. Nutr. 143, 1474–1480.

274. Matsumotoa, C., Petrone, A.B., Sesso, H.D., et al., 2015. Am. J. Clin. Nutr. 101, 362–367.

275. Hooper, L., Kay, C., Abdelhamid, A., et al., 2012. Am. J. Clin. Nutr. 95, 740–751.

276. Liu, K., Zhou, R., Wang, B., et al., 2014. Am. J. Clin. Nut. 99, 1510–1519.

277. Li, D., Zhang, Y., Liu, Y., et al., 2015. J. Nutr. 145, 742–748.

278. Kwon, O., Eck, P., Chen, S., et al., 2007. FASEB J. 21, 366–377; Kobori, M., Masumoto, S., Akimoto, Y., et al., 2009. Mol. Nutr. Food Res. 53, 859–868; Takikawa, M., Inoue, S., HOrios, F., et al., 2010. J. Nutr. 140, 527–533; Youl, E., Bardy, G., Magous, R., et al., 2010. Br. J. Pharmacol. 161, 799–814; Babujanarthanam, R., Kavitha, P., Pandian, M.R., et al., 2010. Fundam. Clin. Pharmacol. 24, 357–364.

279. Knekt, P., Kumpulainen, J., Jävinen, R., et al., 2002. Am. J. Clin. Nutr. 76, 560–568; Arts, I.C., Jacobs, Jr., D.R., Gross, M., et al., 2002. Cancer Causes Control 13, 373–382; Nagata, Y., Sonoda, T., Mori, M., et al., 2007. J. Nutr. 137, 1974–1979; Shimazu, T., Inoue, M., Sasazuki, S., et al., 2010. Am. J. Clin. Nutr. 91, 722–728.

280. Cassidy, A., Huang, T., Rice, M.S., et al., 2014. Am. J. Clin. Nutr. 100, 1344–1351.

be any of several metabolic effects that have been demonstrated for various flavonoids[281]:

- inhibition of some enzymes (e.g., protein kinase C, inhibition of nuclear poly[ADP-ribose] polymerase-1, Akt to cause apoptosis, topoisomerase I, prolyl hydroxylase II to increase hypoxia-inducible factor-1α to impair cell proliferation);
- induction of other activities (e.g., estrogen receptor-β, p38 and the MAPK pathway, DNA repair, cytochrome *P*450-dependent carcinogen metabolism, phase 2 enzymes that conjugate carcinogens);
- preservation of intracellular NAD^+;
- alteration of DNA methylation patterns.

Other effects. It has been suggested that health benefits attributed to traditional, herbal medicaments may be due to bioactive flavonoids. Evidence supporting such hypotheses includes the findings that bilberry anthocyanins may reduce retinal hemorrhage in T2D patients. Some studies indicate that consumption of cranberry juice may reduce risk for recurrent urinary tract infections in women.[282] Such protection may be due to the effects of cranberry A-type proanthocyanidins, or their microbial metabolites, which were found capable of blocking bacterial binding to bladder epithelial cells.[283] Studies with animal models have shown effects of small magnitude on exercise performance associated with mitochondrial biogenesis; however, a meta-analysis of human trials concluded that any such effects are of minimal physiological significance.[284] Still, a study with multiple sclerosis patients found EGCG to increase working efficiency during moderate exercise, particularly in men.[285] High intakes of black tea, flavonols, and flavones have been associated with low risk to osetoporotic fracture.[286]

It was once thought that the effect of grapefruit juice consumption in increasing drug biopotency was due to its dominant flavonoid, the flavanone naringin (the content of which varies widely, 200–2000 μM), which was found to inhibit in vitro CYP3A, the cytochrome *P*450 enzyme that metabolizes more than 60% of commonly prescribed drugs.[287] However, further studies found that the administration of naringin or comparable amounts of grapefruit juice do *not* affect the pharmacokinetics of CYP3A metabolites.[288] This drug–food interaction is now thought to be due to another class of compounds in grapefruit juice, **furanocoumarins**, which are metabolized to reactive forms that irreversibly bind the CYP apoprotein and eliminate its enzymatic activity.[289]

Biomarkers of Flavonoid Status

Status with respect to some flavonoids can be assessed on the basis of **urinary flavonoid excretion**. That parameter can indicate the minimum amount of particular flavonoids that were absorbed. However, it is not useful for assessing quercetin status, as that flavonoid is excreted primarily in the bile.

Flavonoid Safety

While the toxicology of the flavonoids has not been investigated, those in foods are considered safe. There are, however, interactions that have been observed but not well explained. For example, a large, 10-year prospective study found women in the highest quintile of soy isoflavones intake (median 53.6 mg/day) to have small (17–26%) but significant increases in risk of ischemic stroke compared with those with lower intakes.[290]

9. OROTIC ACID

Orotic acid was isolated in the late 1940s from distillers' dried solubles.[291] For a while, it was called "*vitamin* B_{13}"; however, when studies failed to confirm vitamin activity, that designation was dropped. It is recognized as a normal metabolite; however, evidence shows that orotic acid supplementation can promote the synthesis of uracil nucleotides, and produce some health benefits, making it a **beneficial dietary bioactive**.

Benefits of Dietary Orotic Acid

Orotic acid supplementation of experimental animals has been found to increase the utilization of fatty acids by the heart,

281. Miles, S.L., McFarland, M., Niles, R.N., 2014. Nutr. Rev. 72, 720–734.
282. Maki, K.C., Kaspar, K.L., Khoo, C., et al., 2016. Am. J. Clin. Nutr. 103, 1434–1442.
283. Howell, A.B., Vorsa, N., Der Marerosian, A., et al., 1998. N. Engl. J. Med. 339, 1085–1086.
284. Pelletier, D.M., Lacerte, G., Goulet, E.D.B., 2013. Int. J. Prev. Med. 23, 73–82.
285. Mähler, A., Steiniger, J., Bock, M., et al., 2015. Am. J. Clin. Nutr. 101, 487–495.
286. Myers, G., Prince, R.L., Kerr, D.A., et al., 2015. Am. J. Clin. Nutr. 102, 958–965.
287. Owira, P.M., Ojewole, J.A., 2010. Cardiovasc. J. Africa 21, 280–285; Hanley, M.J., Cancalon, P., Widmer, W.W., et al., 2011. Expert Opin Drug Metab. Toxicol.7, 276–286.
288. Bailey, D.G., Arnold, J.M., Strong, H.A., et al., 1993. Clin. Pharmacol. Ther. 53, 589–594; Bailey, D.G., Arnold, J.M., Munoz, C., et al., 1993. Clin. Pharmacol. Ther. 53, 637–642; Rashin, J., McKinstray, C., Renwick, A.G., et al., 1993. Br. J. Clin. Pharmacol. 36, 460–463.
289. Lin, H.L., Kent, U.M., Hollenberg, P.F., 2005. J. Pharmacol. Exp. Ther. 313, 154–164.
290. Yu, D., Shu, X.O., Li, H., et al., 2015. Am. J. Clin. Nutr. 102, 680–686.
291. This feedstuff consists of the dried aqueous residue from the distillation of fermented corn. It is used mainly as a component of diets for poultry, swine, and dairy calves. It is rich in several B vitamins and has been valued as a source of UGFs, particularly for growing chicks and turkey poults.

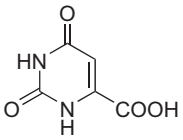

FIGURE 19.20 Orotic acid.

increase the activity of lipoprotein lipase, and increase expression of peroxisome proliferator-activated receptor α (PPARα) and its affected enzymes.[292] Supplemental orotic acid also increases hepatic levels of uracil nucleotides, presumably by increasing the flux through the pyrimidine pathway. Orotate treatment has been shown to be more effective than uracil in stimulating adaptive growth of the rat jejunum after massive small bowel resection.[293] Studies in cultured cells have found orotic acid to stimulate cell proliferation by downregulating AMPK, which activates mTORC1.[294] Magnesium orotate has been used to improve ventricular function and exercise tolerance in cardiac patients[295]; however, those effects would appear to be due to correction of magnesium depletion and not to the orotate moiety per se.

Benefits of dietary orotic acid are as follows:
- Increased pyrimidine production.

Nature of Orotic Acid

Orotic acid is a substituted pyrimidine: 1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid (Fig. 19.20).

Sources of Orotic Acid

The most important dietary sources of orotic acid are milk, milk products, and root vegetables (beets, carrots). Notably, human milk lacks orotic acid.

Metabolism of Orotic Acid

Cellular uptake of orotic acid appears to be facilitated by an organic ion transporter OAT2 driven by glutamate antiport.[296] Orotic acid is an intermediate in the biosynthesis of **pyrimidines** (UTP, CTP, TTP). It is synthesized in the mitochondria from *N*-carbamylphosphate by dehydration (via dihydroorotase) and oxidation via **orotate reductase** to orotate, which is subsequently converted to UMP. An inborn error in UMP synthase, which catalyzes the last step, is characterized by orotic acid accumulating in the plasma and appearing in increased amounts in the urine. Orotic acid excretion is also increased in disorders of the urea cycle and has been proposed as an indicator of arginine depletion. It has been found to be increased in cases of subclinical mastitis in dairy cows.

Safety of Orotic Acid

Orotic acid supplements (0.1%) to the diets of rats have been found to reduce the conversion of tryptophan to niacin,[297] to induce hepatic steatosis and hepatomegaly.[298] The latter effects were associated with increases in the sterol regulatory element binding protein-1c, the target gene for which is involved in fatty acid synthesis. Orotic acid supplementation of the rat appeared to constitute an oxidative stress, as it reduced hepatic superoxide dismutase activity and increased the contents of conjugated dienes and protein carbonyls.[299]

10. UNIDENTIFIED FACTORS

Since the discovery of vitamin B_{12}, experimental nutritionists have observed beneficial effects, particularly stimulated growth, of natural materials added to purified diets. Many such responses have been found to involve interrelationships of known nutrients.[300] Some have involved diet palatability and, thus, the rate of food intake of experimental animals. One resulted in the discovery of an unrecognized essential nutrient, selenium. Other responses remain to be elucidated. For young monogastrics (particularly poultry), several feedstuffs that have such effects are regarded as having "**unidentified growth factor"** (**UGF**) activity (Table 19.11). Elucidating the nature of UGFs has been frustrated by the fact that growth is a nonspecific response and that the observed growth responses are often small and not reproducible, suggesting roles of the environment, the gut microbiome, etc.

The classical vocabulary of nutrition is not well suited to accommodate such cases. How should UGFs and other vitamin-like factors beneficial to health be described? What are the proper descriptors for

(1) Lycopene for reducing cancer risk?
(2) Xanthophylls for reducing risk to AMD?
(3) Carnitine for an individual with an OCTN2 deficit?
(4) Flavonoids or soluble fiber supporting colon health?[301]

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300. e.g., At least part of the beneficial effect of including corn distillers' dried solubles in a soybean meal-based diet for chicks is due to its natural chelating activity, which increases the utilization of zinc.
301. Or soluble fiber, for that matter?

TABLE 19.11 Sources of UGF (unidentified growth factor) Activity for Poultry
Condensed fish solubles
Fish meal
Dried whey
Brewers' dried grains
Brewers' dried yeast
Corn distillers' dried solubles
Other fermentation residues

11. CASE STUDY

Instructions

Review each of the following case reports, paying special attention to the diagnostic indicators on which the treatments were based. Then answer the questions that follow.

In 1989, Killgore and colleagues demonstrated that mice fed a refined diet containing very low concentrations of a tricarboxylic acid with a fused quinone ring, pyrroloquinoline quinone (PQQ) (Fig. 19.21)[302] developed skin lesions that were prevented by supplementation with PQQ.[303] Not only did PQQ-fed mice grow faster, but one-fourth of the PQQ-deprived animals showed friable skin, mild alopecia, and a hunched posture, and one-fifth died by 8 weeks with aortic aneurysms or abdominal hemorrhages. The most frequent sign, friable skin, suggested an abnormality of collagen metabolism; PQQ-deprived animals showed abnormally low activities of lysyl oxidase and increased collagen solubility indicating reduced cross-linking.[304] PQQ is known to be a cofactor in several enzymes (now called quinoproteins) in bacteria, yeasts, and plants. It is ubiquitously present in common bacteria, soil, and plants and has been found in all foods examined. Mice fed the low-PQQ diet for 8–9 weeks produced either no litters, or litters in which the pups were immediately cannibalized at birth. Subsequent work showed deprivation of PQQ to alter mitogenic responses, reduce interleukin 2 levels, elevate plasma levels of glucose and several amino acids, and reduce mass of functional hepatic mitochondria.[305] PQQ has been shown to affect cell signaling.[306] Studies in cultured cells have demonstrated its effects in stimulating the activity of the ras oncogene in signal transduction pathways

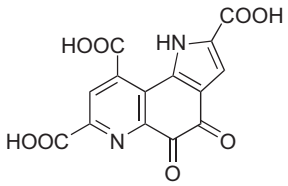

FIGURE 19.21 Pyrroloquinoline quinone.

involved in growth and development. PQQ has also been found to affect the activity of the "Parkinson disease protein," DJ-1, a peptidase involved in androgen receptor-regulated transcription leading to mitochondrial biogenesis. PQQ has been shown to stimulate the activation, by phosphorylation, of the promoter of PPAR-γ-coactivator-1α (PGC-1α) in the stimulation of mitochondrial biogenesis.[307]

PQQ has been found in several foods including all plants analyzed and at relatively high levels in fermented foods (60–800 μg/kg) and human milk (140–180 μg/kg). It has been estimated that most people consume 1–2 mg PQQ per day from foods.

The ability to redox cycle makes PQQ an antioxidant with capable of both one and two electron transfers. **S**tudies in animal models have shown PQQ treatment to have antioxidant-like effects in protecting against carbon tetrachloride hepatotoxicity and inhibiting glucocorticoid-induced lenticular glutathione depletion and cataract.

Case Questions

1. Should PQQ be designated a vitamin? A conditionally essential nutrient? A beneficial dietary bioactive? Something else? Justify your answer.
2. Propose a series of experiments plan to clarify the status of PQQ as a putative nutrient.

12. STUDY QUESTIONS AND EXERCISES

1. List the questions that must be answered in determining the eligibility of a substance for vitamin status.
2. For each of the substances discussed in this chapter, list the available information that would support its designation as a vitamin, and that which would refute such a designation.
3. Outline the general approaches one would need to take to characterize the UGF activity of a natural material such as fish meal for the chick.
4. Prepare a concept map of the relationships of micronutrients and physiological function, including the specific relationships of the traditional vitamins, the quasivitamins, and ineffective factors.

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Part III

Using Current Knowledge of the Vitamins

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Chapter 20

Sources of the Vitamins

Chapter Outline

Anchoring Concepts

1. Estimates of vitamin contents of many foods and feedstuffs are available.
2. For some vitamins, only a portion of the total present in certain foods or feedstuffs is biologically available.
3. The total vitamin intake of an individual is the sum of the amounts of bioavailable vitamins in the various foods, feedstuffs, and supplements consumed.

The intakes of vitamins into the body calculated from standard tables are rarely accurate.

John H. Marks[1]

LEARNING OBJECTIVES

1. To understand the sources of error in estimates of vitamin contents of foods and feedstuffs
2. To understand the concept of a core food and to know the core foods for each of the vitamins
3. To understand the sources of potential losses of the vitamins from foods and feedstuffs
4. To understand which of the vitamins are most likely to be in insufficient supply in the diets of humans and livestock
5. To understand the means available for the supplementation of individual foods and total diets with vitamins.

VOCABULARY

Bioavailability
Biofortification
Core foods
Enrichment
Food labels
Fortification
Genetic engineering
Golden rice
National nutrient database
Nutrition Labeling and Education Act (NLEA)
Revitaminization
Selective breeding
Supplementation
Vitamin–mineral premix
Vitaminization

1. VITAMINS IN FOODS AND FEEDSTUFFS

Vitamin Content Data

Collation of best estimates of the nutrient composition of foods and feedstuffs has been an ongoing activity by several groups in the United States since the early 1900's.[2] Nutrient

1. ohn Henry Marks (b. 1925) is a prominent British physician who was a professor at Cambridge University. He is the author of well-written books on the vitamins, including "*The Vitamins: Their Role in Medical Practice*" (1985), Springer, New York, 224 pp.

2. The formal compilation of food composition data was initiated by the USDA food chemist W.O. Atwater in 1896. Since that time, developing information on the nutrient composition of foods has been an ongoing program of the USDA. Development of nutrient composition information for feedstuffs started in the United States in the early 1900s at several land grant colleges; in more recent times, those activities have passed largely into the private sector, being in the interest of corporate feed producers to have reliable data for contents in feedstuffs of those nutrients that most directly affect the cost of their formulations (e.g., metabolizable energy, protein, indispensable amino acids, calcium, and phosphorus).

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00020-4

composition data for foods and feedstuffs are now available in many forms and from many sources. However, most compilations derive from relatively few primary sources. This is particularly true for the nutrient composition data for foods. For US foods, almost all current versions are renditions of the USDA **National Nutrient Database**[3] developed through an ongoing program of the U.S. Department of Agriculture. A similar database, the **Canadian Nutrient File** has been developed by that country.[4] Less extensive databases have been developed for other countries,[5] and efforts are being made to standardize the collection, compilation, and reporting of food nutrient composition data on a global basis.[6] Variances are to be expected in national food composition databases due to differences in analytical methodologies (a particular problem for folate) and data presentation, e.g., whether tocotrienols are included in the calculation of α-tocopherol equivalents.[7]

The nutrient composition of feedstuffs has, with few exceptions,[8] been developed less systematically and extensively. Data sets presently in the public domain have been compiled largely from original reports in the scientific literature. Therefore, effects of uncontrolled sampling, multiple and often old analytical methods, multiple analysts, unreported analytical precision, unreported sample variance, etc., are likely to be far greater for the nutrient databases for feedstuffs than for the corresponding databases for foods.

Use of any database for estimating vitamin intake is limited for reasons of accuracy and completeness of the data. Although the USDA National Nutrient Database is much more complete than most feed tables with respect to data for vitamins, it is least complete with respect to vitamins D, E, and K, as well as pantothenic acid.

Core Foods for Vitamins

Foods are the most important sources of vitamins in the daily diets of humans. However, the vitamins are unevenly distributed among the various foods that comprise human diets (Table 20.1). Therefore, evaluations of the degree of vitamin adequacy of diets and meal patterns are served by knowing which foods are likely to contribute significantly to the total intake of each particular vitamin, by virtue of the frequency and amounts of each food consumed as well as the probable concentrations of that vitamin in those foods. Identifying such **core foods** is difficult because both the voluntary intakes of foods by free-living people and the concentrations of vitamins in foods are difficult to estimate quantitatively. Nevertheless, attempts to do that have indicated a manageable number of core foods for each of the vitamins, e.g., for Americans, it has been estimated that 80% of the total intakes of several vitamins are provided by 50–200 foods.[9]

Vitamins in Staple Foods

Much of the world's poor, particularly those in resource-poor countries, rely on diets based largely on starchy foods that fail to provide adequate amounts of vitamins. This is evidenced by an analysis of the world's five leading staples (Table 20.2).[10] Therefore, individuals without access to diverse diets and reliant on staple foods are at risk for vitamin deficiencies.

3. This database is used for U.S. national food consumption surveys. Data are obtained from scientific publications, food processors, food industry groups, and USDA-contracted analyses. They are available for public use in the USDA National Nutrient Database for Standard Reference, Release 28, which can be accessed online (http://www.ars.usda.gov/ba/bhnrc/ndl). Additional data relevant to the vitamin status and intakes of Americans can be found in databases maintained by the USDA and the U.S. Food and Drug Administration (FDA).

- *USDA Nutrient Content of the U.S. Food Supply Series* (http://www.cnpp.usda.gov/USfoodsupply)—Historical data (since 1909) for amounts of nutrients available per capita per day, by major food group.
- *What We Eat in America* (http://www.ars.usda.gov/services/docs.htm?docid=13793)—the dietary intake component of the National Health and Nutrition Examination Survey (NHANES).
- *FDA Total Diet Studies* (http://www.fda.gov/Food/FoodScienceResearch/TotalDietStudy/)—ongoing monitoring of levels of various nutrients, pesticide residues, and contaminants in the US food supply.

4. http://www.hc-sc.gc.ca/fn-an/nutrition/fiche-nutri-data/cnf_downloads-telechargement_fcen-eng.php.

5. Europe (http://www.fao.org/infoods/infoods/tables-and-databases/europe/en/); Latin America (http://www.fao.org/infoods/infoods/tables-and-databases/latin-america/en/); Asia (http://www.fao.org/infoods/infoods/tables-and-databases/asia/en/); Africa (http://www.fao.org/infoods/infoods/tables-and-databases/africa/en/); Middle East (http://www.fao.org/infoods/infoods/tables-and-databases/middle-east/en/); Oceana (http://www.fao.org/infoods/infoods/tables-and-databases/oceania/en/); Canada, the Caribbean, and the US (http://www.fao.org/infoods/infoods/tables-and-databases/canada-caribbean-and-united-states/en/).

6. This is the purpose of the INFOODS project of the United Nations University Food and Nutrition Program (http://www.fao.org/infoods/).

7. Uusitaio, U., Kronberg-Kippila, C., Aronsson, C.A. et al., 2011. J. Food Compost. Anal. 24, 494–505.

8. The notable exception in the public domain was the program at the University of Maryland, which involved the ongoing analysis of feedstuffs commonly used in feeding poultry in the United States. That program focused on macronutrients. It was discontinued in the late 1970s; the last version of the data (*1979 Maryland Feed Composition Data*) was published as a supplement in the Proceedings of the Maryland Nutrition Conference in that year. Other widely used feed tables were derived in part from this source, e.g., Scott, M.L., Neshiem, M.C., Young, R.J., 1982. Nutrition of the Chicken. M.L. Scott Assoc., Ithaca, NY, p. 482.

9. Using data from the 1976 Nationwide Food Consumption Survey and the Continuing Survey of Food Intakes by Individuals, USDA nutritionists estimated that Americans obtained 80% of their total intakes of the following vitamins from the following numbers of foods: vitamin A, 60; vitamin E, 100; thiamin, 168; riboflavin, 165; niacin, 159; pyridoxine, 175; folate, 129; and vitamin B_{12}, 58.

10. The same situation pertains to other staples, such as plantain, the major component of diets in >50 countries.

TABLE 20.1 Core Foods for the Vitamins

Vitamin A	Vitamin D	Vitamin E	Vitamin K	Vitamin C	Thiamin	Riboflavin	Niacin	Vitamin B_6	Biotin	Pantothenic Acid	Folate	Vitamin B_{12}
As retinol	Milk[a]	Vegetable oils	Broccoli	Tomatoes	Meats	Eggs	Meats	Meats	Liver	Liver	Tomatoes	Liver
Milk (breast, animal[a])	Ghee		Asparagus	Potatoes	Potatoes	Liver	Eggs	Cabbage	Egg yolk	Milk	Beets	Fish
Butter, ghee, margarine[a]	Margarine[a]		Lettuce	Pumpkins	Whole grains	Meats	Fish	Potatoes	Cauliflower	Meats	Potatoes	Eggs
Liver	Cheese		Cauliflower	Citrus fruits	Some fish	Some fish	Whole grains	Liver	Kidney	Eggs	Wheat germ	Milk
Eggs	Chicken (skin)		Cabbage	Other fruits	Legumes	Asparagus	Legumes	Beans	Peanuts	Fish	Cabbage	
Small fish (eaten whole)	Liver		Brussels sprouts	Yams	Oilseeds	Milk	Milk	Whole grains	Soybeans	Grains	Eggs	
As carotene	Fatty fish		Turnip greens	Cassava	Milk	Whole grains	Liver		Peanuts	Wheat germ	Legumes	Meats
Red palm oil	Cod liver oil		Liver	Milk	Eggs	Green leaves	Peanuts		Soybeans	Oatmeal	Spinach	Peanuts
Dark/ medium-green leaves	Egg yolk		Spinach	Legumes					Some fish	Carrots	Asparagus	Whole grains
Yellow/ orange vegetables									Milk		Milk	Beans
Yellow/ orange fruits												
Yellow maize												

[a]*The high vitamin content is due to fortification.*

TABLE 20.2 Vitamin Adequacy of Major Staple Foods

Vitamin[a]	% RDA (Recommended Dietary Allowance) Provided by Staple[b]				
	Wheat	Rice	Corn[c]	Potatoes	Cassava
Vitamin A	4	0	6	0	4
Vitamin D	0	0	0	0	0
Vitamin E	9	0	3	1	10
Vitamin K	1	0	0	43	21
Vitamin C	0	0	0	199	316
Thiamin	25	18	33	139	114
Riboflavin	17	12	10	23	30
Niacin	26	29	28	156	92
Vitamin B_6	8	31	28	275	44
Pantothenic acid	28	78	30	137	15
Folate	29	5	5	37	54
Vitamin B_{12}	0	0	0	0	0

[a]*This listing does not include biotin, which is not included in the USDA National Nutrient Database.*
[b]*Consumed to provide 80% of calories for lactating adult females.*
[c]*i.e., Maize.*
Fitzpatrick, T.B., Basset, G.J.C., Borel, P., et al., 2012. Plant Cell 24, 395–414.

Predicting Vitamin Contents of Foods and Feedstuffs

Data for the nutrient contents of foods and feedstuffs can be useful in judging the adequacy of food supplies and feedstuff inventories. However, estimates of the nutrient intakes of individuals as determined on the basis of these data are seldom accurate, owing to different factors that may alter the nutrient composition of a food or feedstuff before it is actually ingested. The errors associated with such estimates are particularly great for the vitamins. To accommodate these sources of error, most of which inflate estimates of nutrient intake, it is a common practice to discount by 10–25% the analytical values in the databases. It is likely that such modest discounts may still yield overestimates of intakes of at least some of the vitamins.

There are several sources of error in estimating vitamins in foods and feedstuffs:

- **Errors in sampling and analysis** contribute to inaccuracy of predicted values. Analytical errors are less likely to be problematic for vitamin E, thiamin, riboflavin, niacin, and pyridoxine, for which robust analytical techniques not prone to analyst effects are available.[11]
- **Variation among cultivars** in vitamin contents of plant foods can be as great as several orders of magnitude (Fig. 20.1). In some cases, these differences correspond to identifiable characteristics of the plant. For example, the ascorbic acid contents of lettuce, cabbage, and asparagus tend to be relatively high in the colored and darker green varieties; darker orange varieties of carrot tend to have greater provitamin A contents than do lighter-colored carrots. Still, vitamin contents are not necessarily related to such physical traits or to each other.
- **Variation among tissues** occurs, as most vitamins are not uniformly distributed among the various edible tissues of plants (Figs. 20.2–20.4). Gradients in the tissue contents of several nutrients correspond to the distribution of the phloem and xylem vascular network. Thus, relatively high concentrations have been observed for ascorbic acid in the stem end of oranges and pears; the top ends of pineapples; the apical ends of potatoes, the tuber end of sweet potatoes; both the lower and upper portions of carrots, the top ends of turnips; and the stem tips of asparagus, bamboo shoots, and cucumbers. In general, exposed tissues, i.e., skin/peel and outer leaves, tend to contain greater concentrations of vitamins, particularly ascorbic acid, which is found mostly in chloroplasts in tissues exposed to light.[12] In cereal grains, thiamin and niacin tend to be concentrated in tissues[13] that are removed in milling; therefore, breads made from refined wheat flour are much lower in those vitamins than are products made from maize, which is not milled. Mobilization of seed vitamin stores and, in some cases, biosynthesis of vitamins occur during germination, such that young seedlings (sprouts) tend to have relatively high vitamin contents.
- **Local agronomic factors and weather** conditions that affect growth rate and yield can also affect vitamin contents of plants (Tables 20.3A and 20.3B). For many plants, conditions that favor the production of lush vegetation will result in increased concentrations of several vitamins. Low temperatures have been shown to increase the ascorbic acid contents of beans and potatoes; the thiamin contents of broccoli and cabbage; the riboflavin contents of spinach, wheat, broccoli, and cabbage; and the niacin contents of spinach and wheat. However, low

11. Most nutrient analytical methods have been standardized by the Association of Official Analytical Chemists.
12. As much as 35–40% of the ascorbic acid in green plants may be present in chloroplasts, where its concentration can be as great as 50 mM. In the case of citrus fruits, three-quarters of the ascorbic acid is located in the peel.
13. That is, the scutellum and aleurone layer.

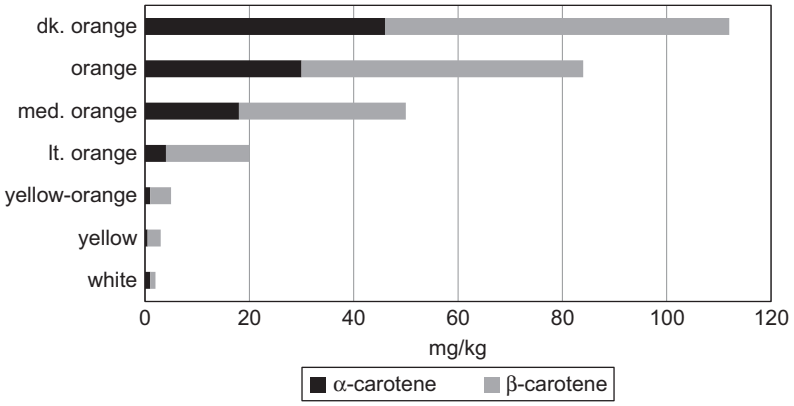

FIGURE 20.1 Variation in carotene contents among carrot cultivars. *After Leferriere, L., Gabelman, W.H., 1968. Proc. Am. Soc. Hortic. Sci. 93, 408–415.*

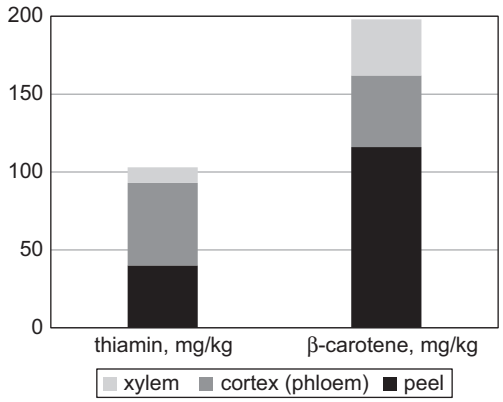

FIGURE 20.2 Distribution of vitamins in carrot tissues. *After Yamaguchi, Y., 1952. Proc. Am. Soc. Hortic. Sci. 60, 351–358.*

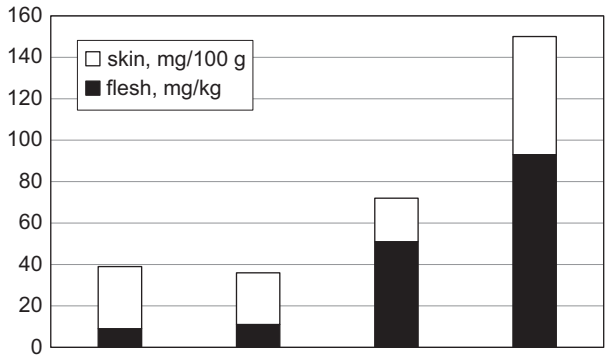

FIGURE 20.4 Distribution of ascorbic acid in tissues of four apple cultivars. *After Gross, E., 1943. Garterbauwissenschaften 17, 500–506.*

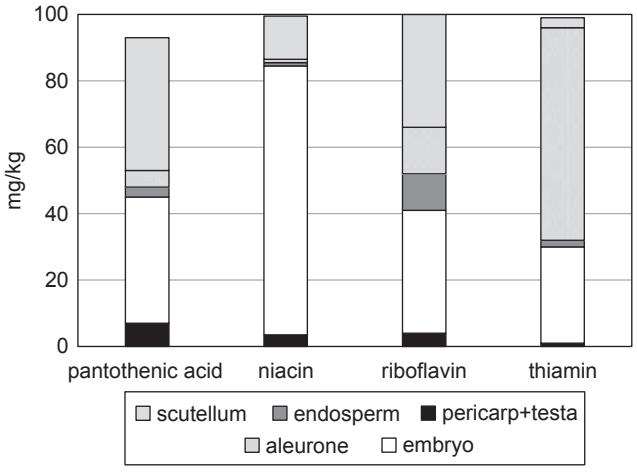

FIGURE 20.3 Distribution of vitamins in wheat tissues. *From Hinto, I., 1953. Nature (London) 173, 993–1000.*

temperatures decrease thiamin in beans and tomatoes; riboflavin in beans; niacin in tomatoes; and carotenoids in carrots, sweet potatoes, and papayas. Conditions at harvest can affect the vitamin content of some crops, e.g., the vitamin E content of fungus-infected corn grain can be less than half that of nonblighted corn. Legumes such as alfalfa and soybeans contain the enzyme lipoxygenase, which, if not inactivated (by drying) soon after harvest, catalyzes lipid oxidation reactions resulting in massive destruction of carotenoids and vitamin E. Accordingly, the vitamin contents of plant-based foods can be markedly different in different parts of the world, show both seasonal and annual changes. These fluctuations can be as great as 8-fold for the α-tocopherol content of alfalfa hay within a single season, and 11-fold for the ascorbic acid content of apples produced in different years.

TABLE 20.3A Fold Variations in Reported Vitamin Contents of Fruit and Vegetable Cultivars: β-Carotene, Ascorbic Acid, α-Tocopherol, Thiamin, and Riboflavin

Food	β-Carotene	Ascorbic acid	α-Tocopherol	Thiamin	Riboflavin
Apple		29		3.0	10
Apricot	2.9			1.5	1.3
Banana		9			
Barley				2.3	
Bean	2.3	2.9		2.7	3.7
Blueberry	17	3.0		1.3	1.8
Cabbage		3.8		2.5	2.8
Carrot	80	1.4		6.9	5.5
Cassava	113	1.9			
Cauliflower		1.7		1.4	1.4
Cherry	3.5	4.2		2.0	1.5
Collard	1.4	1.6			2.1
Cowpea				2.9	3.0
Grape		3.0		7.5	3.4
Grapefruit	9.3	1.3			
Guava		11			
Lemon		1.2			
Lemon		1.2			
Maize	24		2.0	1.8	
Mango	3.8	91			2.0
Muskmelon		20			
Nectarine	4.8	4.7		1.0	1.3
Oat				1.8	
Orange	6.8	1.5			
Palm, oil	5.1				
Papaya	5.7	2.7		1.3	1.5
Pea	4.3	3.4		5.2	1.7
Peach	6.0	4.2		2.0	1.7
Peanut				1.4	1.9
Pear		16		7.0	5.0
Pepper, green	1.3	1.8	18		
Pepper, chili	46	10			
Plum	3.2	1.5			1.2
Potato		5.1		2.5	6.2
Rapeseed, oil			3.4		
Raspberry		2.3			
Soybean		2.4	1.2		

TABLE 20.3A Fold Variations in Reported Vitamin Contents of Fruit and Vegetable Cultivars: β-Carotene, Ascorbic Acid, α-Tocopherol, Thiamin, and Riboflavin—cont'd

Food	β-Carotene	Ascorbic acid	α-Tocopherol	Thiamin	Riboflavin
Spinach		1.6			
Squash					
Summer		9.4			
Winter		3.5			
Strawberry		4.3			
Sunflower			2.7		
Soybean		2.4	1.2		
Sweet potato	89	3.1		2.9	3.1
Taro		3.2		4.9	2.5
Tomato	20	15		1.6	
Turnip, greens		1.1			
Watermelon	15				
Wheat			29	7.9	5.2
Yam		1.9		3.0	3.9

Mozafar, A., 1994. Plant Vitamins: Agronomic, Physiological and Nutritional Aspects. CRC Press, New York, p. 43.

- **Agronomic practices** can affect the vitamin contents of plant tissues. These relationships are complex, varying according to the soil type, plant species, and vitamin in question. In general, mineral fertilization can increase plant contents of ascorbic acid (P, K, Mn, B, Mo, Cu, Zn, Co); carotenes (N, Mg, Mn, Cu, Zn, B); thiamin (N, P, B); and riboflavin (N). However, nitrogen fertilization tends to decrease ascorbic acid concentrations despite increased yields. Organic fertilizers can increase the concentrations of some vitamins, particularly thiamin. These effects may be due to the lower nitrate contents in organic fertilizers compared with inorganic ones. Organic fertilizers may also contain vitamins in forms that plant roots can absorb. Practices that affect light exposure and plant growth rate can affect plant vitamin contents. This is especially true for ascorbic acid and the biosynthesis of which is related to plant carbohydrate metabolism. For example, field-grown tomatoes can have twice the ascorbic acid content of greenhouse-grown tomatoes, shaded fruits having less than those directly exposed to light.[14] Relatively high ascorbic acid contents have been found in peas, grapes, and tomatoes grown at lower planting densities; in lower yielding or smaller apples; and in field-ripened compared to artificially ripened apples.[15]
- **Conditions of feeding** can affect the vitamin contents of foods of animal origin. These can be highly variable according to country of origin, season of the year, age at slaughter, the composition of the diets used, etc. For example, the vitamin E content of poultry meat is greater from chickens fed supplements of the vitamin than from those that are not.[16]

Accounting for Variation in Vitamin Contents

Natural variation in the nutritional composition of foods and feedstuffs has generally been accommodated by the analysis of multiple representative samples of each material of interest. Nevertheless, most databases include only a single value, the mean of all analyses, and fail to indicate the variance around that mean. The practical necessity of using databases so constructed means that the nutritionist is faced with the dilemma of estimating vitamin intake through the use of data that are likely to be inaccurate but to an uncertain and unascertainable degree. Thus, if an average value of 150 mg/kg is used to represent the ascorbic acid concentration of potatoes, as is frequently the case, then it must be recognized that half of all samples will exceed that value (thus yielding an underestimate)

14. In fact, vitamin C levels can differ between exposed and shaded sides of the same fruit.
15. By exposure to ethylene either in storage or in transit to market.
16. A practical example of this comparison is the intensively managed commercial poultry flock fed formulated feeds in the United States *versus* the small courtyard flock largely subsisting on table scraps, insects, and grasses.

TABLE 20.3B Fold Variations in Reported Vitamin Contents of Fruit and Vegetable Cultivars: Niacin, Pyridoxine, Biotin, Pantothenic Acid, and Folate

Food	Niacin	Vitamin B_6	Biotin	Pantothenic Acid	Folate
Apple	2.0		1.1	4.0	
Apricot	1.3				
Avocado	1.5	1.6		13	
Barley	1.1			1.2	
Bean	3.8	2.2			4.6
Blueberry	1.7				
Cherry	1.5				
Cowpea	2.2	1.5	1.5	1.3	
Grape	2.4				
Maize	5.5			1.3	
Mango	18				
Nectarine	1.3				
Oat	1.4				
Papaya	2.3				
Pea	1.2	1.3			2.2
Peach	1.2				
Peanut	1.5				
Pear	4.0		1.1	2.5	
Pepper, green	1.2				
Plum	4.5				
Potato	2.7	3.2			
Rye	1.3				
Strawberry	1.3				
Sweet potato	3.4			2.2	
Taro	4.9				
Wheat	5.0	8.6		2.6	
Yam	2.7				

Mozafar, A., 1994. Plant Vitamins: Agronomic, Physiological and Nutritional Aspects. CRC Press, New York, p. 43.

while half will contain less than that value (thus, yielding an overestimate). In constructing databases for use in meal planning or feed formulation, a better way to accommodate such natural variation is to enter into the database values discounted by a multiple of the standard deviation that would yield an acceptably low probability of overestimating actual nutrient amounts.[17] That approach, however, requires a fairly extensive body of data from which to generate meaningful estimates of variance. Few, if any sets of food/feedstuff vitamin composition data are that extensive.

2. VITAMIN BIOAVAILABILITY

Chemical analyses of vitamin contents may overestimate the amounts of vitamin that are bioavailable in a food or feedstuff (Table 20.4).[18] In the cases of niacin, biotin, pyridoxine, vitamin B_{12}, and choline, which in certain foods and

17. This approach was originated in the 1950s by G.F. Combs, Sr. at the University of Maryland in developing the Maryland Feed Composition Table. Those data were based on replicate analyses from multiple samples of each feedstuff and were expressed as the mean—0.9 SD units. That adjustment allowed a likelihood of overestimating actual nutrient concentration of $p = .20$.

18. See Chapter 3 for a discussion of the concept of nutrient bioavailability.

TABLE 20.4 Foods and Feedstuffs With Low Vitamin Bioavailabilities

Vitamin	Form	Food/Feedstuff
Vitamin A	Provitamins A	Corn
Vitamin E	Nontocopherols	Corn oil, soybean oil
Ascorbic acid	Ascorbinogen	Cabbage
Niacin	Niacytin	Corn, potatoes, rice, sorghum grain, wheat
Pyridoxine	Pyridoxine 5´-β-glucoside	Corn, rice bran, unpolished rice, peanuts, soybeans, soybean meal, wheat bran, whole wheat
Biotin	Biocytin	Barley, fishmeal, oats, sorghum grain, wheat

feedstuffs can be poorly utilized, only the biologically available amounts have nutritional relevance. The bioavailability of a vitamin in a particular food depends on several factors extrinsic and intrinsic to the individual consuming that food:

Extrinsic factors

- **Chemical form**—can affects vitamin solubility, absorption, and/or metabolism.
- **Physical form** (including emulsifiers, coatings, etc.)—can affect vitamer interactions with other food components.
- **Concentration**—can affect vitamer solubility and absorption kinetics.
- **Food/diet composition**—can affect gastrointestinal transit time and vitamer digestion, absorption, and/or synthesis by gut microbiota.
- **Nonfood agonists** (e.g., cholestyramine, alcohol, drugs)—may impair vitamin absorption and/or metabolism.

Intrinsic factors

- **Age**—many older individuals have poor vitamin B_{12} absorption associated with loss of gastric production of intrinsic factor.
- **Health status**—impaired gastrointestinal function can reduce vitamin absorption and synthesis by gut microbiota.

For most vitamins, bioavailability must be determined using animal models. An in vitro method is available to measure niacin bioavailability; it involves comparing the amounts of niacin determined chemically before (free niacin) and after (total niacin) alkaline hydrolysis. The free niacin thus determined correlates with the bioavailable niacin determined using the growth response of niacin-deficient rats fed a low-tryptophan diet.

3. VITAMIN LOSSES IN FOODS

Every step in the handling of a food in the locally, nationally, and globally interrelated food system (Fig. 20.5) has the potential of contributing to losses of vitamins (Table 20.5). In theory, these losses can be modeled and, thus, predicted. However, in practice, the variation in the actual conditions of handling foods through each of these steps is so great that the only way to estimate vitamin intakes of people is to analyze the vitamin contents of foods as they are eaten.

Vitamin losses in foods can be of several types:

- **Storage losses** can occur due to postharvest oxidation and enzymatic decomposition. For example, the ascorbic acid contents of cold-stored apples and potatoes can drop by two-thirds and one-third, respectively, within 1–2 months. Those of some green vegetables can drop to 20–78% of original levels after a few days of storage at room temperature. Such losses can vary according to specific techniques of food processing and preservation.
- **Milling losses** can occur in removing the bran and germ portions of grains to produce flours.[19] Because those portions are typically rich in vitamin E and many of the water-soluble vitamins, highly refined flours are low in these vitamins (Fig. 20.6).
- **Processing losses** can occur during thermal processing in the preservation of foods. Blanching (mild heating to inactivate enzymes, reduce microbial numbers, and decrease interstitial gases) is usually minimally destructive, although it can result in the leaching of water-soluble vitamins from foods blanched in hot water. Otherwise, blanching usually improves vitamin stability. In contrast, canning and other forms of high-temperature treatment can accelerate reactions of vitamin degradation, depending on the chemical nature of the food (i.e., its pH, dissolved oxygen and moisture contents, presence of transition metals and/or other reactive compounds; Tables 20.5 and 20.6). Vitamins A, E, C, thiamin, and folate are sensitive to moist, high-temperature

19. The yield of flour obtained from the milling process is expressed as the extraction rate. A 100% extraction is whole meal flour containing all of the grain; removal of progressively more of the bran and germ produces whiter flours of lower extraction rates; 72% extraction is common white flour; "patent" flours comprised mostly of endosperm are of extraction rates of 30–50%.

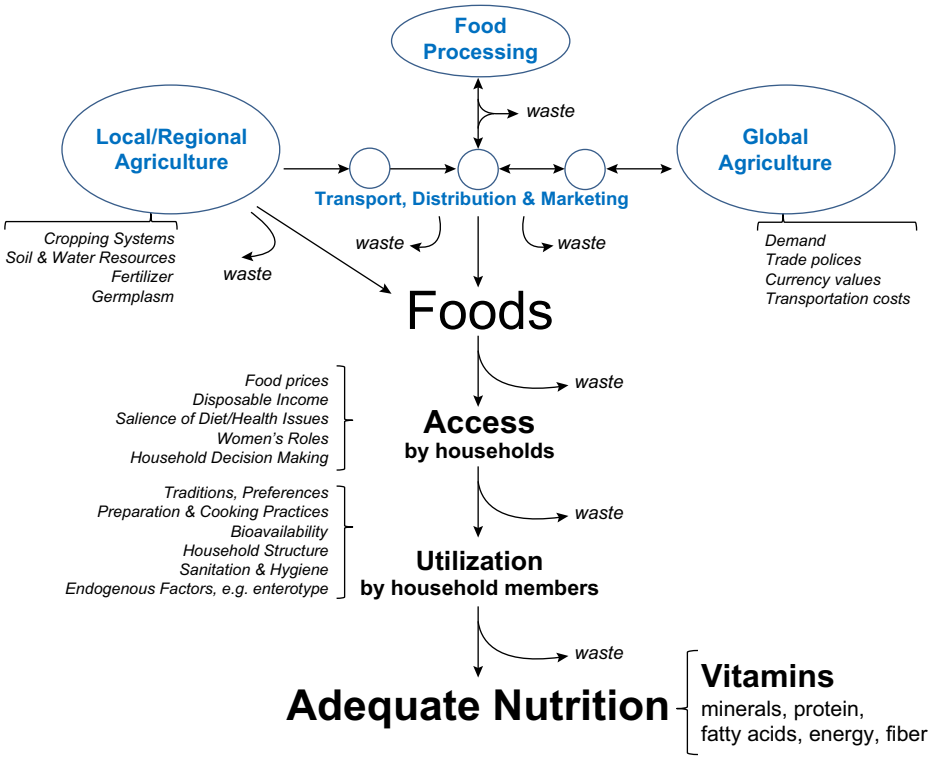

FIGURE 20.5 The food system.

TABLE 20.5 Effects of Food Processing Techniques on Vitamin Contents of Foods

Technique	Main Effects	Vitamins Destroyed[a]
Blanching	Partial removal of oxygen	Vitamin C (10–60%)[b,c]
	Partial heat inactivation of enzymes	Thiamin (2–30%), riboflavin (5–40%), niacin (15–50%), carotene (<5%)[c]
Pasteurization	Removal of oxygen[d]	Thiamin (10–15%)
	Inactivation of enzymes	Minor losses (1–5%) of niacin, vitamin B_6, riboflavin, and pantothenic acid
Canning	Exclusion of oxygen	Highly variable losses[e,f]
Freezing[h]	Inhibition of enzyme activity[g]	Very slight losses of most vitamins
Frozen storage[h]		Substantial losses of vitamin C and pantothenic acid; moderate losses of thiamin and riboflavin
Freeze drying	Removal of water	Very slight losses of most vitamins
Hot air drying	Removal of water	10–15% losses of vitamin C and thiamin
γ–Irradiation	Inactivation of enzymes	Some losses (about 10%) of vitamins C, E, K, and thiamin

[a]*Actual losses are variable, depending on exact conditions of time, temperature, etc.*
[b]*Loss of vitamin C is due to both oxidation and leaching.*
[c]*Losses of oxidizable vitamins can be reduced by rapid cooling after blanching.*
[d]*Vitamin losses are usually small, owing to the exclusion of oxygen during this process.*
[e]*Losses in addition to those associated with heat sterilization before canning.*
[f]*For example, 15% loss of vitamin C after 2 years at 10°C.*
[g]*While enzymatic decomposition is completely inhibited in frozen vegetables, reactivation occurs during thawing such that significant vitamin losses can occur. This is avoided by rapidly blanching before freezing.*
[h]*Thawing losses are associated with vitamin leaching into the syrup.*

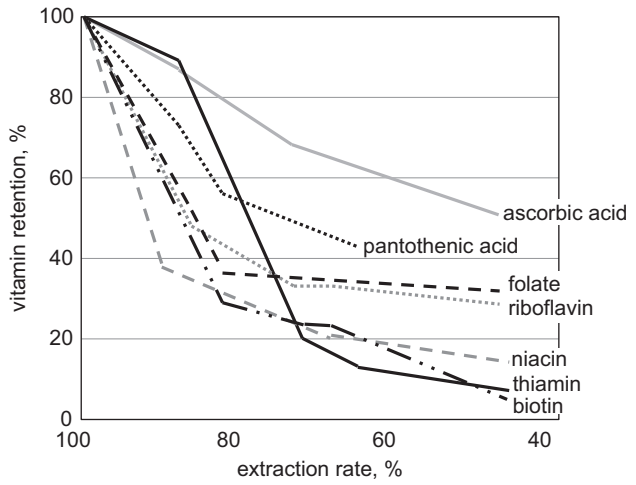

FIGURE 20.6 Loss of vitamins in the milling of wheat flour. Note: extraction rates of about 72% are typical for common bread flours. *From Moran, T., 1959. Nutr. Abstr. Rev. 29, 1–16.*

conditions such as those occurring during extrusion. Losses as great as 80% can occur, depending on the amount of water added to the food mixture and the temperature of the system. Freezing and drying usually result in only minor losses of most vitamins. Losses associated with ionizing (γ) irradiation vary according to the energy dose but are generally low (less than 10%).

- **Cooking losses** can result in further losses of vitamins from foods. However, methods used for cooking vary widely between different cultures and among different individuals, making vitamin losses associated with cooking highly variable. Washing fruits and vegetables in water before cooking can result in the extraction of water-soluble vitamins, particularly if they are soaked for long periods of time. Peeling fruits and vegetables can remove vitamins associated with the outer tissues.[20] Vitamin losses associated with cooking processes are also highly variable, but generally amount to about 50% for the less stable vitamins. The greatest losses are associated with long cooking times under conditions of exposure to air. Vitamin losses are less when food is cooked rapidly, as in a pressure cooker or a microwave oven, or by high-temperature stir frying. The baking of bread can reduce the thiamin content of flour by about 25% without affecting its contents of niacin or riboflavin. The susceptibilities of vitamins to processing and cooking losses are summarized in Table 20.7.
- **Waste** occurs throughout the food system due to such factors as spoilage, damage, pests, and discard. The United Nation has estimated that at least one-third of all food produced globally gets wasted.[21] In North America, food wastage has been estimated to reduce the food supplies by 30–40%. The food wastes of industrialized countries are estimated to be ~220 M tons.

Vitamin losses from foods can be minimized using the following:

- fresh instead of stored food
- minimum amounts of water in preparing and cooking
- minimum cooking, using high temperatures for short times
- minimum storage

4. VITAMIN FORTIFICATION

Availability of Purified Vitamins

All of the vitamins are produced commercially in pure forms. Most are produced by chemical synthesis, but some are also isolated from natural sources (e.g., vitamin A from fish liver, vitamin D_3 from liver oil or irradiated yeast, vitamin E from soybean or corn oils, and vitamin K from fish meal) and some are produced microbiologically (e.g., thiamin, riboflavin, folate, pyridoxine, biotin, pantothenic acid, and vitamin B_{12}[22]). Before their commercial synthesis became feasible, which began only in the 1940s, vitamins were extracted from such natural sources as fish oils and rose hip syrup. Today, the production of vitamins is based predominantly on their chemical synthesis and/or microbiological production, the latter having been greatly impacted by the emergence of new techniques in biotechnology.[23] With the notable exception of the tocopherols,[24] there is no basis to the notion that biopotencies of vitamins prepared by chemical/microbiological synthesis are at least as great as those of vitamins isolated from natural sources. In some cases, synthetic vitamins may be appreciably more bioavailable than the vitamin from natural sources (e.g., purified niacin versus protein-bound niacytin; purified biotin versus protein-bound biocytin).

The use of purified vitamins offers obvious advantages for purposes of ensuring vitamin potency in a wide

20. e.g., Peeling potatoes can substantially reduce their ascorbic acid content.

21. Lipinski, B., Hanson, C., Lomax, J., et al., 2013. World Resources Institute Working Paper (http://www.wri.org/sites/default/files/reducing_food_loss_and_waste.pdf).

22. The commercial production of vitamin B_{12} is strictly from microorganisms.

23. The industrial production of the vitamins has been nicely reviewed: O'Leary, M.J., 1993. Industrial production. In: Ottaway, P.B. (Ed.), The Technology of Vitamins in Food. Chapman & Hall, London, p. 63.

24. Vitamers E produced by chemical synthesis vary in biopotency (*see* Chapters 3 and 8); the most potent is *RRR*-α-tocopherol.

TABLE 20.6 Typical Losses of Vitamins Through Canning (%)

Food	Vitamin A	Vitamin C	Thiamin	Riboflavin	Niacin	Vitamin B_6	Biotin	Pantothenic Acid	Folate
Asparagus	43	54	67	55	47	64	0		75
Lima bean	55	76	83	67	64	47		72	62
Green bean	52	79	62	64	40	50		60	57
Beet	50	70	67	60	75	9		33	80
Carrot	9	75	67	60	33	80	40	54	59
Corn	32	58	80	58	47	0	63	59	72
Mushroom		33	80	46	52		54	54	84
Green pea	30	67	74	64	69	69	78	80	59
Spinach	32	72	80	50	50	75	67	78	35
Tomato	0	26	17	25	0		55	30	54

Lund, D., 1988. Nutritional Evaluation of Food Processing. In: Karmas. E., Harris, R.S. (Eds.), third ed. Van Nostrand Reinhold, New York, 319 pp.

variety of formulated products, including fortificants for foods, premixed supplements for feeds, nutritional supplements, pharmaceuticals, and ingredients in cosmetics. Commercial vitamin production has grown steadily since the discovery of vitamin B_{12}. At that time, annual world vitamin production was estimated to be only <1500 metric tons; by 2005, it exceeded 20,000 metric tons. Vitamins are produced by at least 30 firms in some 17 countries; but a half-dozen companies presently dominate the world market.

Addition of Vitamins to Foods

The addition of vitamins to certain foods is a common practice in most countries. Vitamins are added for several purposes:

- **Fortification**—ensuring vitamin adequacy of populations, e.g., white flour (folate), milk (vitamins A and D), margarine (vitamin A), formula foods (multiple vitamins in infant formulas, liquid nutrient supplements, enteral formulas used for tube feeding, and parenteral formulas used for intravenous feeding).
- **Vitaminization**—making foods carriers of vitamins not normally present, e.g., many breakfast cereals (multiple vitamins), orange juice (vitamin D), wheat (vitamin A), table salt (multiple vitamins), and parental feeding solutions (multiple vitamins).
- **Revitaminization**—restoring the vitamin content to that originally present before processing, e.g., white flour (thiamin, riboflavin, niacin).
- **Enrichment**—increasing the amounts of vitamins already present.

These processes are subject to regulation by national food authorities. In the United States, the addition of nutrients to foods is regulated by the Food and Drug Administration (FDA), which has identified as candidates for addition to foods 22 nutrients including 12 vitamins (Table 20.8).[25] Fortification of wheat flour with folate has been mandatory in the United States since 1998 and in Ireland since 2006. Since 1966, the USDA and USAID[26] have also routinely fortified or enriched foods provided as foreign aid under Public Law 480 (Table 20.9).[27] In addition, many antixerophthalmia programs have used vitamin A fortification of such foods as dried milk, wheat flour, sugar, tea, margarine, and monosodium glutamate.[28] As a consequence, processed foods provide one-third of the vitamin D, two-thirds of the folate, and almost half of the vitamin B_{12} consumed by Americans.[29]

Stabilities of Vitamins Added to Foods

The stabilities and bioavailabilities of vitamins added to foods depend on the form of vitamin used, the composition of the food to which it is added, and the absorption status of the individual ingesting that food. The less stable vitamins can be lost from foods during storage, depending

25. In addition to these vitamins, other nutrients are approved: protein, calcium, phosphorus, magnesium, potassium, manganese, iron, copper, zinc, and iodine.
26. United States Agency for International Development.
27. The cost of this fortification is very low relative to the total value of the commodities. The ingredients (vitamins and minerals) used to enrich the processed and soy-fortified commodities cost less than 2.5% of the value of the product; those used to enrich the more expensive blended food supplements cost less than 5% of the product value.
28. MSG, used as a seasoning.
29. Weaver, C.M., Dwyer, J., Fulgoni, V.L., et al., 2014. Am. J. Clin. Nutr. 99, 1525–1542.

TABLE 20.7 General Susceptibilities of Vitamins to Processing and Cooking Losses

Vitamin	Conditions that Enhance Loss
Vitamin A	Highly variable but significant losses during storage and preparation
Vitamin D	(Stable to normal household procedures)
Vitamin E	Frying can result in losses of 70–90%; bleaching of flour destroys 100%; other losses in preparation or baking are small
Vitamin K	(Losses not significant due to synthesis by intestinal microflora)
Ascorbic acid	Readily lost by oxidation and/or extraction in many steps of food preparation, heat sterilization, drying, and cooking
Thiamin	Readily lost by leaching, by removal of thiamin-rich fractions from native foods (e.g., flour milling), and by heating; losses as great as 75% may occur in meats and 25–33% in breads
Riboflavin	Readily lost on exposure to light (90% in milk exposed to sun light for 2 h, 30% from milk exposed to room light for 1 day) but very stable when stored in dark; small losses (12–25%) on heating during cooking
Niacin	Leached during blanching of vegetables (≤40%) but very stable to cooking
Vitamin B_6	Leached during food preparation; pasteurization causes losses of 67%; roasting of beef causes losses of about 50%
Biotin	(Apparently very stable; limited data)
Pantothenic acid	Losses of 60% by milling of flour and of about 30% by cooking of meat; small losses in vegetable preparation
Folate	(Data not available)
Vitamin B_{12}	Only small losses on irradiation of milk by visible or ultraviolet light

on the conditions (time, temperature, and moisture) of that storage (Fig. 20.7).

5. BIOFORTIFICATION

The term "biofortification" was coined in the 1990s to describe the use of intrinsic metabolic capacities of plants to enhance their contents of micronutrients in major staple crops.[30] Increasing vitamin contents had not been an explicit goal of crop improvement, which had centered on traits directly related to yield and disease resistance. That attitude started to change

30. The term "**field fortification**" has also been used.

TABLE 20.8 Vitamins Approved by the FDA for Addition to Foods

Vitamin	Recommended Level of Addition (per 100 kcal)
Vitamin A	250 IU
Vitamin D	20 IU
Vitamin E	1.5 IU
Vitamin C	3 mg
Thiamin	75 µg
Riboflavin	85 µg
Niacin	1.0 mg
Vitamin B_6	0.1 mg
Biotin	15 µg
Pantothenic acid	0.5 mg
Folate	20 µg; wheat flour products: [a] 140 µg/100 g
Vitamin B_{12}	0.3 µg

[a]*Mandated in the United States for most enriched flour, breads, corn meals, rice, noodles, macaroni, and other grain products.*

with the recognition of the "**hidden hunger**"[31] of micronutrient malnutrition, i.e., the persistent, debilitating shortages of vitamins and essential minerals in the face of remarkable gains in the global production of total staple foods and total calories. That one-sixth of the world's population does not have access to the foods necessary for nutritionally balanced diets has made it impossible to overlook shortages of vitamin A, vitamin C, folate, vitamin B_{12}, iron, iodine, and zinc in the diets of the world's poor. Even in the industrialized world, where diet-related chronic diseases are substantial problems, opportunities exist to develop vitamin/mineral-rich fruits and vegetables and to use these aspects of specific, good nutrition as marketing "hooks." The international agricultural community has responded with a number of coordinated efforts using modern agricultural technologies to enhance the micronutrient contents of major staple foods eaten widely by the poor (rice, wheat, corn [maize], cassava, beans, sweet potato, and pearl millet).[32] These efforts, in effect, treat agriculture as an instrument of public health.

31. "Hidden hunger" should in the lexicon of every person interested global health and well-being. It differs from other familiar terms: "**undernourishment**" and "**undernutrition**," which describe insufficient intakes of *energy and protein* to sustain growth and maintenance; and "**hunger**" and "**food insecurity**," which describe insufficient access to sufficient *quantities* of food.

32. The motive force for this effort has been the Consultative Group for International Agricultural Research (CGIAR) led by the International Food Policy Research Institute (IFPRI). With support from the Bill and Melinda Gates Foundation, IFPRI put together a global program, Harvest Plus (HarvestPlus@cgiar.org and www.HarvestPlus.org).

TABLE 20.9 Vitamins Added to P.L. 480 Title II Commodities Amount Added per 100 g[a]

Vitamin	Wheat–Soy Blend	Corn–Soy Blend	S-fortified Cereals	Nonfat Dry Milk	Others
Vitamin A (IU)	2314	2314	2204–2645	5000–7000	2204–2645
Vitamin D (IU)	198	198			
Vitamin E[b] (IU)	7.5	7.5			
Vitamin C (mg)	40.1	40.1			
Thiamin[c] (mg)	0.28	0.28	0.44–0.66		0.44–0.66
Riboflavin (mg)	0.39	0.39	0.26–0.40		0.26–0.40
Niacin (mg)	5.9	5.9	3.5–5.3		3.5–5.3
Vitamin B_6[d] (mg)	0.165	0.165			
Pantothenic acid (mg)	2.75	2.75			
Folate (μg)	198	198			
Vitamin B_{12} (μg)	3.97	3.97			

[a]*Processed blended foods are also fortified with Ca, P, Fe, Zn, I, and Na; Soy-fortified cereals and other processed foods are fortified with Ca and Fe.*
[b]*As all-rac-α-tocopheryl acetate.*
[c]*As thiamin mononitrate.*
[d]*As pyridoxine hydrochloride.*

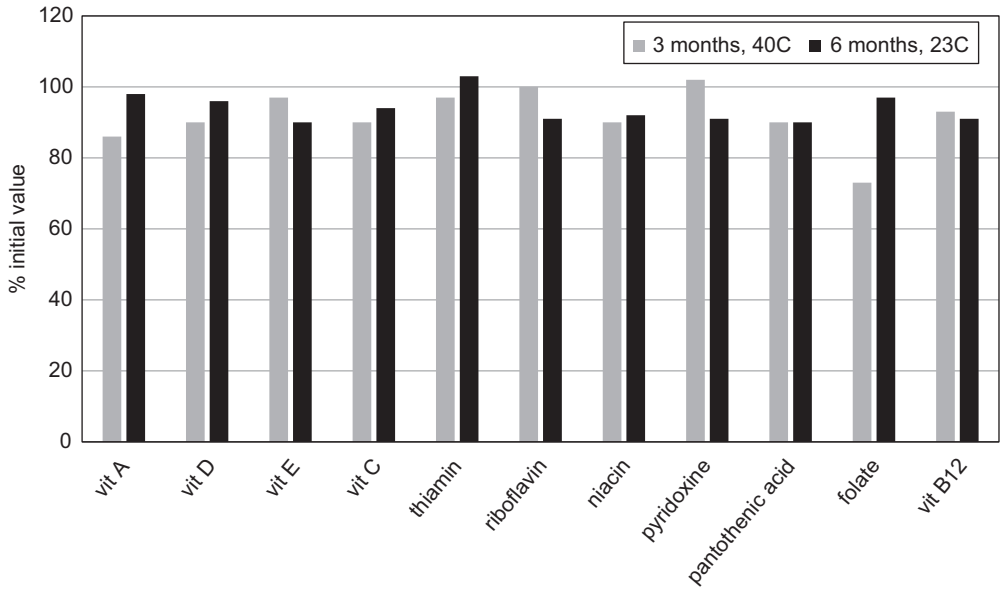

FIGURE 20.7 Stabilities of vitamins added to a breakfast cereal. *After Anderson, A.K., 1976. Food Technol. 30, 110–117.*

Biofortification involves the use of both conventional techniques of plant breeding as well as genetic modification of plant genomes to increase levels of key vitamins and essential minerals in crops. In cases of significant heterogeneity in tissue micronutrient content (provitamin A carotenoids in sweet potato, corn [maize], and cassava), conventional plant breeding techniques have been used to move high-micronutrient traits into agronomically superior varieties. In cases without such trait variability (provitamin A carotenoids and folate in rice), and for plants the breeding of which is prohibitively difficult or impossible (plantain, cassava, and potato), genetic engineering offers opportunities to improve tissue micronutrient contents. In both cases, improved germplasm can be moved into the respective crop breeding programs of local countries' national agricultural research systems to be incorporated into high-yielding cultivars well-suited to local agroecological conditions. This utilization of local agricultural systems to improve micronutrient nutrition amortizes the original costs of developing

high-micronutrient traits over years-to-decades of crop production in nations of need.

Conventional selective breeding combined with marker-assisted selection has produced germplasm with enhanced vitamin content. Such efforts by collaborators in the HarvestPlus program exploited heterogeneity in the β-carotene contents among available varieties to produce new, high-β-carotene varieties:[33]

- **Orange-fleshed sweet potato**—Forty-six improved varieties with β-carotene contents as great as 24,900 μg/g (compared to 30–100 μg/g in common cultivars) have been released in throughout Africa, South America, and China.[34]
- **Yellow cassava**—Screening of germplasm revealed a range of β-carotene contents of 0–19 μg/g. Three hybrids with β-carotene contents of 6–8 μg/g have been released in Nigeria.[35]
- **High-β-carotene corn (maize)**—Screening revealed a range of β-carotene contents of 0–19 μg/g in existing lines. Genetic loci associated with β-carotene level were identified, and DNA markers were developed that facilitated breeding using marker-assisted selection to take advantage of rare genetic variation in the β-carotene hydroxylase-1 (*CRTRB1*) gene, and increase expression of *PSY1*, which encodes for phytoene synthase, the rate-limiting step in the carotenoid biosynthetic pathway.[36] Hybrids were produced with β-carotene contents of 6–8 μg/g; five have been released in Zambia, Nigeria, and Ghana.[37]
- **High-β-carotene plantain**[38]—Screening of >300 genotypes revealed a range of β-carotene contents of 1–345 μg/g (fresh weight). Five varieties with β-carotene contents of 17–106 μg/g have been identified for dissemination in Burundi and the Democratic Republic of the Congo.[39]

Others have used selective breeding to produce high-β-carotene carrots[40] and tomatoes[41] and high-anthocyanin carrots.[42] Proof-of-concept experiments have been done to increase other vitamins species used as models in plant research: tocopherols[43] and vitamin B_6[44] in *Arabidopsis thaliana*; tocopherols in *Brassica napus*.[45] Strain selection and optimization of culture conditions of baker's yeast (*Saccharomyces cerevisiae*) have been used to make three- to fivefold increases in the folate contents of breads.[46]

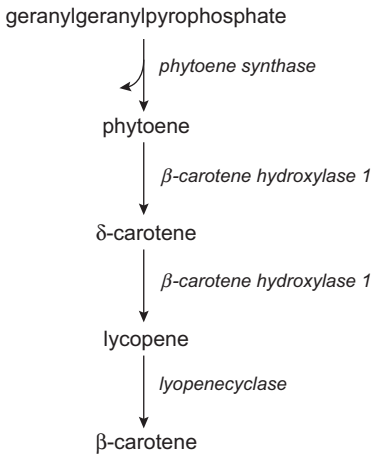

FIGURE 20.8 β-carotene biosynthetic pathway in plants.

Genetic engineering has been shown to be useful in adding vitamin biosynthetic capacity to plant species with incomplete biosynthetic pathways. This concept was demonstrated by the pioneering work of producing "**Golden Rice**,"[47] i.e., various rice containing ~35 μg/g β-carotene in the endosperm. This was achieved by inserting into the rice genome two missing genes needed for β-carotene synthesis (Fig. 20.8): *PSY* (encoding phytoene synthase), initially from daffodil (*Narcissus pseudonarcissus*) and subsequently from corn (maize); and *CRT1* (encoding β-carotene hydroxylase-1) from a soil bacterium (*Erwinia uredovora*). As the rice genome already contained the third gene required for this pathway, LYC (encoding *lycopene* cyclase), these additions completed β-carotene biosynthetic capacity.

Others have used genetic engineering to increase folate in lettuce[48] and rice.[49] Unlike conventional breeding, the

33. Increases in other micronutrients have also been achieved: high-iron pearl millet (India), bean (D.R. Congo, Rwanda), and high-zinc rice (Bangladesh, India) and wheat (India, Pakistan).
34. Andrade, M., 2014. Biofortification Progress Briefs, p. 13–15.
35. Kulakow, P., Parkes, E., 2014. Biofortification Progress Briefs, p.11–12.
36. Yan, J., Kandiani, C.B., Harjes, C.E., et al., 2010. Nature Genetics 42, 322–327; Toledo-Ortiz, G., Huq, E., Rodriguez-Concepcíon, M., 2010. PNAS 107, 11626–11631; Messias, R., Galli, V., Silva, S.D., et al., 2014. Nutrients 6, 546–563.
37. Dhliwayo, T., Palacios, N., Babu, R., et al., 2014. Biofortification Progress Briefs, p.9–10.
38. i.e., Cooking banana.
39. Ekesa, B., 2014. Biofortification Progress Briefs, p.15–16.
40. Mills, J.P., Simon, P.W., Tanumihardjo, S.A., 2008. J. Nutr. 138, 1692–1698.
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44. Raschke, M., Boycheva, S., Crèvecoeur, M., et al., 2011. Plant J. 66, 414–432.
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47. Ye, X. Al-Babili, S., Klöti, A., et al., 2000. Science 287, 303–305; Paine, J.A., Shipton, C.A., Chaggar, S., et al., 2005. Nat. Biotechnol. 23, 482–487.
48. Nunes, A.C.S., Kalkmann, D.C., Aragão, F.J.L., 2009. Transgenic Res. 18, 661–667.
49. Storozhenko, S., De Brouwer, V., Volkaert, M., et al., 2007. Nat. Biotechnol. 25, S212–S240.

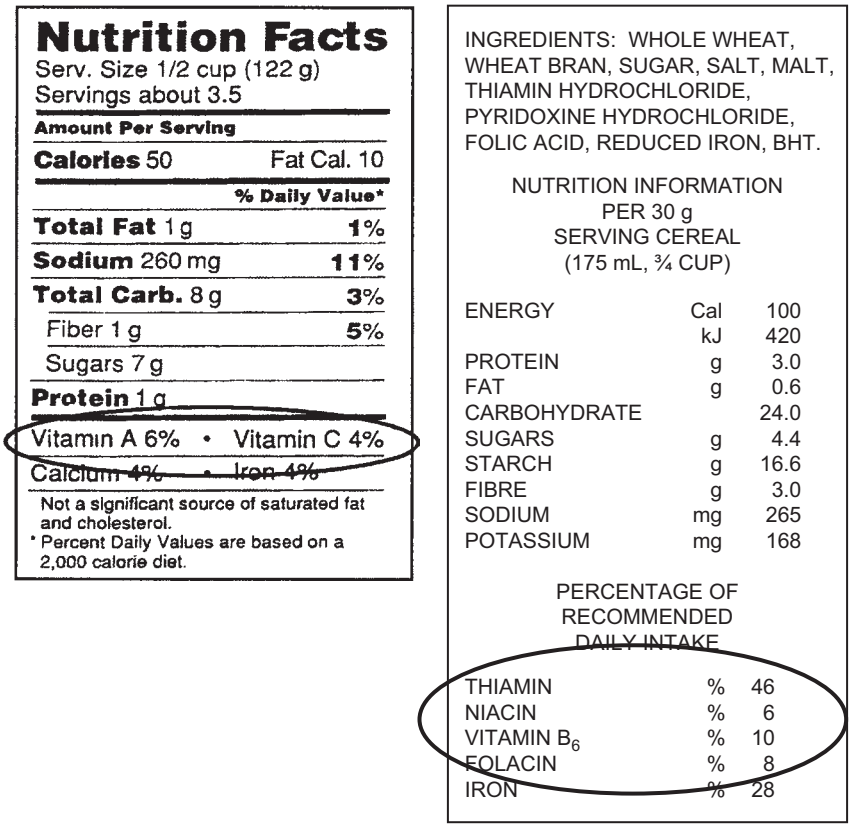

FIGURE 20.9 Nutrition information food labels, United States (left) and Canada (right). Note vitamin contents (*circled*).

products of genetic engineering are subject to regulatory legislation in each country. This has delayed the release of these enhanced varieties for use by farmers and, thus, to be available to consumers.[50]

6. VITAMIN LABELING OF FOODS

The labeling of nutrient contents of foods is a relatively new practice, having been instituted in the United States in 1972. The US regulations were respecified by the **Nutrition Labeling and Education Act** (**NLEA**) of 1990, the purpose of which was to provide, through a consistent food label format, useful information to consumers about the foods they eat in the context of their daily diets. The nutrition labeling of foods has the potential to influence consumer food use choices to the extent that the label information is accessible and can be acquired, processed, and used.

This US program involves compulsory labeling for most prepared and packaged foods.[51] It encourages voluntary labeling, either for individual products or at the point of purchase; for the most frequently consumed fresh fruits,[52] vegetables,[53] or seafood,[54] and at the point of purchase for fresh poultry and meats; and for prepared foods served in restaurants. In addition to information about the name of the product and its manufacturer and the measure/count of food contents, the act requires the food label to carry information about the ingredients, serving size and number of servings, and quantities of specified food components and nutrients (Fig. 20.9). Vitamin and mineral content information must be presented in comparison with a standard, the Reference Daily Intakes (RDIs) (Table 20.10).[55] For the information they present, nutrition labels draw on the USDA National Nutrient Data Bank, or an alternative data bank developed by the Produce Marketing Association.

50. Potrykus, I., 2010. New Biotechnol. 27, 466–472.
51. The act excludes foods containing few nutrients (e.g., plain coffee, tea, spices); foods produced by small businesses; and foods prepared and served by the same establishment.
52. Bananas, apples, watermelons, oranges, cantaloupe, grapes, grapefruits, strawberries, peaches, pears, nectarines, honeydew melons, plums, avocados, lemons, pineapples, tangerines, cherries, kiwi fruits, and limes.
53. Potatoes, iceberg lettuce, tomatoes, onions, carrots, celery, corn, broccoli, cucumbers, bell peppers, leaf lettuce, sweet potatoes, mushrooms, green onions, green beans, summer squash, and asparagus.
54. Shrimp, cod, pollock, catfish, scallops, salmon, flounder, sole, oysters, orange roughy, mackerel, ocean perch, rockfish, whiting, clams, haddock, blue crabs, rainbow trout, halibut, and lobster.
55. Most labels use the RDIs developed for adults and children 4 years of age or older; foods targeted to a certain age group must use the RDI developed for that group (see Chapter 5 for a discussion of DRIs).

TABLE 20.10 US RDAs (Recommended Dietary Allowances) Used in Food Labeling

Nutrient	Amount
Protein[a]	50 g
Minerals	
Calcium	1000 mg
Iron	18 mg
Iodine	150 µg
Copper	2 mg
Vitamins	
Vitamin A	5000 IU
Vitamin D	400 IU
Vitamin E	30 IU
Vitamin C	60 mg
Thiamin	1.5 mg
Riboflavin	1.7 mg
Niacin	20 mg
Vitamin B_6	2 mg
Pantothenic acid	10 mg
Biotin	300 µg
Folate	400 µg

The NLEA requires that information about vitamin A and vitamin C be carried on all food labels. It makes optional the disclosure of contents of other nutrients including vitamins for which RDAs (Recommended Dietary Allowances) have been established. In all cases, information must be presented according to the specified format.

7. VITAMINS IN HUMAN DIETS

Trends in US food intake patterns. Historical records of the American food supply show increases in the amounts of most of the vitamins available for consumption (Table 20.11).[56] Whether such increases have been reflected in the actual intakes of vitamins, or whether they have been distributed equitably across the American population is not indicated by such gross evaluations of the food supply. It is clear that people with low incomes tend to consume less food, although their food tends to have greater nutritional value per calorie than that consumed by people with greater incomes. While differences in diet quality due to income status appear to be small on average, variation in nutrient intake within groups of individuals appears to be very large. On average, at least, the vitamin intakes of Americans would appear to be generally adequate (Table 21.8). However, studies indicate that the vitamin intakes of many Americans may not meet the RDAs.

Despite an emerging picture of health benefits of diets richer in fruits and vegetables, surveys have shown that the regular intakes of fruits and vegetables of many Americans continue to fall short of the 5-A-Day goals. During the last decade, these intakes have increased by nearly 29% for vegetables and 38% for noncitrus fruits; however, the list of most frequently consumed fruits and vegetables continues to be short, with lowest consumption observed among lower socioeconomic groups and among individuals unaware of the health benefits attached to fruits and vegetables.

Vitamin Intakes From Foods

Food intake patterns are determined by many factors (e.g., tradition, taste, access, cost, and ease of preparation) but seldom nutrient content. In addition, patterns of food intake change. The most current estimates indicate that most Americans obtain most of their vitamins from their foods (Table 20.12); however, the intakes of vitamins A, D, E, K, B_6, B_{12}, thiamin, riboflavin, niacin, and folate were found to be generally lower among individuals living under 131% of poverty compared to other economic groups.[57] Individuals consuming strict vegetarian diets will not obtain adequate amounts of vitamins D and B_{12} through food sources alone.[58] Foods of both plant and animal origin provide vitamins in mixed diets for humans (Fig. 20.10; Table 20.13):

- **Meats and meat products**—Generally excellent sources of thiamin, riboflavin, niacin, pyridoxine, and vitamin B_{12}. Liver (including that from poultry or fish) is a very good source of vitamins A, D, E, and B_{12}, as well as folacin. Eggs are good sources of biotin. Animal products, however, are generally not good sources of vitamin C, K (except pork liver), or folate.
- **Beans, peas and lentils**—Generally good sources of thiamin, riboflavin, niacin, vitamin B_6, biotin, pantothenic acid, and folate.
- **Milk products**—Important sources of vitamins A and C, thiamin, riboflavin,[59] pyridoxine, and vitamin B_{12}. Because milk is widely enriched with irradiated ergosterol (vitamin D_2), it is also an important source of vitamin D.[60]

56. These result from increases in the availability of vegetables (+26%), fruits (+22%), grains (+44%), added fats and oils (+56%), and meats (+13%); and decreases in milk (−34%), eggs (−19%) over that same period of time (1970–2007) (Barnard, N.D., 2010. Am. J. Clin. Nutr. 91, 1530S–1536S).

57. USDA, Agricultural Research Service, 2010. What We Eat in American, NHANES 2007–2008 www.ars.usda.gov/ba/bhnrc/fsrg.

58. As well as calcium and long chain n-3 fatty acids.

59. In the United States, milk products supply an estimated 40% of the required riboflavin.

60. This practice has practically eliminated rickets in countries that use it.

TABLE 20.11 Vitamins Available for Consumption (per Person per Day) by Americans

Vitamin	1909–19	1920–29	1930–39	1940–49	1950–59	1960–69	1970–79	1980–89	1990–99	2000	2005
Vitamin A (IU)	1040	1090	1070	1210	1140	1150	1260	1230	1270	1260	1030
Vitamin E (mg)[a]	7.7	8.5	9.2	10.3	10.6	11.7	13.9	15.6	16.8	20.0	21.4
Vitamin C (mg)	95	100	104	112	98	93	112	119	127	130	115
Thiamin (mg)	1.5	1.5	1.4	1.9	1.8	1.9	2.3	2.6	3.0	3.0	2.9
Riboflavin (mg)	1.8	1.8	1.8	2.3	2.3	2.2	2.5	2.8	2.9	2.9	2.8
Niacin (mg)	18	17	16	20	20	20	25	29	32	33	33
Vitamin B_6 (mg)	2.1	2.0	1.9	2.0	1.8	1.8	2.0	2.2	2.4	2.5	2.5
Folate (μg)	309	305	309	325	297	284	326	356	449	706	682
Vitamin B_{12} (μg)	7.8	7.6	7.2	8.6	8.6	8.9	8.9	8.1	7.9	8.2	8.5

[a]*α-Tocopherol equivalents.*
Hiza, H.A.B., Bente, L., Fungwe, T., 2008. "Nutrient Content of the U.S. Food Supply", Home Economics Rept. 58, USDA, Washington, 72 pp.

TABLE 20.12 Average Daily Intakes of Vitamins From Foods by Americans, by Percentage of Usual Intake

Vitamin	Average Intake From Foods	% Consumed in				% Consumed Away From Home
		Breakfast	Lunch	Dinner	Snacks	
Vitamin A (μg)	607 ± 15	29	21	32	18	27
Vitamin D (μg)	4.6 ± 0.1	36	18	28	18	23
Vitamin E (mg)	7.2 ± 0.2	17	24	34	25	35
Vitamin K (mg)	88.9 ± 4.2	8	29	52	11	38
Vitamin C (mg)	84.2 ± 3.5	22	20	30	28	27
Thiamin (mg)	1.59 ± 0.03	25	24	34	17	31
Riboflavin (mg)	2.16 ± 0.04	30	20	28	22	30
Niacin (mg)	23.9 ± 0.34	19	25	39	17	34
Vitamin B_6 (mg)	1.91 ± 0.04	24	22	35	19	31
Pantothenic acid (mg)	–	–	–	–	–	–
Folate (μg)	527 ± 10	29	22	42	17	29
Vitamin B_{12} (μg)	5.19 ± 0.12	27	23	34	16	31

US Department of Agriculture, Agricultural Research Service, 2010. What We Eat in American, NHANES 2007–2008. www.ars.usda.gov/ba/bhnrc/fsrg.

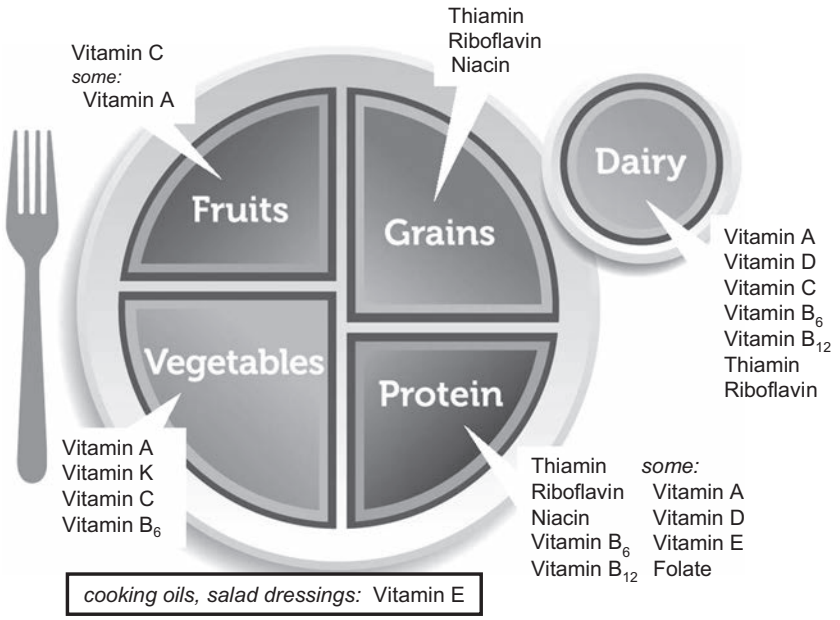

FIGURE 20.10 Vitamins provided by the major food groups, as depicted in ChooseMyPlate.gov.

- **Vegetables**—Generally good sources of vitamins A, K, and C and pyridoxine.
- **Fruits**—Generally good sources of vitamin C; some (e.g., mangoes) are also good sources of vitamin A.
- **Grain products**—Generally good sources of thiamin, riboflavin, and niacin.
- **Plant oils**—Generally good sources of vitamin E. Red palm oil is a particularly good source of vitamin A.

Dinner, typically the largest meal of the day, tends to be the most important for providing vitamins, but breakfast tends to be the most important in providing vitamin D likely due to the consumption of vitamin D-fortified milk. Snacking, now practiced by 97% of Americans,[61] provided nearly one-fifth of vitamin intake. An even greater amount, some 30%, was obtained from meals consumed away from the home, i.e., prepared by others. Studies show that Americans, on average, fall short of the daily recommendations for fruits (by 132%), vegetables (by 31%), whole grains (by 248%), and dairy products (by 66%).[62,63]

Vitamins in Breast Milk and Formula Foods

The vitamin contents of foods that are intended for use as the main or sole components of diets (e.g., human milk, infant formulas, and parenteral feeding solutions) are particularly important determinants of the vitamin status of individuals consuming them. The vitamin contents of human milk can vary, most being reduced under conditions of vitamin deprivation and responding to supplementation. For that reason, the contents of some vitamins in breast milk can vary,[64] even in well-nourished women. In general, the concentrations of all vitamins (except vitamin B_{12}) in human milk tend to increase during lactation. In comparison with cow's milk, human milk contains more of vitamins A, E, C, and niacin, but less vitamin K, thiamin, riboflavin, and pyridoxine (Table 20.14). Reference values for the contents of several vitamins in the breast milk of well-nourished women have been set for the purposes of establishing adequate intake values: thiamin, 0.21 mg/L; riboflavin, 0.35 mg/L; vitamin B_6, 0.13 mg/L, vitamin B_{12}, 0.42 µg/L; and choline, 160 mg/L.[65]

Because infant formulas and parenteral feeding solutions are carefully prepared and quality-controlled products, each is formulated largely from purified or partially refined ingredients to contain known amounts of the vitamins. For parenteral feeding solutions, however, some

61. Piernas, C., Popkin, B.M., 2010. J. Nutr. 140, 325–332.
62. For an individual consuming 2000 cal/day, the USDA Food Guide recommends the following daily servings: fruit, 2 cups; vegetables, 2.5 cups; whole grains, 3 oz.; dairy, 3 cups.
63. In fact, it is not clear whether the US agriculture could meet those recommended needs. In 2006, the USDA estimated that doing so would require doubling the US fruit production; increasing the production of nonstarchy vegetables by ~430% while reducing the production of starchy vegetables by 35%; and increasing dairy production by 66%. In contrast, increasing whole grain consumption at the expense of milled wheat would reduce total grain demand by 27% (Buxby, J.C., Wells, H.F., Vocke, G., 2006. Economic Res. Rept. No. 31, 29 pp.).
64. e.g., Riboflavin concentrations in the breast milk of well-nourished women has been found in the range of 180–1800 µg/L (Roughead, Z.K., McCormick, D.B., 1990. Am. J. Clin. Nutr. 52, 854–857).
65. These values compare to those observed for breast milk fed to deficient infants: thiamin, 0.16 mg/L; riboflavin, 0.21 mg/L; vitamin B_6, 0.10 mg/L, vitamin B_{12}, <0.05 µg/L; choline, 90 mg/L (Allen, L.H., 2012. Adv. Nutr. 3, 362–369).

TABLE 20.13 Contributions of Food Groups to the Vitamin Contents of Mixed Diets for Americans

	Vitamin A	Vitamin E	Vitamin C	Thiamin	Riboflavin	Niacin	Vitamin B_6	Folate	Vitamin B_{12}
Per Capita Daily Availability									
	1030 µg	21.4 mg	115 mg	2.9 mg	2.8 mg	33 mg	2.5 mg	682 µg	8.5 µg
% Contributions by Food Group									
Vegetables	**27.1**[a]	5.9	**48.3**	8.6	5.7	9.8	20.1	12.3	0
Legumes, Nuts	0	4.8	0.1	4.5	1.6	4.0	3.6	9.3	0
Fruits	2.5	2.9	**40.5**	3.6	2.3	2.0	9.1	5.9	0
Grain products	5.3	3.9	4.7	**59.3**	**38.5**	**42.3**	18.3	**61.2**[b]	0.1
Meats, fish	**33.2**	3.8	2.3	18.0	17.9	**37.7**	**38.1**	3.8	**77.4**
Milk products	15.7	1.7	2.5	4.3	**25.0**	1.1	6.8	3.3	18.1
Eggs	6.6	1.8	0	0.7	6.3	0.1	1.9	2.5	4.2
Fats, oils	7.4	**74.6**	0	0	0.1	0	0	0	0.1
Other	2.2	0.6	1.7	0.9	1.9	3.2	1.9	1.7	0

[a]*Major contributing foods indicated in bold.*
[b]*Reflects mandatory fortification of wheat flour.*
Hiza, H.A.B., Bente, L., Fungwe, T., 2008. USDA Home Economics Rep. No. 58, 72 pp.

TABLE 20.14 Vitamin Contents of Human and Cow's Milk

Vitamin	Human Milk	Cow's Milk
Vitamin A (retinol) (mg/L)	0.60	0.31
Vitamin D_3 (μg/L)	0.3	0.2
Vitamin E (mg/L)	3.5	0.9
Vitamin K (mg/L)	0.15	0.6
Ascorbic acid (mg/L)	38	20
Thiamin (mg/L)	0.16	0.40
Riboflavin (mg/L)	0.30	1.90
Niacin (mg/L)	2.3	0.8
Pyridoxine (mg/L)	0.06	0.40
Biotin (μg/L)	7.6	20
Pantothenic acid (mg/L)	0.26	0.36
Folate (mg/L)	0.05	0.05
Vitamin B_{12} (μg/L)	<0.1	3

Porter, J.W.G., 1978. Proc. Nutr. Soc. 37, 225–230.

problems related to vitamin nutrition have occurred. One problem involved biotin, which was not added to such solutions before 1981 in the belief that intestinal synthesis of the vitamin was adequate for all patients except children with inborn metabolic errors or individuals ingesting large amounts of raw egg white. When it was found that children supported by total parenteral nutrition (TPN) frequently suffered from gastrointestinal abnormalities that responded to biotin,[66] the vitamin was added to TPN solutions.[67] Another problem with parenteral feeding solutions has been the loss of fat-soluble vitamins and riboflavin either by absorption to the plastic bags and tubing most frequently used, or by decomposition on exposure to light. Such effects can reduce the delivery of vitamins A, D, and E to the patient by two-thirds and to result in the loss of one-third of the riboflavin.

8. VITAMIN SUPPLEMENTATION

More than half of the US population consumes dietary supplements,[68] multivitamin/mineral supplements are the most popular and are used by 40% of American adults and more than 30% of children.[69] Dietary supplement use is greater among women than men and increases with age such that nearly three-quarters of Americans over 70 years. take a supplement. Studies have shown that supplement users tend to be more health conscious and have better diets, more education, and higher incomes than the general population. Supplement use is greater among health professionals, vegetarians, the elderly, and readers of health-focused magazines. Vitamin supplement use is most frequent among individuals who believe that diet affects disease, nondrinkers and lighter drinkers of alcohol, former smokers and individuals who never smoked, and individuals in the lowest three quartiles of BMI. Users of vitamin supplements have markedly greater vitamin intakes than nonusers (Table 20.15). The median levels of supplement use by Americans is one to two times the RDA for vitamins A, D, and B_6; niacin; pantothenic acid; and folate; and greater than twice the RDA for vitamins E, C, and B_{12}; thiamin; and riboflavin.

Studies in the US have shown that regular users of multivitamin/mineral supplements are more likely to have adequate vitamin intakes (Table 20.16), less likely to have suboptimal blood nutrient concentrations and more likely to have optimal levels of biomarkers of chronic disease.[70] However, it is not clear whether those users are actually at reduced chronic disease risk as a result of that practice. This is, in part, because users have generally healthy lifestyles, which independently reduce their low risk. Systematic reviews of the relevant clinical literature are limited by the paucity of rigorous studies conducted to date; most available results do not provide strong evidence for beneficial health effects of multivitamin/mineral supplements for most people.[71] However, there have been indications of some benefits: reduced fracture risks in postmenopausal women, marginal increases in cognitive performance in children.[72]

Interventions with multivitamin supplements have been useful in undernourished populations. For example, antenatal multiple-micronutrient supplementation reduced combined fetal loss and neonatal deaths by 11% and increased birth weight by 14% in Indonesia (particularly in undernourished and/or anemic mothers), and increased birth weight by in Nepal.[73] The use of vitamin supplements among peoples in developed countries has become great enough to make this means a significant contributor to the vitamin nutriture of

66. These patients appeared to have had altered gut microbiota as a result of antibiotic treatment.

67. Although there are no RDAs for biotin, it has been suggested that biotin supplements be given to individuals being fed parenterally (infants, 30 μg/kg/day; adults, 5 μg/kg/day). These levels are consistent with the provisional recommendations (infants, 10 μg/day; adults, 30–100 μg/day) of the National Research Council Food Nutrition Board (1989).

68. i.e., 53.4% of respondents in the NHANES 2003–06 (Bailey, R.L., Gahche, J.J., Lentino, C.V., et al., 2011. J. Nutr. 141, 261–266).

69. Rock, C.L., 2007. Am. J. Clin. Nutr. 85, 277S–279S; Gahche, J., Bailey, R., Burt, V., et al., 2011. NCHS Data Brief No. 61; Picciano, M.F., Dwyer, J.T., Radimer, K.L., et al., 2007. Arch. Pediatr. Adolesc. Med. 161, 978–985.

70. Block, G., Jensen, C.D., Norkus, E.P., et al., 2007. Nutr. J. 6, 30.

71. NIH State-of-the-Science Panel, 2007. Am. J. Clin. Nutr. 85, S257–S264; McCormick, D.B., 2010. Nutr. Rev. 68, 207–213.

72. Eilander, A., Gera, T., Sachdev, H.S., et al., 2010. Am. J. Clin. Nutr. 91, 115–130.

73. The Supplementation with Multiple Micronutrients Intervention Trial (SUMMIT) Study Group, 2008. Lancet 371, 215–222; Vaidya, A., Saville, N., Shresthra, B.P., et al., 2008. Lancet 371, 492–469.

TABLE 20.15 Contributions of Commonly Used Dietary Supplements to Vitamin Intakes of Americans

Vitamin	Sex	EAR[a]	Intake From Food	Intake From Supplement[a]	Total Intake
Vitamin A	Male	625 µg	656 µg	1050 µg	1706 µg
	Female	500 µg	564 µg	1050 µg	1614 µg
Vitamin E	Male	12 µg	8.2 µg	13.5 µg	21.7 µg
	Female	12 µg	6.3 µg	13.5 µg	19.8 µg
Vitamin C	Male	75 mg	105 mg	60 mg	165 mg
	Female	60 mg	84 mg	60 mg	144 mg

[a]*Vitamin content based on that of the most commonly consumed multivitamin/mineral supplement in the NHANES 2001–02.*
Dwyer, J.T., Holden, J., Andrews, K., et al., 2007. Anal. Bioanal. Chem. 389, 37–46.

TABLE 20.16 Effect of Multivitamin (MV) Use on Prevalence of Adequate Vitamin Intakes of Subjects in the Hawaii–Los Angeles Multiethnic Cohort

	Men			Women		
	Nonusers	Users		Nonusers	Users	
Vitamin	From Food	From Food	Total (Food + MV)	From Food	From Food	Total (Food + MV)
Vitamin A	59 ± 42[a]	61 ± 41	87 ± 29	69 ± 39	73 ± 39	89 ± 28
Vitamin E	27 ± 41	28 ± 41	68 ± 43	22 ± 38	23 ± 39	60 ± 45
Vitamin C	72 ± 42	76 ± 40	89 ± 29	82 ± 37	86 ± 33	93 ± 25
Thiamin	82 ± 35	84 ± 33	94 ± 23	79 ± 37	82 ± 35	92 ± 25
Riboflavin	87 ± 30	89 ± 28	95 ± 19	77 ± 20	79 ± 19	90 ± 16
Niacin	89 ± 27	90 ± 25	96 ± 18	84 ± 32	86 ± 30	94 ± 22
Vitamin B_6	79 ± 37	81 ± 36	93 ± 24	73 ± 41	77 ± 39	90 ± 28
Folate	93 ± 23	94 ± 21	97 ± 15	88 ± 30	90 ± 27	95 ± 20
Vitamin B_{12}	90 ± 28	90 ± 27	96 ± 18	83 ± 18	85 ± 34	94 ± 23

[a]*Mean ± S.E.*
Murphy, S.P., White, K.K., Park, S.Y., et al., 2007. Am. J. Clin. Nutr. 85, S280–S284.

those populations. Still, for most of the poor in those countries, access to multivitamin supplements remains limited by costs, making food-based approaches more sustainable for addressing prevalent multimicronutrient shortages.

Retail sales of vitamins and nutritional supplements in the United States are expected to exceed $36 billion by 2017, a doubling since 2001. The drivers of this trend have been identified as increasing awareness of consumers, increasing demand by the food industry facilitated by the GRAS[74] status of vitamins, increasing consumer demand for health-beneficial products, and increasing demand by livestock producers for performance enhancers. The United States is the largest consumer of vitamins, 40% of which is in multivitamin supplements most (>70%) of which are sold in drug stores, supermarkets, and health food stores. In the United States, dietary supplements are regulated by the FDA under the Dietary Supplement Health and Education Act of 1994.[75]

74. Generally Recognized As Safe.

75. This legislation charged the FDA to establish a framework for assuring safety, outline guidelines for literature displayed where supplements are sold, provide for use of claims and nutritional support statements, require ingredient and nutritional labeling, and establish good manufacturing practice regulations. The law changed previous legislation in that dietary supplements are no longer subject to the premarket safety evaluations required of other food ingredients.

Guidelines for Supplement Use

Healthy individuals can and should obtain adequate amounts of all nutrients, including vitamins, from a well-balanced diet based on different foods of good quality. Such an approach minimizes the risks of deficiencies as well as excesses of all nutrients. It also acknowledges that foods can provide health benefits that have yet to be fully elucidated.

For individuals with varied, balanced diets, the benefit of taking vitamin supplements is doubtful. However, certain circumstances may warrant the use of vitamin supplements:

- Folate for women, who may conceive, are pregnant or are lactating;[76]
- Multivitamins for individuals with very low caloric intakes (such that their consumption of total food is insufficient to provide all nutrients);
- Vitamin B_{12} for strict vegetarians, individuals with gastric achlorhydria, gastric resection, and most people over 50 years of age;
- Vitamin D for people living in the northern latitudes;
- Vitamin K (single dose) for newborn infants to prevent abnormal bleeding;
- Patients with diseases or medications that interfere with vitamin utilization.

9. VITAMINS IN LIVESTOCK FEEDING

Vitamins in Animal Feeds

The economic considerations in feeding livestock generally dictate the use of a relatively small number of feedstuffs with few (if any) day-to-day changes in diet formulation.[77] In livestock production, the continued use of the same or very similar diets has resulted in the empirical development of knowledge about the vitamin contents of feedstuffs (Table 20.17).

Unlike human diets, formulated diets for livestock generally do not provide adequate amounts of vitamins unless they are supplemented with either certain vitamin-rich feedstuffs or purified vitamins. In general, the relative vitamin adequacy of unsupplemented animal feeds depends on the relative complexity (the number of feedstuffs used in the mixture) of the diet. The vitamin contents of simple rations tend to be less than those of complex ones (Table 20.18). For example, complex diets such as those used for feeding poultry or swine in the 1950s[78] would be expected to contain in their constituent feedstuffs more than an adequate amount of vitamin B_{12}, and adequate (or nearly so) amounts of vitamin K, vitamin E, thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, folate, and choline. In contrast, the simpler rations (based almost exclusively on corn and soybean meal) that are used today contain lower amounts, if any, of the more costly vitamin-rich feedstuffs previously used. Such simple rations can be expected to contain in constituent feedstuffs adequate levels only of vitamin E, thiamin, pyridoxine, and biotin (Table 20.19). The availability of stable, biologically available, and economical vitamins facilitated this change in complexity of animal feeds by replacing with inexpensive mixtures of vitamins the more costly vitamin-rich feedstuffs used previously.

Vitamins likely to be limiting in (unsupplemented) nonruminant diets:
- Vitamin A.
- Vitamin E
- Niacin
- Pantothenic acid
- Choline (chicks)
- *If raised indoors*—vitamin D
- *If raised on slatted or wire floors*—vitamin K, vitamin B_{12}

Losses of Vitamins From Feedstuffs and Finished Feeds

The vitamin contents of feedstuffs and finished feeds[79] are subject to destruction in ways very similar to those of foods. The storage losses that can occur in particular feedstuffs are dependent on the conditions of temperature and moisture during storage; heat and humidity enhance oxidation reactions of several of the vitamins (vitamins A and E, thiamin, riboflavin, and biotin). Vitamin losses are, therefore, minimized by drying feedstuffs quickly and storing them dry in weather-proof bins. Where the drying of a feedstuff is slow[80] or incomplete,[81] or where leaky bins are used for its storage, vitamin losses are greatest.

Vitamin losses from finished feeds are usually greater than those of individual feedstuffs. Finished feeds are supplemented with essential trace elements, some of which (Cu^{2+}, Fe^{3+}) can act as catalytic centers of oxidation reactions leading to vitamin destruction. Such effects are particularly important in high-energy feeds (e.g., broiler diets), which generally contain significant amounts of polyunsaturated fats. It is a common practice in many countries to

76. Pregnant and lactating women may also need supplements of iron and calcium.
77. For example, broiler chicks are typically fed the same diet from hatching to 3 weeks of age, and laying hens may be fed the same diet for 20 weeks before the formula is changed.
78. Those complex diets contained, in addition to a major grain and soybean meal, small amounts of alfalfa meal, corn distillers' dried solubles, fish meal, and meat-and-bone meal.
79. Complete, blended, ready-to-feed ratios.
80. For example, sun drying enhances the destruction of vitamin E in corn (although sun curing of cut hay is essential to provide vitamin D activity).
81. Where moisture is not reduced to less than about 15%.

TABLE 20.17 Feedstuffs Containing Significant Amounts of Vitamins

Vitamin A	Vitamin D	Vitamin E	Vitamin K	Vitamin C	Thiamin	Riboflavin	Niacin	Vitamin B_6	Biotin	Pantothenic Acid	Folate	Vitamin B_{12}	Choline
None[a]	None[a]	Alfalfa, dehydrated	Alfalfa, dehydrated	None[a,b]	None[a]	Dried skim milk	Barley	Sunflower seed meal	Corn germ meal	Molasses	Dried brewers' grains	Dried fish solubles	Liver/glandular meal
	Alfalfa, sun-cured		Alfalfa, sun-cured			Peanut meal	Cottonseed meal	Sesame meal	Brewers' yeast	Rice polishings	Alfalfa, dehydrated	Liver/glandular meal	Dried fish solubles
	Wheat germ meal					Brewers' yeast	Dried fish solubles	Meat/bone meal	Molasses	Sunflower seed meal	Brewers' yeast	Hydrolyzed feathers	Soybean meal
	Corn germ meal					Dried buttermilk	Rice bran, polishings		Torula yeast	Peanut meal	Soybean meal	Fish meals	Corn distillers' solubles
	Stabilized vegetable oils					Dried whey	Wheat bran		Hydrolyzed feathers	Torula yeast	Torula yeast	Crab meal	
						Torula yeast	Corn gluten feed		Safflower meal	Liver/glandular meal	Meat/bone meal	Dried skim milk	
						Corn distillers' solubles	Fish meals			Brewers' yeast	Corn distillers' solubles	Dried butter milk	
						Liver/glandular meal	Peanut meal				Alfalfa, sun-cured	Meat/bone meal	
							Torula yeast						
							Corn gluten meal						
							Corn distillers' solubles						
							Liver/glandular meal						
							Sunflower seed meal						
							Brewers' yeast						

[a]*Instability of the vitamin in most feedstuffs renders few, if any, predictable sources of appreciable amounts of it.*
[b]*Not required by livestock species.*

TABLE 20.18 Vitamins Provided by Constituent Feedstuffs in Older (Complex) and Modern (Simple) Chick Starter Diets

	1942 Diet[a] (%)	Modern Diet (%)
Ingredients		
Cornmeal	27.5	65.61
Oats	10.0	
Wheat bran	20.0	
Wheat middlings	10.0	
Soybean meal, 49% protein	10.0	19.08
Meat and bone meal	10.0	4.78
Poultry by-product meal		7.00
Dried whey	5.0	
Dehydrated alfalfa meal	5.0	
Blended fat		3.18
Limestone	2.0	
Salt	0.5	0.25
D,L-methionine (98%)		0.10
Trace minerals	+[b]	+[c]
Vitamins	+[d]	+[e]
Vitamins Provided by Feedstuffs		
Vitamin A (IU)	6000 (400)[f]	1360 (91)[f,g]
Vitamin E (IU)	27 (270)	20.5 (205)[g]
Vitamin K (mg)	0.73 (146)	0 (0)[g]
Thiamin (mg)	4.7 (261)	3.1 (172)
Riboflavin (mg)	5.4 (150)	2.3 (64)[g]
Niacin (mg)	69.9 (259)	28.3 (105)[g]
Pyridoxine (mg)	5.7 (190)	5.3 (177)
Biotin (g)	208 (139)	141 (94)
Pantothenic acid (mg)	15.7 (157)	7.4 (74)[g]
Folate (mg)	0.81 (145)	0.32 (58)
Vitamin B_{12} (g)	6.5 (72)	21.0 (233)[g]
Choline (mg)	1115 (86)	1395 (107)[g]

[a]This was a state-of-the-art diet for starting chicks at Cornell University in 1942.
[b]$MnSO_4$, 125 mg/kg.
[c]Provides per kg of diet: ZnO, 66 mg; $MnSO_4$, 220 mg; Na_2SeO_3, 220 g.
[d]Vitamin D_3, 790 IU/kg.
[e]Provides per kg of diet: vitamin A, 4400 IU; vitamin D_3, 2200 IU; vitamin E, 5.5 IU; vitamin K_3, 2 mg; riboflavin, 4 mg; nicotinic acid, 33 mg; pantothenic acid, 11 mg; vitamin B_{12}, 1 g; choline, 220 mg.
[f]Numbers in parentheses give amounts of each vitamin as a percentage of current (1984) recommendations of the U.S. National Research Council.
[g]Included in the vitamin–mineral premix.

TABLE 20.19 Insufficient Amounts of Vitamins in Turkey Starter Diet Feedstuffs

	Level From Feedstuffs, % NRC Requirement	
Vitamin	**Simple Feed[a]**	**Complex Feed[b]**
Vitamin A	20	40
Vitamin E	130	130
Thiamin	170	160
Riboflavin	60	90
Niacin	30	60
Vitamin B_6	130	100
Pantothenic acid	90	110
Biotin	120	140
Folate	50	50
Vitamin B_{12}	0	74
Choline	90	100

[a]Corn, 40.5%; soybean meal, 51.2%; animal fat, 4%; $CaHPO_4$, 3%; limestone, 0.8%; salt, 0.3%; methionine, 0.15%; trace minerals, 0.05%.
[b]Milo, 20.5%; wheat, 20%; soybean meal, 33.9%; poultry meal, 6%; animal fat, 5%; meat and bone meal, 5%; fish meal, 4%; alfalfa meal, 2%; distillers' grains and solubles, 2%; limestone, 0.7%; $CaHPO_4$, 0.5%; salt, 0.3%; methionine, 0.13%; trace minerals, 0.05%.
Anonymous, 1989. "Vitamin Nutrition for Poultry". Hoffman–La Roche, Inc., Nutley, New Jersey, pp.13–14.

compress many of these (and other) feeds into pellet form[82] by processes involving steam, heat, and pressure. Evidence suggests that pelleting can enhance the bioavailability of niacin and biotin, which occur in feedstuffs in bound forms; but it generally results in the destruction of vitamins A, D, E, K_3, C, and thiamin.

Vitamin Premixes for Animal Feeds

As purified sources of the vitamins have become available at low cost, it has become possible to use fewer feedstuffs in less complicated blends to produce diets of high quality that will support efficient and predictable animal performance. Thus, many feedstuffs formerly valuable as sources of vitamins (e.g., brewers' yeast, dried buttermilk, and green feeds[83]) are no longer economical to use in intensive animal management systems. This is most true in the economically developed parts of the world. In the developing world, such factors as the shortage of hard currency may make purified sources of vitamins too expensive to use in animal diets, making natural sources of the vitamins more valuable. Under such circumstances, it is prudent to exploit a wide variety of local feedstuffs, food wastes, and food by-products in the formulation of animal feeds that are adequate in terms of vitamins as well as all other known nutrients.

The use of purified vitamins as supplements to animal feeds has increased the economy of animal feeding by obviating the need to include relatively expensive vitamin-rich feedstuffs in favor of lower-priced feedstuffs that are lower in vitamin content but provide useful energy and protein. In modern practice, the addition of vitamins to animal feeds is accomplished by preparing a mixture of the specific vitamins required with a suitable carrier[84] to ensure homogeneous distribution in the feed as it is mixed. Such a preparation is referred to as **vitamin premix** (Tables 20.20 and 20.21) and is handled in much the same way as other feedstuffs in the blending of animal feeds. Typically, vitamin premixes are formulated to be blended into diets at rates of 0.5–1.0%.[85]

Premixes generally also contain synthetic antioxidants [e.g., ethoxyquin, butylated hydroxytoluene

82. There are many reasons for pelleting finished feeds. Pelleting prevents demixing of the feed during handling. By increasing bulk density, it can improve the economy of feed handling and improve the consumption of bulky, low-density feeds. It can improve the handling of feeds that are otherwise dusty and reduce wastage at the feeder. The metabolizable energy values of some feedstuffs may be improved by the steam treatment used in pelleting (e.g., soybean meal with significant residual antitryptic activity).

83. e.g., Fresh cabbage, grass.

84. e.g., Soybean meal, finely ground corn or wheat, corn gluten meal, wheat middlings.

85. The cost of the vitamin premix is typically <2% of the total cost of most finished feeds. Of that amount, two-thirds of the vitamin cost is accounted for by vitamin E, niacin, vitamin A, and riboflavin (roughly in that order).

TABLE 20.20 Vitamins Generally Included in Vitamin Premixes for Livestock Diets

Vitamin	Poultry	Piglets	Hogs	Calves	Cattle
Vitamin A	+	+	+	+	+
Vitamin D_3	+	+	+	+	+
Vitamin E	+	+	+	+	+[a]
Vitamin K	+	+	+[a]		
Ascorbic acid	+[b]+	+			
Thiamin	+[a]	+[a]			
Riboflavin	+	+	+	+[a]	
Niacin	+	+			
Vitamin B_6	+[a]	+	+[a]	+[a]	
Pantothenic acid	+	+	+	+[a]	
Biotin	+[a]	+[a]	+[a]		
Folate					
Vitamin B_{12}	+	+	+[a]		
Choline	+	+			

[a]*Sometimes added.*
[b]*Added in situations of stress.*

(BHT)] to enhance vitamin stability during storage.[86] In many cases, trace minerals are included in **vitamin–mineral premixes**.[87] It is a standard practice in the formulation of vitamin premixes to use amounts of vitamins that, when added to the expected amounts intrinsic to the component feedstuffs, will provide a comfortable excess above those levels found experimentally to be required to prevent overt deficiency signs. This is done in view of the many potential causes of increased vitamin needs; owing to the low cost of vitamin supplementation, this approach is considered a kind of low-cost nutrition insurance, as vitamin premixes usually account for only 1–2% of the total cost of feeds for nonruminant livestock. It should be remembered, however, that purified vitamins may not always be cheap, particularly in developing countries. Under those circumstances, the appropriate way to assess the value of using vitamin supplements is to compare their market prices with the estimated loss of production realized by not supplementing feeds that can be economically produced using locally available feedstuffs.

Stabilities of Vitamins in Feeds

Vitamins tend to be less stable in vitamin–mineral premixes used for livestock feeds owing to the redox reactions catalyzed by trace elements and physical abrasion of protective coatings (Table 20.22). Vitamin premixes that contain choline chloride typically show accelerated losses of vitamin B_6, which reacts with choline. During the storage of finished feeds, the migration of moisture to the shady, relatively cool side of a feed bin can result in the development of pockets of relatively high moisture, which can enhance both the chemical degradation of vitamins as well as support the growth of vitamin-consuming fungi. Feeds that are pelleted or extruded are also exposed to friction, pressure, heat, and humidity, all of which enhance vitamin loss.

The chemical stabilities of some vitamins can be improved by using a more stable chemical form or formulation. For example, the calcium salt of pantothenic acid is more stable than the free acid form, and esters of vitamins A and E (retinyl acetate, tocopheryl acetate) are much more resistant to

86. Loss of vitamin A from poultry feeds stored at moderate temperatures (ca. 15% in 30 days) can be reduced (to ca. 10%) by the addition of an antioxidant. Under conditions of high temperature and high humidity, vitamin A losses from finished feeds can be much greater (80–95%). Maximal protection by antioxidants is expected under conditions in which vitamin oxidation is moderate, e.g., short-term feed storage in hot, humid environments.

87. Owing to the presence of mineral catalysts in oxidative reactions, the stabilities of oxidant-sensitive vitamins in compound premixes can be expected to be less than in premixes of the vitamins alone.

TABLE 20.21 Examples of Vitamin Premixes for Animal Feeds

Vitamin	Units/1000 kg		
	Practical Diet[a] for Chicks[c]	Diet Semipurified Diet[b] for Chicks[c]	Semipurified Diet[b] for Rats[d]
Vitamin A[e] (IU)	8,800,000	50,000	40,000,000
Vitamin D_3 (IU)	2,200,000	4,500,000	1,000,000
Vitamin E (IU)[f]	5,500	50,000	50,000
Menadione $NaHSO_3$ (g)	2.2	1.5	50
Thiamin HCl (g)	15	6	
Riboflavin (g)	4.4	15	6
Niacin (g)	33	50	30
Pyridoxine HCl (g)	6	7	
d-Calcium pantothenate (g)	11	20	16
Biotin (mg)	0.6	0.2	
Folic acid (g)	6	2	
Vitamin B_{12} (mg)	10	20	10
Choline chloride (g)	220	2000	+[g]
Minerals	+[h]	+[i]	+[i]
Other Ingredients			
Antioxidant[j] (g)	125	100	100
Carrier (g)	To weight[k]	To weight[l]	To weight[l]

[a]*Composed of nonpurified natural feedstuffs (e.g., corn, soybean meal).*
[b]*Composed of purified/partially purified ingredients (e.g., isolated soy protein, casein, sucrose, and starch).*
[c]*From Scott, M.L., Nesheim, M.C., Young, R.J., 1982. "Nutrition of the Chicken", third ed. M.L. Scott & Assoc., Ithaca, New York.*
[d]*AIN-76 diet*
[e]*As all-*trans*-retinyl palmitate.*
[f]*As all-*rac*-tocopheryl acetate.*
[g]*Added as 0.2% choline bitartrate.*
[h]*Includes 66 g of ZnO, 220 g of $MnSO_4$, and 220 mg of Na_2SeO_3.*
[i]*Includes $CaHPO_4 \cdot 2H_2O$, $CaCO_3$, KH_2PO_4, $NaHCO_3$, $KHCO_3$, KCl, NaCl, $MnSO_4 \cdot H_2O$, $FeSO_4 \cdot 7H_2O$, $MgCO_3$, $MgSO_4$, KIO_3, $CuO_4 \cdot 5H_2O$, $ZnCO_3$, $CoCl_2$, $NaMoO_4 \cdot 2H_2O$, and/or Na_2SeO_3 in amounts appropriate for the composition of the particular diet.*
[j]*e.g., Ethoxyquin, BHT (butylated hydroxytoluene).*
[k]*Corn meal.*
[l]*Sucrose.*

oxidation than the free alcohol forms. Vitamin preparations can also be coated or encapsulated[88] in ways that exclude oxygen and/or moisture, thus rendering them more stable. They are often spray-dried, spray-congealed, or prepared as adsorbates to improve their handling characteristics. Owing to such approaches, purified vitamins added to foods have been found to be as stable and bioavailable, if not more so, than the forms of the vitamins intrinsic to foods.

10. CASE STUDY

Cindy Stacey stepped into the center elevator the Longworth Building and punched "3." She was a new staffer for US Congressman Carl Rep. Pomerantz (ND), now in his fourth term. The congressman had the distinction of being a popular urban democrat in an overwhelmingly conservative, republican, agricultural state. His background in agricultural insurance had landed him a seat on the House Agriculture Committee where he was now the ranking minority member. His chief of staff hired Cindy during the intersession; she was just back from the Peace Corps where she taught English in Bangladesh.

Cindy liked the congressman but was less sure about his chief of staff Campbell Hurst. Hurst's job was to make sure that Mr Pomerantz had the information he needed when he needed it, which required him to be on top of the Congressman's political agenda. Campbell saw his own future as tied to Mr Pomerantz's political success. He tried

88. Gelatin, edible fats, starches, and sugars are used for this purpose.

TABLE 20.22 Typical Stabilities of Vitamins in a Broiler Feed

Vitamin	% Retained Activity			
	Premix Storage (2 months)	Pelleting/ Conditioning (93°C, 1 min)	Feed Storage (2 weeks)	Cumulative
Vitamin A[a]	98	90	92	81
Vitamin D_3	98	93	93	85
Vitamin E[b]	99	97	98	94
Vitamin K[c]	92	65	85	51
Thiamin[d]	99	89	98	86
Riboflavin	99	89	97	85
Niacin	99	90	93	83
Vitamin B_6[e]	99	87	95	82
Biotin	99	89	95	84
Pantothenic acid[f]	99	89	98	86
Folate	99	89	98	86
Vitamin B_{12}	100	96	98	86

[a]*all*-trans-*Retinyl acetate.*
[b]*all-rac-α-Tocopheryl acetate.*
[c]*Menadione sodium bisulfite complex.*
[d]*Thiamin mononitrate.*
[e]*Pyridoxine hydrochloride.*
[f]*Calcium pantothenate.*
BASF Keeping Current, No. 9138, 1992.

to steer the congressman into the most politically safe decisions whenever he could.

Two days ago, Campbell had given Cindy her first major assignment: review the needs and opportunities in agriculture-related international markets. This was to be used for Mr Pomerantz's work on the new House Agriculture bill. Mr Pomerantz had asked her specifically to investigate opportunities to address "hidden hunger," as he was interested in redirecting funds presently spent on the US surplus commodities. Campbell had added that she should look at activities of the USDA Agricultural Research Service related to market opportunities for the US commodities. She doubted that Campbell would want to hear much about micronutrients, as he saw political liabilities in the use of USDA budgetary support to address nondomestic problems.

Cindy also knew that Campbell had never seen those problems. Nor had she, until going to Bangladesh. In villages outside of the port city of Chittagong, she had been shocked at the blindness and bone deformities, the goiter, and diarrhea. She had been amazed at the stunted kids and the bent-over old women. They all seemed to have become part of the landscape—as though their infirmities were expected features of the lives of the poor. These conditions, she had learned, were caused by eating rice and little else. She did not like the term "hidden hunger" for something that was so obvious; but she was glad it was something Mr Pomerantz wanted to address.

Cindy suspected, though she had never discussed the matter with him, that the congressman's interest related to the fact that his own daughter had cerebral palsy. One could not miss the passion with which he talked about "hidden hunger" as "preventable sources of disability." Others in the office dated his interest in this area to an official trip he had made to Mozambique in his second term. Campbell had not had such experiences; for him, "hidden hunger" was something abstract.

So, for the last 2 days, Cindy had lived in the Library of Congress. She had poured over everything she could find publications from UNICEF and FAO and scores of research papers in plant breeding and nutritional surveillance in Africa and south Asia. She had called one of her old professors and had talked to a friend at USDA. During her investigations, she had come across "Golden Rice," a transgenic rice into which genes for the biosynthesis of beta-carotene had been added from other species. Rice that could be a source of vitamin A. This rice, still in the regulatory process in several countries, was being promoted as the solution to global vitamin A deficiency by its developers at the International Rice Research Institute in the Philippines.

Golden Rice fascinated Cindy. She saw opportunities to Bangladesh, where each day a child goes blind due to insufficient vitamin A. She saw opportunities to help millions of children in places where rice is a dietary staple. But she wondered about its politically sensitive GMO status, when it might be approved for *trans*national distribution, and whether it might carry any risks. She wondered what kind of political resistance there might be. And, if it were effective, she wondered whether that efficacy might be used as an excuse not to fund other international efforts related to food, agriculture, and market development. In short, she could see more questions than answers.

The elevator door opened on the third floor and Cindy stepped out, starting down the long corridor to the Pomerantz offices. Campbell had asked her to present her initial findings to Mr Pomerantz just before lunch, so she would spend the morning for preparing her recommendation.

Case question: How should Cindy present the Golden Rice case? Outline a presentation, highlighting the pros and cons of introducing genetically modified foods into developing nations with the intent of preventing the health and economic effects of "hidden hunger."

11. STUDY QUESTIONS AND EXERCISES

1. For a core food for any particular vitamin, construct a flow diagram showing all of the processes, from the growing of the food to the eating of it by a human, that might reduce the useful amount of that vitamin in the food.
2. In consideration of the core foods for the vitamins and your personal food habits, which vitamin(s) might you expect to have the lowest intakes from your diet? Which might you expect to be low in the typical American diet? Which might you expect to be low in vegetarian and low-meat diets?
3. Use a concept map to show the relationships of vitamin supplementation of animal feeds to the concepts of chemical stability, bioavailability, and physiological utilization.
4. Prepare a flow diagram to show the means by which you might first evaluate the dietary vitamin status of a specific population (e.g., in an institutional setting), and then improve it, if necessary.
5. What principles should be used in planning diets to ensure adequacy with respect to the vitamins (and other nutrients)?

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Chapter 21

Assessing Vitamin Status

Chapter Outline

Anchoring Concepts

1. Detection of suboptimal vitamin status at early stages (before manifestation of overt deficiency disease) is desirable for the reason that vitamin deficiencies are most easily correctable in their early stages.
2. Vitamin status can be estimated by evaluating diets and food habits, but these methods are not precise.
3. Vitamin status can be determined by measuring the concentrations of vitamins and metabolites, and the activities of vitamin-dependent enzymes, in samples of tissues and urine.
4. Suboptimal status is more probable for some vitamins, and in some population demographics, than for others.

...the old idea, that the state of nutrition of a child could be at once established by mere cursory inspection by the doctor, has to be abandoned....[Such methods] gave us very little information about the occurrence of the milder degrees of deficiency, or of the earlier stages of their development.

L.J. Harris[1]

LEARNING OBJECTIVES

1. To understand the requirements of valid methods for assessing vitamin status.
2. To understand the methods available for assessing the vitamin status of humans and animals.
3. To be familiar with available information regarding the vitamin status of human populations.

1. Leslie, J., 1955. Harris was a professor of nutrition at Cambridge University; his "Vitamins in Theory and Practice", fourth ed. Cambridge Univ. Press, 366 pp., is a classic.

VOCABULARY

Anthropometric assessment
Biochemical assessment
Bioindicator
Biomarker
Clinical assessment
Dietary assessment
Dietary records
Food frequency questionnaires (FFQ)
Hidden hunger
Micronutrient malnutrition
Nutrient loading
Nutrition screening
Nutrition surveillance
Nutrition surveys
Nutritional assessment
Nutritional status
24-h recalls sociologic assessment

1. NUTRITIONAL ASSESSMENT

The need to understand and describe the health status of individuals, a basic tenet of medicine, spawned the development of methods to assess nutrition status as appreciation grew for the important relationship between nutrition and health. The first applications of nutritional assessment were in investigations of feed-related health and production problems of livestock and, later, in examinations of human populations in developing countries. Activities of the latter type, consisting mainly of organized nutrition surveys, resulted in the first efforts to standardize both the methods employed to collect such data and the ways in which those

The Vitamins. http://dx.doi.org/10.1016/B978-0-12-802965-7.00021-6

data are interpreted.[2] Ultimately, nutritional assessment became an essential part of the nutritional care of hospitalized patients and an important means of evaluating the impacts of public nutrition intervention programs.

Purposes of nutritional assessment are as follows:
- detection of deficiency states;
- evaluation of nutritional qualities of diets, food habits, and/or food supplies;
- prediction of health effects.

Approaches and Methods of Nutritional Assessment

Three nutritional assessment systems have been employed both in population-based studies and in the care of hospitalized patients.

- **Nutrition surveys** conducted to generate baseline nutritional data, to learn overall nutrition status, and to identify subgroups at nutritional risk. These are typically cross-sectional evaluations of selected population groups.
- **Nutrition surveillance** conducted to identify possible causes of malnutrition. These involve continuous monitoring of the nutritional status of selected population groups (e.g., at-risk groups) for extended periods of time.
- **Nutrition screening** conducted to identify malnourished individuals requiring nutritional interventions. These rely on the use of biomarkers of nutritional status.

Nutritional assessments employ any of five methodologies:

Dietary assessment—estimating nutrient intakes from evaluations of diets, food availability, and food habits. Several methods can be used for dietary assessment:

- **24-h recalls**—The USDA has improved interview-based methods with the development of a five-step computerized dietary recall instrument, the USDA Automated Multiple-Pass Method (AMPM)[3]; this is available as a web-based, self-administered instrument.
- **Dietary records**—Written records (food diaries) have been used; new approaches include smart phone-based methods with food image processing and voice and/or text input to capture eating episodes.[4] Studies have found these methods to yield useful results for vitamin intakes if conducted for several days.[5]
- **Food frequency questionnaires (FFQ)**—This approach depends on respondent memory and uses fixed lists of foods. FFQs yield information about the usual diet of the past at relatively low cost,[6] for which reasons they are the methods of choice for epidemiological studies. FFQs have been found to yield useful estimates of biomarkers of some vitamins.[7] A systematic review found that vitamin intake estimated from FFQs and 24-h recall methods showed correlation coefficients of 0.26–0.38, and that those from FFQs and dietary record methods showed correlation coefficients of 0.41–0.53.[8]

Due to the cumulative predictive uncertainties of quantitative food intakes and of vitamin contents of foods (see Chapter 20), these methods yield imprecise estimates of vitamin intakes. Nevertheless, these methods can identify features of diets and food habits that are likely to provide insufficient amounts of bioavailable vitamins:

- **monotonous diets** with little food variety,[9] particularly those based on milled cereal grains;
- **low caloric intakes**, i.e., low intakes of total food[10];
- **enteric malabsorption** due to deficiencies and/or imbalances, e.g., fat.

Biochemical assessment—estimating nutritional status from measurements of stores, functional forms, excreted forms, and/or metabolic functions of specific nutrients. Vitamin status refers to the functional reserve capacity provided by vitamins in tissue. This approach offers the best opportunity for detecting early-stage (i.e., subclinical) vitamin deficiencies. However, direct measurement of

2. In 1955, the US government organized the Interdepartmental Committee on Nutrition for National Defense (ICNND) to assist developing countries in assessing the nutritional status of their peoples, identifying problems of malnutrition, and developing practical ways of solving their nutrition-related problems. The ICNND teams conducted nutrition surveys in 24 countries. In 1963, the ICNND published the first comprehensive manual (ICNND, 1963. Manual for Nutrition Surveys, second ed. U.S. Government Printing Office, Washington, DC) in which analytical methods were described and interpretive guidelines were presented.

3. Thompson, F.E., Subar, A.F., Loria, C.M., et al., 2010. J. Am. Diet. Assoc. 110, 48–51.

4. Wharton, C.M., Johnston, C.S., Cunningham, B.K., et al., 2014. J. Nutr. Educ. Behav. 46, 440–444.

5. Presse, N., Payette, H., Shatenstein, B., et al., 2011. J. Nutr. 141, 341–346.

6. Block, G., Thompson, F.E., Hartman, A.M., et al., 1990. J. Am. Diet. Assoc. 92, 686–693.

7. Tangney, C.C., Bienias, J.L., Evans, D.A., et al., 2004. J. Nutr. 134, 927–934.

8. Serra-Majem, L., Anderson, F.L., Henríque-Sánchez, P., et al., 2009. Br. J. Nutr. 102, S3–S9.

9. This may include a strict vegetarian diet that does not include some source of vitamin B_{12}.

10. Reduced food intake is thought to be a major cause of subadequate nutrient intakes of the elderly. Studies have shown that Americans ≥71 years frequently have intakes less than the estimated average requirement: vitamin A, 50%; vitamin E, 75%; vitamin K, 49% of women and 34% of men; vitamin C, 40%; and folate, 40% of women and 16% of men (Marriott, B.P., Olsho, L., Hadden, L., et al., 2010. Crit. Rev. Food Sci. Nutr. 50, 228–258).

TABLE 21.1 Relevance of Nutritional Assessment Methodologies to the Stages of Vitamin Deficiencies

	Most Informative Methods				
Stage of Deficiency[a]	Dietary	Biochemical	Clinical	Anthropometric	Sociologic
1. Depletion of stores	+	+			
2. Cellular metabolic changes		+	+	+	
3. Clinical defects		+	+	+	
4. Morphological changes			+	+	
5. Behavioral signs					+

[a]*See Chapter 4.*

vitamin function is limited by the availability of functional biomarkers,[11] the existence of more than one metabolic function with different sensitivities to vitamin supply,[12] and/or the function of the vitamin in a loosely bound fashion unstable to methods of tissue preparation.[13]

Clinical assessment—estimating nutritional status from medical histories and physical examinations by a qualified observer to detect signs and symptoms associated with malnutrition. Diagnoses of vitamin deficiencies are generally most possible in the latter stages when physiologic dysfunction and/or morphological changes can be detected. Clinical evaluation can identify pathophysiological factors that may limit vitamin utilization:

- **enteric malabsorption** due to acquired or innate problems affecting the absorptive surface of the gut, e.g., enteritis, helminth infection, gastrointestinal surgery;
- **impaired vitamin retention/utilization** due to acquired or innate problems of hepatic or renal vitamin metabolism, e.g., hepatitis, nephritis.

Anthropometric assessment—estimating nutritional status from measurements of the physical dimensions and gross composition of an individual's body.

Sociologic assessment—collecting information on other variables known to affect or be related to nutritional status, e.g., socioeconomic status, food habits and beliefs, food prices and availability, food storage and cooking practices, drinking water quality, alcohol use, immunization records, incidence of low-birth weight infants, breast-feeding and weaning practices, age- and cause-specific mortality rates, birth order, family structure, etc.

2. BIOMARKERS OF VITAMIN STATUS

Risk of suboptimal vitamin status is determined largely by factors that limit access to a diet providing adequate amounts of vitamins and other essential nutrients, as well as factors that limit the body's ability to utilize them after ingestion. These factors can be best ascertained by assessing dietary practices, clinical status, and biochemical indicators (**biomarkers**) of vitamin status (Table 21.1).

Biomarker Definition

The term "biomarker" is used in the field of nutrition to describe a trait that can be measured objectively and used as an indicator of the status of normal or pathogenic biological processes, or responses to therapeutic interventions.[14] A useful biomarker of vitamin status must

- relate to the rate of vitamin intake, particularly within the nutritionally significant range, and respond to deprivation of the vitamin;
- relate to normal physiologic function and a meaningful period of time;
- be measurable in an accessible specimen, technically feasible, reproducible, and affordable; and
- have an available base of normative data.

11. For example, while vitamin E functions as a biological lipid antioxidant, measuring that function is not possible with any physiological relevance because all of the known products of lipid peroxidation (e.g., malonaldehyde, alkanes) are metabolized, making results difficult to interpret with respect simply to vitamin E status.

12. For example, pyridoxal phosphate (PalP) is an essential cofactor for each of two enzymes involved in the metabolic conversion of tryptophan to niacin: kynureninase and a transaminase. However, because kynureninase has a much greater affinity for PalP ($K_m = 10^{-3}$ M) than does the transaminase ($K_m = 10^{-8}$ M), under conditions of pyridoxine deprivation the transaminase activity can be reduced while kynureninase activity remains unaffected.

13. For example, the metabolically active forms of niacin, NAD(P)H, function as the cosubstrates of many redox enzymes. These enzyme–cosubstrate complexes are only transiently associated; therefore, dilution of biological specimens results in their dissociation and usually in the oxidation of the cosubstrate.

14. This distinguishes a biomarker from a "bioindicator," which is typically used to describe a measure of processes used to assess the quality of an individual's or community's the environment and its changes over time (Raiten, D.J., Combs Jr., G.F., 2015. Sight Life 29, 39–44).

TABLE 21.2 Tissues Accessible for Assessing Biomarkers of Vitamin Status

Tissue or Cell Type	Relevance
Blood	
Plasma/serum	Contains newly absorbed vitamins being transported to other tissues; therefore, tends to reflect recent vitamin intake; this effect can be reduced by collecting fasting blood
Erythrocytes	With a half-life of about 120 days, they tend to reflect chronic nutrient status; analyses can be technically difficult
Leukocytes	Have relatively short half-lives and, therefore, can be used to monitor short-term changes in nutrient status
Tissues	
Liver, adipose, muscle, marrow	Sampling is invasive, requiring research or clinical settings
Hair, nails	Easily collected and stored specimens offer advantages for studies of trace element status; not useful for assessing vitamin status
Skin, macula	Can be scanned noninvasively by resonance Raman spectroscopy for assessing carotenoids

Biomarkers of Vitamin Status

In some cases, it is possible to assess the current and stored metabolic function of a vitamin.[15] In many cases, however, direct measurement of vitamin function may not be possible, making other biomarkers useful: measurements of the vitamin, particular metabolites, and other enzymes in accessible tissues or urine (Tables 21.2 and 21.3). In this regard, biomarker data of informative value for individuals will call for combining it with genetic information regarding inborn metabolic errors. In addition, the emerging field of **metabolomics**, i.e., the measurement of multiple small molecular weight metabolites in biological specimens, is likely to offer useful new tools that will facilitate gaining insight into metabolic pathways affected by specific vitamins.[16] This approach has been used to identify metabolic profiles characteristic of general dietary patterns.[17]

15. e.g., Measuring prothrombin time to assess vitamin K status, and stimulation coefficients of erythrocyte transketolase and glutathione reductase to assess thiamin and riboflavin status, respectively.
16. Guertin, K.A., Moore, S.C., Sampson, J.N., et al., 2014. Am. J. Clin. Nutr. 100, 208–217.
17. e.g., High serum levels of acylcarnitines and acylalkylphosphatidylcholines were related to consumption of high amounts of butter; high serum hexose and phosphatidylcholines were related to consumption of red meat and fish with low amounts of whole grains and tea; high serum methionine and branched-chain amino acids were related to consumption of potatoes, dairy products, and cornflakes (Floegel, A., von Ruesten, A., Drogan, D., et al., 2013. Eur. J. Clin. Nutr. 67, 1100–1108).

Interpreting Biomarker Data

The relevance of biomarkers of vitamin status of individuals is not straightforward, owing to issues of intraindividual variation and confounding effects, which may be quantitatively more significant for individuals than for populations. For example, intraindividual variation is frequently noted in serum analytes. Therefore, a measurement of a single blood sample may not be appropriate for estimating the usual circulating level of the analyte of an individual, even though it may be useful in estimating the mean level of a population. Several factors can confound the interpretation of parameters of vitamin status: those affecting the response parameters directly, drugs that can increase vitamin needs, seasonal effects related to the physical environment[18] or food availability,[19] use of parenteral feeding solutions,[20] and use of vitamin supplements,[21] smoking,[22] etc. (Table 21.4). The guidelines originally developed by the ICNND remain useful for the interpretation of the results of biomarkers of vitamin status (Table 21.5). It is important to note, however, that those interpretive guidelines were developed for use in surveys of populations.

3. VITAMIN STATUS OF HUMAN POPULATIONS

Reserve Capacities of Vitamins

The reserve capacities of the vitamins vary. Each is affected by the history of vitamin intake, the metabolic needs for the vitamin, and the general health status of the individual. Typical reserve capacities of a healthy, adequately nourished human adult to meet normal metabolic needs are as follows:

- **4–10 days**—thiamin, biotin, and pantothenic acid
- **2–6 weeks**—vitamins D, E, K, and C; riboflavin, niacin, and vitamin B_6

18. For example, individuals living in northern latitudes typically show peak plasma levels of 25-OH-D_3 around September and low levels around February, with inverse patterns of plasma parathyroid hormone concentrations, owing to the seasonal variation in exposure to UV light.
19. For example, residents of Finland showed peak plasma ascorbic acid levels in August–September and lowest levels in November–January, owing to seasonal differences in the availability of vitamin C-rich fruits and vegetables.
20. Individuals supported by total parenteral nutrition (TPN) have frequently been found to be of low status with respect to biotin (due to their abnormal intestinal microflora) and the fat-soluble vitamins (due to absorption by the plastic bags and tubing and to destruction by UV light used to sterilize TPN solutions).
21. The NHANES I survey showed that more than 51% of Americans over 18 years of age used vitamin/mineral supplements, with 23.1% doing so on a daily basis. Multivitamins are the most commonly used supplements, followed by vitamin C, calcium, and vitamins E and A. The use of vitamin supplements has been found to have greater impact than that of vitamin-fortified food on both the mean and coefficient of variation (CV) of estimates of vitamin intake in free-living populations.
22. Smokers have been found to have abnormally low-plasma levels of ascorbic acid (with a corresponding increase in dehydroascorbic acid), pyridoxal, and pyridoxal phosphate.

TABLE 21.3 Biomarkers of Vitamin Status

Vitamin	Functional Parameters	Tissue Levels	Urinary Excretion
Vitamin A		Serum retinol[a]	
		Change in serum retinol after oral load[b]	
		Liver retinyl esters	
Vitamin D		Serum 25-$(OH)_2$-vitamin D_3[a]	
		Serum vitamin D_3	
		Serum 1,25-$(OH)_2$-D_3	
		Serum alkaline phosphatase	
Vitamin E	Erythrocyte hemolysis	Serum tocopherols[a]	
		Serum malondialdehyde	
		Serum 1,4-isoprostanes	
		Breath alkanes	
Vitamin K	Clotting time		
	Prothrombin time[a]		
Vitamin C		Serum ascorbic acid	Ascorbic acid
		Leukocyte ascorbic acid[a]	Ascorbic acid after load[c]
Thiamin	Erythrocyte transketolase	Blood thiamin	Thiamin (thiochrome)
	Stimulation[a]	Blood pyruvate	Thiamin after load[c]
Nacin		RBC NAD[a]	1-Methylnicotinamide
		RBC NAD:NADP ratio	1-Methyl-6-pyridone-3-carboxamide
		Plasma tryptophan	
Riboflavin	RBC glutathione reductase	Blood riboflavin	Riboflavin
	Stimulation[a]		Riboflavin after load[c]
Vitamin B_6	RBC transaminase	Plasma pyridoxal phosphate	Xanthurenic acid after tryptophan load[a,c]
		RBC transaminase stimulation	Quinolinic acid
		RBC pyridoxal phosphate	4-Pyridoxic acid
		Plasma pyridoxal	
Biotin		Blood biotin[a]	Biotin
Pantothenic acid		RBC sulfanilamide acetylase	Pantothenic acid
		RBC pantothenic acid	
		Blood pantothenic acid[a]	
Folate		Serum folates[a]	FIGLU[c] after histidine load[a,c]
		RBC folates[a]	
		Leukocyte folates	Urocanic acid after histidine load[c]
		Liver folates	
Vitamin B_{12}		Serum vitamin B_{12}[a]	FIGLU[d]
		RBC vitamin B_{12}	Methylmalonic acid[a]

[a]*Most useful parameter.*
[b]*Relative dose–response test.*
[c]*Single large oral dose.*
[d]*FIGLU, formiminoglutamic acid.*

- **3–4 months**—folate
- **1–2 years**—vitamin A
- **3–5 years**—vitamin B_{12}.

Differences in reserve capacities reflect differential abilities to retain and store vitamins. Such differences lead, therefore, to differential sensitivities to vitamin deprivation. For example, individuals with histories of generally adequate vitamin nutriture can be expected to sustain longer periods of deprivation of vitamins A or B_{12} than they could of thiamin, biotin, or pantothenic acid. Similarly, metabolic and physiologic lesions caused by deficiencies of thiamin, biotin, or pantothenic acid can be expected to appear much sooner than those of vitamins A or B_{12}, which may remain occult. Because nutritional intervention is typically most efficacious and cost-effective in earlier stages of vitamin

TABLE 21.4 Limitations of Some Biomarkers of Vitamin Status

Vitamin	Biomarker	Limitations
Vitamin A	Plasma[a] retinol	Reflects body vitamin A stores only at severely depleted or excessive levels; confounding effects of protein and zinc deficiencies and renal dysfunction
Vitamin D	Plasma[a] alkaline phosphatase	Affected by other disease states
Vitamin E	Plasma[a] tocopherol	Affected by blood lipid transport capacity
Thiamin	Plasma[a] thiamin	Low sensitivity to changes in thiamin intake
Riboflavin	Plasma[a] riboflavin	Low sensitivity to changes in riboflavin intake
Vitamin B_6	RBC glutamic–pyruvic	Genetic polymorphism transaminase
Folate	RBC folates	Also reduced in vitamin B_{12} deficiency
	Urinary FIGLU[b]	Also increased in vitamin B_{12} deficiency
Vitamin B_{12}	Urinary FIGLU[b]	Also increased in folate deficiency

[a]*Or serum.*
[b]*FIGLU, formiminoglutamic acid.*

TABLE 21.5 Interpreting Biomarkers of Vitamin Status

Vitamin	Parameter	Age Group	Values, by Category of Status[a]		
			Deficient (High Risk)	Low (Moderate Risk)	Acceptable (Low Risk)
Vitamin A	Plasma[b] retinol (μg/dL)	<5 months	<10	10–19	>20
		0.5–17 years	<20	20–29	>30
		Adult	<10	10–19	>20
Vitamin D	Plasma[b] 25-(OH)-D_3[c] (ng/mL)	All ages	<20[v]	20–29[v]	≥30[v]
	Plasma[b] alkaline phosphatase[c] (U/mL)	Infants	>390	298–390	99–298
		Adults	<40	40–56	57–99
Vitamin E	Plasma[b] α-tocopherol (mg/dL)	All ages	<0.35	0.35–0.80	>0.80
Vitamin K	Clotting time (min)	All ages	>10	~10	
	Prothrombin time (min)	—[d]	—[d]	—[d]	
Vitamin C	Plasma[b] ascorbic acid (mg/dL)	All ages	<0.20	0.20–0.30	>0.30
	Leukocyte ascorbic acid (mg/dL)	All ages	<8	8–15	>15
	Whole blood ascorbic acid (mg/dL)	All ages	<0.30	0.30–0.50	>0.50

TABLE 21.5 Interpreting Biomarkers of Vitamin Status—cont'd

Vitamin	Parameter	Age Group	Values, by Category of Status[a]		
			Deficient (High Risk)	Low (Moderate Risk)	Acceptable (Low Risk)
Thiamin	Urinary thiamin (μg/g creatinine)	1–3 years	<120	120–175	>175
		4–6 years	<85	85–120	>120
		7–9 years	<70	70–180	>180
		10–12 years	<60	60–180	>180
		13–15 years	<50	50–150	>150
		Adults	<27	27–65	>65
		Pregnant 2nd trim.	<23	23–55	>55
		Pregnant 3rd trim.	<21	21–50	>50
	Urinary thiamin				
	μg/24 h	Adults	<40	40–100	>100
	μg/6 h	Adults	<10	10–25	>25
	Urinary thiamin after load[e] (μg/4 h)	Adults	<20	20–80	>80
	RBC transketolase stimulated by TPP[f,g] (%)	Adults	>25	15–25	<15
Riboflavin	Urinary riboflavin (μg/g creatinine)	1–3 years	<150	150–500	>500
		4–6 years	<100	100–300	>300
		7–9 years	<85	85–270	>270
		10–15 years	<70	70–200	>200
		Adults	<27	27–80	>80
		Pregnant 2nd trim.	<39	39–120	>120
		Pregnant 3rd trim.	<30	30–90	>90
	Urinary riboflavin (μg/24 h)	Adults	<40	40–120	>120
	Urinary riboflavin (μg/6 h)	Adults	<10	10–30	>30
	Urinary riboflavin load[h] (μg/4 h)	Adults	<1000	1000–1400	>1400
	RBC riboflavin (μg/day)	Adults	<10.0	10.0–14.9	>14.9
	RBC glutathione reductase FAD[i] stimulation (%)	Adults	>40	20–40	<20
Niacin	Urinary *N*′-methylnicotinamide (μg/g creatinine)	Adults	<0.5	0.5–1.6	>1.6
		Pregnant 2nd trim.	<0.6	0.6–2.0	>2.0
		Pregnant 3rd trim.	<0.8	0.8–2.5	>2.5
	Urinary *N*′-methylnicotinamide (μg/6 h)	Adults	<0.2	0.2–0.6	>0.6
	Urinary 2-pyridone[j]:*N*′-methylnicotinamide	All ages	—[k]	<1.0	≥1.0
Vitamin B_6	Plasma PalP[l] (nM)	All ages	—[k]	<60[m]	≥60[m]
	Urinary vitamin B_6 (μg/g creatinine)	1–3 years	—[k]	<90[m]	≥90[m]
		4–6 years	—[k]	<75[m]	≥75[m]
		7–9 years	—[k]	<50[m]	≥50[m]
		10–12 years	—[k]	<40[m]	≥40[m]
		13–15 years	—[k]	<30[m]	≥30[m]
		Adults	—[k]	<20[m]	≥20[m]

Continued

TABLE 21.5 Interpreting Biomarkers of Vitamin Status—cont'd

Vitamin	Parameter	Age Group	Values, by Category of Status[a]		
			Deficient (High Risk)	Low (Moderate Risk)	Acceptable (Low Risk)
	Urinary 4-pyridoxic acid (mg/24 h)	Adults	<0.5[m]	0.5–0.8[m]	>0.8[m]
	Urinary xanthurenic acid after tryptophan load[h] (mg/24 h)	Adults	>50[m]	25–50[m]	<25[m]
	Urinary 3-OH-kynurenine after tryptophan load[h] (mg/24 h)	Adults	>50[m]	25–50[m]	<25[m]
	Urinary kynurenine after tryptophan load[h] (mg/24 h)	Adults	>50[m]	10–50[m]	<10[m]
	Quinolinic acid after tryptophan load[h] (mg/24 h) adults	Adults	>50[m]	25–50[m]	<25[m]
	Erythrocyte alanine aminotransferase stimulation by PalP[l] (%)	Adults	—[k]	>25[m]	≤25[m]
	Erythrocyte aspartate aminotransferase stimulation by PalP[l] (%)	Adults	—[k]	>50[m]	≤50[m]
Biotin	Urinary biotin (μg/24 h)	Adults	<10[m]	10–25[m]	>25[m]
	Whole blood biotin (ng/mL)	Adults	<0.4[m]	0.4–0.8[m]	>0.8[m]
Pantothenic acid	Plasma[b] pantothenic acid (μg/dL)	Adults	—[k]	<6[m]	≥6[m]
	Blood pantothenic acid (μg/dL)	Adults	—[k]	<80[m]	≥80[m,n]
	Urinary pantothenic acid (mg/24 h)	Adults	—[k]	<1[m]	≥1[m,o]
Folate	Plasma[b] folates (ng/mL)	All ages	<3	3–6	>6
	RBC folates (ng/mL)	All ages	140	140–160	>160
	Leukocyte folates (ng/mL)	All ages	—[k]	<60	>60
	Urinary FIGLU[p] after histidine load[q] (mg/8 h)	Adults	>50[m]	5–50	<5[r]
Vitamin B_{12}	Plasma[b] vitamin B_{12} (pg/mL)	All ages	100	100–150	>150[s]
	Urinary methylmalonic acid after valine load[t] (mg/24 h)	Adults	≥300	2–300	≤2
	Urinary excretion of labeled B_{12} after a flushing dose[u] (%)	Adults	<3	3–8	>8

[a] *ICNND., 1963. Manual for Nutrition Surveys, second ed., U.S. Government Printing Office, Washington, DC; Sauberlich et al., 1974. Laboratory Tests for the Assessment of Nutritional Status. CRC Press, Cleveland, Ohio; Gibson, R.S., 1990. Principles of Nutritional Assessment. Oxford University Press, New York.*
[b] *Or serum.*
[c] *Subject to effects of season and sex.*
[d] *Results vary according to assay conditions; most assays are designed such that normal prothrombin times are 12–13 sec, with greater values indicating suboptimal vitamin K status.*
[e] *Single oral 2-mg dose.*
[f] *TPP, thiamin pyrophosphate.*
[g] *The* TPP effect.
[h] *Single oral 2-g dose.*
[i] *FAD, flavin adenine dinucleotide, reduced form, 1–3 μM.*
[j] *N′-methyl-2 pyridone-5-carboxamide.*
[k] *Database is insufficient to support a guideline.*
[l] *PalP, pyridoxal phosphate.*
[m] *These values have only a small database and, therefore, are considered as tentative.*
[n] *Normal values are about 100 μg/dL.*
[o] *Normal values are 2–4 mg/24 h.*
[p] *FIGLU, formiminoglutamic acid.*
[q] *Single oral 2- to 20-mg dose.*
[r] *Normal adults excrete 5–20 mg/8 h.*
[s] *Most healthy individuals show 200–900 pg/mL.*
[t] *Single oral 5- to 10-g dose.*
[u] *This is the Schilling test; it involves measurement of labeled vitamin B_{12} excreted from a 0.5- to 2-μg tracer dose after a large flushing dose (e.g., 1 mg) given 1 h after the tracer.*
[v] *Modified according to discussion in Chapter 5.*

deficiencies, the early detection of occult deficiencies is important for designing effective therapy and prophylaxis programs.

National Nutrition Surveillance

The United States has had a series of programs to track the nutritional adequacy of the food supply and/or the nutritional status of people. These efforts are now consolidated into the ongoing survey ***What We Eat in America***,[23] the dietary intake component of the National Health and Nutrition Examination Survey.[24] Other countries have conducted similar studies,[25] as well as regular food reporting.

Vitamins Status of Americans

The comprehensive review by the 2015 Dietary Guidelines Scientific Advisory Committee found that Americans widely underconsume vitamins D, E, and thiamin (Fig. 21.1), as well as calcium, magnesium, potassium, and fiber, and that adolescents and women also underconsume vitamin A, vitamin C, folate, and iron (Table 21.6).[26] This reflects the underconsumption of nutrient-rich foods; three-quarters of food energy comes from only half (16) of the subcategories of available foods. The vast majority of Americans does not consume the recommended amounts of fruit, vegetables, whole grains, and dairy foods, with lowest consumptions occurring in lower socioeconomic groups and the elderly.

It is estimated that at least one-fifth of heart disease and one-third of all cancers could be prevented by improving the American diet, specifically by increasing the consumption of fruits and vegetables and reducing intakes of saturated and total fat. Dietary patterns with the highest Healthy Eating Index (HEI) scores are generally those that emphasize seafood and plant proteins and dairy foods and have relatively few highly refined grains and "empty" calories. Vegetarian, but not fruitarian, diets can be nutritionally adequate if sensibly selected and appropriately supplemented (e.g., vitamin B_{12}).[27] Problems can arise in any type of diet if the variety of food is restricted and, particularly, if the consumption of dairy products is low. Therefore, emphases on increasing fruits and vegetables at the expense of meats (important sources of vitamins A, B_6, and B_{12}; thiamin; and niacin), and replacing vegetable oils (important sources of vitamin E) with reduced- and no-fat substitutes should be balanced to avoid reduced intakes of B vitamins (Table 21.7).

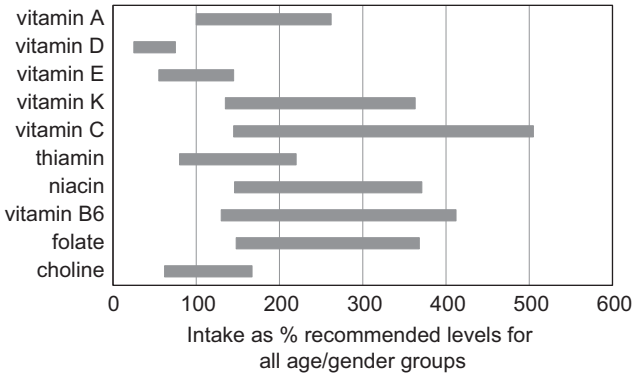

FIGURE 21.1 Estimated vitamins provided by American diets. *After Dietary Guidelines Advisory Committee, 2015. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. US Government Printing Office, 571 pp.*

Nutritional Surveillance Reveals Vitamin Deficiencies

Data on food-nutrient supplies and apparent nutrient consumption are necessary for national food and health policy planning; but they yield no information useful in addressing questions of nutritional status of individuals within populations. Nutrition surveys were initiated to produce such data. The NHANES surveys have revealed the following[28]:

- **Vitamin A status**—Fewer than 1% of Americans had deficient levels of serum retinol (<20 μg/dL), but ~2% had levels indicative of risk to excess vitamin A (>100 μg/dL). Serum retinol concentrations increased with age. Serum β-carotene levels decreased throughout childhood and then increased, with females showing greater levels than males and non-Hispanic whites showing greater levels than other groups.
- **Vitamin E status**—About 2% of Americans had deficient levels of serum α-tocopherol (<500 μg/dL), although that portion was greater (2–4%) for adolescents. Serum α-tocopherol levels decreased throughout childhood and then increased with age. Serum γ-tocopherol levels were relatively stable throughout the life cycle.
- **Vitamin D status**—Results from 2003 to 2006 showed that 17% Americans had low-serum 25-OH-D_3 levels (<40 nM), with 8% being deficient (serum 25-OH-D_3 levels <30 nM) and <1% had excessive levels (>125 nM). Low-vitamin D status was more prevalent among females (10%) than males (6%) and among non-Hispanic blacks (31%) compared to other groups (non-Hispanic whites, 4%; Mexican Americans, 11%).
- **Vitamin C status**—Serum vitamin C concentrations showed a U-shaped distribution according to age, lowest

23. http://www.ars.usda.gov/services/docs.htm?docid=13793.
24. http://www.cdc.gov/nchs/nhanes/.
25. e.g., New Zealand National Nutrition Survey; Luxembourg Nutritional Surveillance System; United Kingdom Expenditure and Food Survey, National Food Survey and School Nutrition Dietary Assessment.
26. Dietary Guidelines Advisory Committee, 2015. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. US Government Printing Office, 571 pp.
27. Craig, W.J., 2009. Am. J. Clin. Nutr. 89, S1627–S1633.
28. National Center for Health Statistics, 2012. Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population, 450 pp. http://www.cdc.gov/nutritionreport/report.html.

TABLE 21.6 Usual Intakes of American Women, 19–50 years, of Vitamins From Foods and Beverages Compared to Recommendations

Vitamin	Mean Intake[a]	EAR[b]	% Below EAR	UL[c]	% Above UL
Vitamin A, μg RE/d	549	500	48	3000	<3
Vitamin D, μg/d	3.9	10	>97	100	<3
Vitamin E, mg/d	6.9	12	95	–	–
Vitamin C, mg/d	76.6	60	45	2000	<3
Folate, μg/day	470	320	15	1000	<3

[a]n = *2957*.
[b]*Estimated average requirement.*
[c]*Tolerable upper limit.*
Adapted from Dietary Guidelines Advisory Committee, 2015. Scientific Report of the 2015 Dietary Guidelines Advisory Committee. US Government Printing Office, 571 pp.

TABLE 21.7 Three Healthy Dietary Patterns

Food Group	Healthy US Style	Healthy Vegetarian	Healthy Mediterranean Style
Fruit	2 cups/day	2 cups/day	2.5 cups/day
Vegetables	2.5 cups/day	2.5 cups/day	2.5 cups/day
Legumes	1.5 cups/week	3 cups/week	1.5 cups/week
Whole grains	3 oz. equiv./day	3 oz. equiv./day	3 oz. equiv./day
Dairy	3 cups/day	3 cups/day	2 cups/day
Protein foods	5.5 oz. equiv./day	3.5 oz. equiv./day	6.5 oz. equiv./day
Meat	12.5 oz. equiv./day		12.5 oz. equiv./day
Poultry	10.5 oz. equiv./day		10.5 oz. equiv./day
Seafood	8 oz. equiv./day		15 oz. equiv./day
Eggs	3 oz. equiv./day	3 oz. equiv./day	3 oz. equiv./day
Nuts/seeds	4 oz. equiv./day	7 oz. equiv./day	4 oz. equiv./day
Processed soy	0.5 oz. equiv./day	8 oz. equiv./day	0.5 oz. equiv./day
Oils	27 g/day	27 g/day	27 g/day

Adapted from USDA Food Modeling Report, 2015.

levels occurring in persons 20–59 years. The prevalence of deficiency (serum vitamin C <11.4 μM) varied from 3% (Mexican Americans) to 7% (non-Hispanic whites) in 2003–06, with males, smokers, and individuals of low-socioeconomic status at elevated risk to being deficient.

- **Vitamin B_6 status**—The prevalence of low-vitamin B_6 status (plasma pyridoxal 5′-phosphate <20 nM) was 11% across age groups in 2005–06, with older individuals and women of childbearing age or using oral contraceptives and smokers being greatest risk of being deficient.
- **Folate status**—Since the introduction of folate fortification in 1998, serum folate concentrations of Americans have doubled and erythrocyte folate concentrations have increased by 50%. During the period of 1999–2006, fewer than 1% of Americans were of low-folate status (i.e., serum folate <2 ng/mL and erythrocyte folate <95 ng/mL), compared to 30 in 1988–94.[29] Serum and erythrocyte folate levels showed U-shaped distributions according to age, lowest levels occurring in adolescents and young adults.
- **Vitamin B_{12} status**—Results in 2003–06 showed <1–3% of children and 3–6% of adults to be of deficient

29. National Center for Health Statistics, 2012. Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population, 450 pp.

vitamin B_{12} status (serum B_{12} levels <200 pg/mL), with 20% showing marginal status.[30] The prevalence of low status was greater in older Americans (4%) and those with vegetarian (particularly vegan) dietary practices.[31] Vitamin B_{12} deficiency was indicated among subjects with low-serum vitamin B_{12} levels, by the fact that 17–19% of subjects also had elevated concentrations of methylmalonic acid and/or homocysteine, both of which variables increased with age.

- **Non-provitamin A carotenoids status**—Serum levels of α-carotene, lutein, zeaxanthin and cryptoxanthin decreased throughout childhood and then increased with age. Serum lycopene levels were greatest among young adults but decreased with age. Women showed greater α-carotene levels than men, but men showed greater lycopene levels than women.

4. GLOBAL UNDERNUTRITION

Under the auspices of national programs, bilateral programs, and international agencies, many nutrition surveys have been conducted in developing countries where malnutrition continues to be a problem. These have shown that, globally, more than 795 million people—one in nine of the world's people—do not to have access to enough food to meet their basic daily needs.[32] Malnutrition is an underlying cause of nearly half of all deaths and accounts for 11% of the global burden of disease.[33] The root causes of malnutrition, and underlying the food insecurity, are poverty and conflict.

Significant reductions have been made in global poverty. In fact, the first Millennium Development Goal (MDG) target, to cut the 1990 poverty rate in half by 2015, was accomplished in 2010, with the greatest reductions occurring in east Asia.[34] By 2015, the World Bank estimated the global prevalence of extreme poverty to be under 10% for the first time in recorded history. Still, on 2012, 896 M people (13% of the world's population) lived on no more than $1.90/day and 2.1 B lived on less than $3.10/day. These changes have been accompanied by a 42% reduction in the prevalence of undernourished people; the MDG targets for developing countries, of cutting in half the prevalence of undernourished people by 2015 (i.e., from 23% in 1990–92 to 12% by 2015) has almost been met. The least progress has been made in sub-Saharan Africa where 23% are undernourished; despite progress south Asia has 276 M undernourished people.

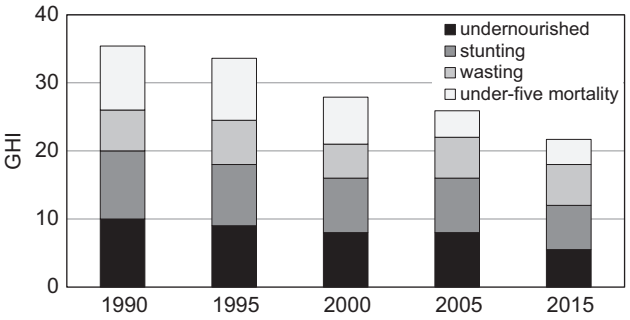

FIGURE 21.2 Trends in Global Hunger. The Global Hunger Index (GHI) was developed by the International Food Policy Institute (http://ghi.ifpri.org/results/).

Undernutrition has its most visible effects on children (Figs. 21.2 and 21.3). With diets inadequate in quantity and quality, 15–25% of the children in developing countries are stunted or wasted.[35] Undernourished children can experience as much as 160 days of illness in a year. At least half of the 11 million child deaths that occur each year are because of malnutrition and its potentiating effects on infectious disease[36]—the childhood mortality rate in developing countries is 10 times that of developed countries.

Hidden hunger. It is clear that the view of malnutrition resulting mainly from insufficient supplies of macronutrients (i.e., energy and protein) has led to gross underestimates of problems caused by deficiencies of critical micronutrients (i.e., vitamins and trace elements). The recognition of those problems due to micronutrient deficiencies generated the need for a new descriptor, "**hidden hunger**."[37] Two billion people live at risk of diseases resulting from deficiencies of vitamin A, iodine, and iron. Most are women and children living in the less-developed

30. Allen, L.H., 2009. J. Nutr. 89, S693–S696.
31. Elmadfa, I., Singer, I., 2009. Am. J. Clin. Nutr. 89, S1693–S1698.
32. Widespread malnutrition exists despite impressive gains in global agricultural production. In the last five decades, cereal yields have more than doubled and per capita supplies of food energy are at all-time high levels—exceeding present global needs. However, the high-yielding, "***green revolution***" varieties of major staple grains, being more profitable than traditional crops (including pulses), have displaced the latter and led to substantial reductions in the diversity of cropping systems. This has contributed to micronutrient malnutrition while increasing caloric output.
33. Malnutrition is considered the number 1 risk to health globally. Adults malnourished as children face 20% deficits in their earning potentials, reducing the gross domestic products of countries with prevalent malnutrition by 2–3%. It is estimated that each dollar spent on alleviating malnutrition yields $138 in benefits.
34. The World Bank estimated that 78% of the world's extremely poor live in south Asia (Pakistan, India, Bangladesh).
35. That is, more than two standard deviations below the median for height-for-age and weight-for-age, respectively. In 2010, the WHO estimated that 171 M children (98% in developing countries) were stunted. This trend shows improvement, particularly in Asia where the percentage has been cut in half since 1990. Still, stunting remains a major public health problem in many developing countries.
36. i.e., Diarrheal diseases (61% of deaths), malaria (57%), pneumonia (52%), and measles (45%) (Bryce, J., Boschi-Pinto, C., Shibuya, K., et al., 2005. Lancet 365, 1147–1152).
37. Kul Gautam, the former deputy executive director of UNICEF, described it well. He said, "*The 'hidden hunger' due to micronutrient deficiency does not produce hunger as we know it. You might not feel it in the belly, but it strikes at the core of your health and vitality.*"

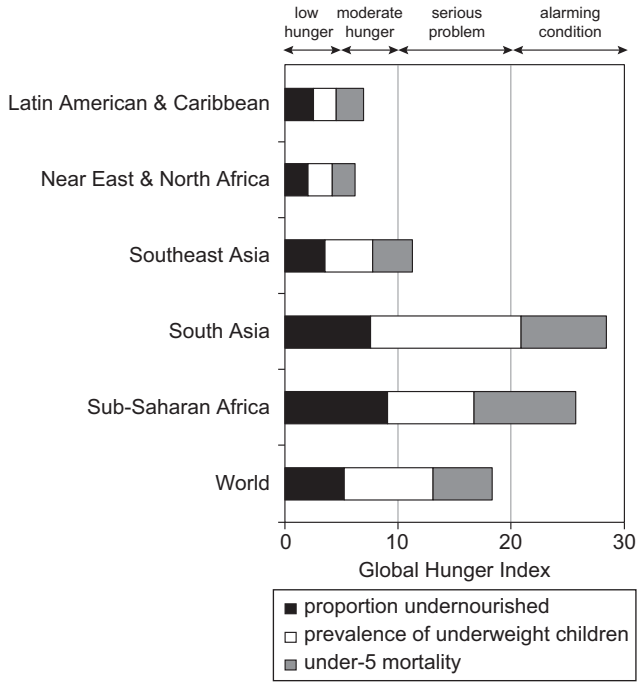

FIGURE 21.3 Regional rankings according to the 2010 Global Hunger Index. *After von Grebmer, K., Ruel, M.T., Menon, P., et al., 2010. 2010 Global Hunger Index, The Challenge of Hunger: Focus on the Crisis of Child Undernutrition. IFPRI, Washington.*

countries of sub-Saharan Africa, the eastern Mediterranean, southern and southeast Asia, Latin America, the Caribbean and the western Pacific.[38] The micronutrient deficiencies contributing most to hidden hunger are as follows:

- **Vitamin A deficiency**, affecting 190 M preschool age children and 19 M pregnant women, remains the single most important cause of childhood blindness in developing countries. **Frank vitamin A deficiency causes visual impairment and blindness**. Subclinical vitamin A deficiency **increases risk to infections including diarrhea and measles in children and** is associated with increased child mortality. Providing vitamin A can reduce child mortality by about 25% and birth-related, maternal mortality by 40%. One-third of vitamin A-deficient pregnant women are affected by night blindness.
- **Iodine deficiency**, affecting ~1.8 B, causes brain damage in newborns and reduced mental capacity and goiter in adults
- **Iron deficiency**, affecting ~1.6 B, causes anemia and reduced work capacity, impairing motor and cognitive development, and increasing maternal perinatal mortality and premature births.[39] Iron deficiency is thought to be responsible for at least half of the anemia affecting more than 40% of the world's women.[40,41]
- **Zinc deficiency**, affecting ~1.2 B, causes stunting in children and a weakened immune system.
- **Other deficiencies** affect some groups who consume insufficient amounts of riboflavin, folate, vitamin B_{12}, and calcium.

The Challenge

As global poverty is reduced and incomes increase, diets change. They tend to shift from cereals and tubers to meats, fats, and sugars, increasing total caloric consumption.[42] In addition, the forces of globalization appear to be leading to what has been called a "creeping homogenization"[43] in diets. These forces will put upward pressures on the prices of animal products and feed grains.

By 2050, the world population will exceed 9 billion, one-third more than today. Virtually all of this increase will occur in developing countries, most in their urban areas where it will be accompanied by continued increases in consumer purchasing power. Meeting this growing demand for food will call for increasing food production by ~70% above present levels. Experts believe that this can be achieved by increasing yields, reducing food wastes, and facilitating international trade. They agree that world has or can develop the requisite resources and technologies.

It is a practical and moral imperative that future demands for food be achieved in ways that will eliminate all types of hunger, including that of micronutrient malnutrition. This will require holistic approaches mindful of the inherent dependence of health on the balanced nutrition provided by access to diverse foods. The opportunities for food systems to advance public health are clear. They must inform the agendas not only of public health professionals but also of political leaders and other decision-makers in agriculture and trade.

38. An estimated 1600 M people live in iodine-deficient areas. The most prevalent outcome of iodine deficiency is goiter, affecting some 200 M people. In addition, some 6 million infants born annually to iodine-deficient mothers develop severe mental and neurological impairment known as *cretinism* (half of this number is in southern Asia). The deficiency also increases the rates of stillbirths, abortions, and infant deaths.

39. It is estimated that one-fifth of maternal mortality is due to the direct (heart failure) or indirect (inability to tolerate hemorrhage) effects of anemia; severe anemia is responsible for nearly one-third of fatalities among children who are not given immediate transfusions.

40. This ranges from a high in southern Asia (64%) to the lowest but still surprisingly high rates (>20%) in industrialized countries.

41. Anemia can have multiple causes including malaria and intestinal parasitism.

42. An estimated 1.46 B of the world's people are overweight/obese. In the developing world, that number increased from 250 M in 1980 to 904 M in 2008.

43. Keats, S., Wiggins, S., 2014. Future Diets Report, ODI, 116 pp.

5. STUDY QUESTIONS AND EXERCISES

1. Give an example of a situation wherein a particular biochemical test may be necessary for the diagnosis of a vitamin-related disorder detected by clinical examination.
2. Devise a system of biochemical measurements that could be performed on a 7-mL sample of fresh blood to yield as much information as possible about the vitamin status of the donor. (Assuming enzyme activities can be assayed using no more than 20 μL of plasma or erythrocyte lysate and other biochemical measurements can be made using no more than 100 μL each.)
3. In general terms, discuss the advantages and disadvantages of the various biomarkers for assessing vitamin status (e.g., functional tests, load tests, urinary excretion tests, circulating metabolite tests).

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Appendix A

Current and Obsolete Designations of Vitamins (Bolded) and Other Vitamin-Like Factors

Name	Explanation
Aneurin	Infrequently used synonym for thiamin
A-N factor	Obsolete term for the *"antineuritic factor"* (thiamin)
Bios factors	Obsolete terms for yeast growth factors now known to include biotin
Citrovorum factor	Infrequently used term for a naturally occurring form of folic acid (N^5-formyl-5,6,7,8-tetrahydropteroylmonoglutamic acid), which is required for the growth of *Leuconostoc citrovorum*
Extrinsic factor	Obsolete term for the antianemic activity in liver, now called vitamin B_{12}
Factor U	Obsolete term for chick anti-anemic factor now known as a form of folate
Factor R	Obsolete term for chick antianemic factor now known as a form of folate
Factor X	Obsolete term used at various times to designate the rat fertility factor now called vitamin E and the rat growth factor now called vitamin B_{12}
Filtrate factor	Obsolete term for the antiblack tongue disease activity, now known to be niacin that could be isolated from the *"B_2 complex"* by filtration through fuller's earth; also used to describe the chick antidermatitis factor, now known to be pantothenic acid, isolated from acid solutions of the *"B_2 complex"* by filtration through fuller's earth
Flavin	Term originally used to describe the water-soluble fluorescent rat growth factors isolated from yeast and animal tissues; now, a general term for isoalloxazine derivatives including riboflavin and its active forms, FMN and FAD
Hepatoflavin	Obsolete term for the water-soluble rat growth factor, now known to be riboflavin, isolated from liver
Intrinsic factor	Accepted designation for the vitamin B_{12}-binding protein produced by gastric parietal cells and necessary for the enteric absorption of the cobalamins
Lactoflavin	Obsolete term for the water-soluble rat growth factor, now known to be riboflavin, isolated from whey
LLD factor	Obsolete term for the activity in liver that promoted the growth of *Lactobacillus lactis* Dorner, now known to be vitamin B_{12}
Norit eluate	Obsolete term for *Lactobacillus casei* growth promotant, factor now known as folic acid, that could be isolated from liver and yeasts by adsorption on norit
Ovoflavin	Obsolete term for the water-soluble rat growth factor, now known to be riboflavin, isolated from egg white
P–P factor	Obsolete term for the thermostable "*pellagra-preventive*" component, now known as niacin, of the "*water-soluble B*" activity of yeast

Continued

Name	Explanation
Rhizopterin	Obsolete synonym for the "*SLR factor*", i.e., a factor from *Rhizobium* sp. fermentation that stimulated the growth of *Streptococcus lactis* R. (now called *Streptococcus faecalis*), which is now known to be a folate activity
SLR factor	Obsolete term for the *Streptococcus lactis* R. (now called *S. faecalis*) growth promotant later called "*rhizopterin*" and now known to be a folic acid activity
Streptogenin	A peptide present in liver and in enzymatic hydrolysates of casein and other proteins which promotes growth of mice and certain microorganisms (hemolytic streptococci and lactobacilli); not considered a vitamin
Vitamin A	Accepted designation of retinoids that prevent xerophthalmia and nyctalopia, and are essential for epithelial maintenance
Vitamin B	Original antiberiberi factor; now known to be a mixture of factors and designated as the vitamin B complex
Vitamin B complex	Term introduced when it became clear that "*water-soluble B*" contained more than one biologically active substance (such preparations were subsequently found to be mixtures of thiamin, niacin, riboflavin, pyridoxine, and pantothenic acid); the term has contemporary lay use as a nonspecific name for all of the B-designated vitamins
Vitamin B_1	Synonym for thiamin
Vitamin B_2	Synonym for riboflavin
Vitamin B_2 complex	Obsolete term for the thermostable "second nutritional factor" in yeast, which was found to be a mixture of niacin, riboflavin, pyridoxine, and pantothenic acid
Vitamin B_3	Infrequently used synonym for pantothenic acid; was also used for nicotinic acid
Vitamin B_4	Unconfirmed activity preventing muscular weakness in rats and chicks; believed to be a mixture of arginine, glycine, riboflavin, and pyridoxine
Vitamin B_5	Unconfirmed growth promotant for pigeons; probably niacin
Vitamin B_6	Synonym for pyridoxine
Vitamin B_7	Unconfirmed digestive promoter for pigeons; may be a mixture; also "*vitamin I*"
Vitamin B_8	Adenylic acid; no longer classified as a vitamin
Vitamin B_9	Unused designation
Vitamin B_{10}	Growth promotant for chicks; likely a mixture of folic acid and vitamin B_{12}
Vitamin B_{11}	Apparently the same as "*vitamin B_{10}*"
Vitamin B_{12}	Accepted designation of the cobalamins (cyano- and aquocobalamins) that prevent pernicious anemia and promote growth in animals
Vitamin B_{12a}	Synonym for aquacobalamin
Vitamin B_{12b}	Synonym for hydroxocobalamin
Vitamin B_{12c}	Synonym for nitritocobalamin
Vitamin B_{13}	Synonym for orotic acid, an intermediate of pyrimidine metabolism; not considered a vitamin
Vitamin B_{14}	Unconfirmed
Vitamin B_{15}	Synonym for "*pangamic acid*"; no proven biological value
Vitamin B_{17}	Synonym for laetrile, a cyanogenic glycoside with unsubstantiated claims of anticarcinogenic activity; not considered a vitamin
Vitamin B_c	Obsolete term for pteroylglutamic acid
Vitamin B_p	Activity preventing perosis in chicks; replaceable by choline and Mn
Vitamin B_t	Activity promoting insect growth; identified as carnitine
Vitamin B_x	Activity associated with pantothenic acid and *p*-aminobenzoic acid
Vitamin C	Accepted designation of the antiscorbutic factor, ascorbic acid
Vitamin C_2	Unconfirmed antipneumonia activity; also called "*vitamin J*"
Vitamin D	Accepted designation of the antirachitic factor (the calciferols)
Vitamin D_2	Accepted designation for ergocalciferol (a vitamin D-active substance derived from plant **sterols**)

Name	Explanation
Vitamin D_3	Accepted designation for cholecalciferol (a vitamin D-active substance derived from animal sterols)
Vitamin E	Accepted designation for tocopherols active in preventing myopathies and certain types of infertility in animals
Vitamin F	Obsolete term for essential fatty acids; also an abandoned term for thiamin activity
Vitamin G	Obsolete term for riboflavin activity; also an abandoned term for the "*pellagra-preventive factor*" (niacin)
Vitamin H	Obsolete term for biotin activity
Vitamin I	Mixture also formerly called "*vitamin B_7*"
Vitamin J	Postulated antipneumonia factor also formerly called "*vitamin C_2*"
Vitamin K	Accepted designation for activity preventing hypoprothrombinemic hemorrhage shared by related naphthoquinones
Vitamin K_1	Accepted designation for phylloquinones (vitamin K-active substances produced by plants)
Vitamin K_2	Accepted designation for prenylmenaquinones (vitamin K-active substances synthesized by microorganisms and produced from other vitamers K by animals)
Vitamin K_3	Accepted designation for menadione (synthetic vitamin K-active substance not found in nature)
Vitamin L_1	Unconfirmed liver filtrate activity, probably related to anthranilic acid, proposed as necessary for lactation
Vitamin L_2	Unconfirmed yeast filtrate activity, probably related to adenosine, proposed as necessary for lactation
Vitamin M	Obsolete term for antianemic factor in yeast now known to be pteroylglutamic acid
Vitamin N	Obsolete term for a mixture proposed to inhibit cancer
Vitamin O	Unused designation
Vitamin P	Activity reducing capillary fragility related to citrin, which is no longer classified as a vitamin
Vitamin Q	Unused designation (the letter was used to designate coenzyme Q)
Vitamin R	Obsolete term for folic acid; from Norris' chick antianemic "*factor R*"
Vitamin S	Chick growth activity related to the peptide "*streptogenin*"; the term was also applied to a bacterial growth activity probably related to biotin
Vitamin T	Unconfirmed group of activities isolated from termites, yeasts, or molds and reported to improve protein utilization in rats
Vitamin U	Unconfirmed activity from cabbage proposed to cure ulcers and promote bacterial growth; may have folic acid activity
Vitamin V	Tissue-derived activity promoting bacterial growth; probably related to NAD
Wills' factor	Obsolete term for the antianemic factor in yeast now known to be a form of folate
Zoopherin	Obsolete term for a rat growth factor now known as vitamin B_{12}

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Appendix B

Original Reports for Case Studies

Chapter 6	Case 1 McLaren, D.S., Ahirajian, E., Tchalian, M., et al., 1965. Xerophthalmia in Jordan. Am. J. Clin. Nutr. 17, 117–130.
	Case 2 Wechsler, H.L., 1979. Vitamin A deficiency following small-bowel bypass surgery for obesity. Arch. Dermatol. 115, 73–75.
	Case 3 Sauberlich, H.E., Hodges, R.E., Wallace, D.L., et al., 1974. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vit. Horm. 32, 251–275.
Chapter 7	Marx, S.J., Spiegel, A.M., Brown, E.M., et al., 1978. A familial syndrome of decrease in sensitivity to 1,25-dihydroxyvitamin D. J. Clin. Endocrinol. Metab. 47, 1303–1310.
Chapter 8	Case 1 Boxer, L.A., Oliver, J.M., Spielberg, S.P., et al., 1979. Protection of Granulocytes by vitamin E in glutathione synthetase deficiency. N. Eng. J. Med. 301, 901–905.
	Case 2 Harding, A.E., Matthews, S., Jones, S., et al., 1985. Spinocerebellar degeneration associated with a selective defect in vitamin E absorption. N. Eng. J. Med. 313, 32–35.
Chapter 9	Case 1 Colvin, B.T., Lloyd, M.J., 1977. Severe coagulation defect due to a dietary deficiency of vitamin K. J. Clin. Pathol. 30, 1147–1148.
	Case 2 Corrigan, J. and Marcus, F.I., 1974. Coagulopathy associated with vitamin E ingestion. J. Am. Med. Assoc. 230, 1300–1301.
Chapter 10	Case 1 Hodges, R.E., Hood, J., Canham, J.E., et al., 1971. Clinical manifestations of ascorbic acid deficiency in man. Am. J. Clin. Nutr. 24, 432–443.
	Case 2 Dewhurst, K., 1954. A case of scurvy simulating a gastric neoplasm. Br. Med. J. 2, 1148–1150.
Chapter 11	Case 1 Burwell, C.S., Dexter, L., 1947. Beriberi heart disease. Trans. Assoc. Am. Physiol. 60, 59–64.
	Case 2 Blass, J.P., Gibson, G.E., 1977. Abnormality of a thiamin-requiring enzyme in patients with Wernicke-Korsakoff syndrome. New Eng. J. Med. 297, 1367–1370.
Chapter 12	Dutta, P., Gee, M., Rivlin, R.S., et al., 1988. Riboflavin deficiency and glutathione metabolism in rats: possible mechanisms underlying altered responses to hemolytic stimuli. J. Nutr. 118, 1149–1157.
Chapter 13	Vannucchi, H., Moreno, F.S., 1989. Interaction of niacin and zinc metabolism in patients with alcoholic pellagra. Am. J. Clin. Nutr. 50, 364–369.

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Chapter 14	Case 1 Barber, G.W., Spaeth, G.L., 1969. The successful treatment of homocystinuria with pyridoxine. J. Pediatr. 75, 463–478.
	Case 2 Schaumberg, H., Kaplan, J., Windebank, A., et al., 1983. Sensory neuropathy from pyridoxine abuse. New Eng. J. Med. 309, 445–448.
Chapter 15	Mock, D.M., DeLorimer, A.A., Liebman, W.M., et al., 1981. Biotin deficiency: An unusual complication of parenteral alimentation. New Eng. J. Med. 304, 820–823.
Chapter 16	Lacroix, B., Didier, E., Grenier, J.F., 1988. Role of pantothenic and ascorbic acid in wound healing processes: in vitro study on fibroblasts. Int. J. Vit. Nutr. Res. 58, 407–413.
Chapter 17	Freeman, J.M., Finkelstein, J.D., Mudd, S.H., 1975. Folate-responsive homocystinuria and schizophrenia. A defect in methylation due to deficient 5,10-methylenetetrahydrofolic acid reductase activity. New Eng. J. Med. 292, 491–496.
Chapter 18	Higginbottom, M.C., Sweetman, L., Nyhan, W.L., 1978. A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurological abnormalities of a vitamin B_{12}-deficient breast-fed infant of a strict vegetarian. New Eng. J. Med. 299, 317–323.
Chapter 19	Killgore, J., Smidt, C., Duich, L., et al., 1989. Nutritional Importance of Pyrroloquinoline Quinone, Science 245, 850–852.
	Rucker, R., Chowanadisai, W., Nakano, M., 2009. Potential physiological importance of Pyrroloquinoline Quinone. Altern. Med. Rev. 14, 268–277.

Appendix C

A Core of Current Vitamin Literature

The following tables list journals publishing original research and reviews (Table 1) about the vitamins, as well as website (Table 2) and reference texts (Table 3) useful to students, researchers, and clinicians.

TABLE C-1 Journals Presenting Original Research and Reviews

Title	Publisher/URL[a,b]
Advances in Nutrition	American Society for Nutrition *www.advances.nutrition.org*
American Journal of Clinical Nutrition	American Society for Nutrition *www.ajcn.nutrition.org*
American Journal of Epidemiology	Oxford Journals *www.aje.oxfordjournals.org*
American Journal of Medicine	Alliance for Academic Internal Medicine *www.www.amjmed.com*
American Journal of Public Health	American Public Health Association *www.ajph.aphypublications.org*
Annals of Internal Medicine	American College of Physicians *www.annals.org*
Annals of Nutrition and Metabolism	Karger *www.karger.com/Journal/223977*
Annual Review of Biochemistry	Annual Reviews *www.annualreviews.org/journal/biochem*
Annual Review of Medicine	Annual Reviews *www.annualreviews.org/loi/med*
Annual Review of Nutrition	Annual Reviews *www.annualreviews.org/journal/nutr*
Australian Journal of Nutrition and Dietetics	Dietetics Association of Australia *www.ajnd.org.au*
BBA (Biochemica et Biophysical Acta)	Elsevier *www.journals.elsevier.com/bba-general-subjects*
Biochemical and Biophysical Research Communications	Elsevier *www.journals.elsevier.com/biochemical-and-biophysical-research-communications*

Continued

TABLE C-1 Journals Presenting Original Research and Reviews—cont'd

Title	Publisher/URL[a,b]
Biochemistry	American Chemical Society *www.pubs.acs.org/journal/bichaw*
Biofactors	ILR Press, Oxford *www.onlinelibrary.wiley.com/journal/10.1002/(ISSN)1872-8081*
British Journal of Nutrition	Nutrition Society *www.journals.cambridge.org/action/displayJournal?jid=BJN*
British Medical Journal	BMJ Publishing Group *www.bmj.com*
Cancer Epidemiology, Biomarkers and Prevention	American Association for Cancer research *www.cebp.aacrjournals.org/*
Cell	Cell Press *www.cell.com*
Clinical Biochemistry	Canadian Society of Clinical Chemists *www.journals.elsevier.com/clinical-biochemistry*
Clinical Chemistry	American Association of Clinical Chemists *www.clinchem.org*
Critical Reviews in Biochemistry and Molecular Biology	Taylor & Francis *www.tandfonline.com/loi/ibmg20#.V2mIL7grlfo*
Critical Reviews in Food Science and Nutrition	Taylor & Francis Group *www.tandfonline.com/loi/bfsn20#.V2mIL7grlfo*
Current Nutrition and Food Science	Bentham Science Publishers *www.eurekaselect.com/612*
Current Opinion in Clinical Nutrition and Metabolic Care	Lippincott Williams & Wilkins *www.journals.lww.com/co-clinicalnutrition/Pages/default.aspx*
Epidemiology	Lippincott Williams & Wilkins *www.journals.lww.com/epidem/Pages/default.aspx*
European Journal of Biochemistry (FEBS Journal)	Federation of European Biochemical Societies *www.febs.onlinelibrary.wiley.com/hub/journal/10.1111/(ISSN)1742–4658/issues/*
European Journal of Clinical Nutrition	Stockton Press *www.nature.com/ejcn/index.html*
FASEB Journal	Federation of American Societies for Experimental Biology *www.fasebj.org*
FEBS Letters	Federation of European Biochemical Societies *www.febs.onlinelibrary.wiley.com/hub/journal/10.1002/(ISSN)1873–3468/*
Gastroenterology	American Gastroenterology Association Institute *www.gastrojournal.org*
International Journal of Epidemiology	Oxford Journals *www.ide.oxforedjournjals.org*
International Journal of Food Sciences and Nutrition	Taylor & Francis *www.tandfonline.com/loi/iijf20#.V2mMeLgrlf0*
International Journal for Vitamin and Nutrition Research	Hogrefe & Huber Publishers *www.econtent.hogrefe.com/loi/vit*
International Journal of Nutrition and Metabolism	Academic Journals *www.academicjournals.org/IJNAM*
International Journal of Obesity	Nature Publishing Group *www.nature.com/ijo/index.html*

TABLE C-1 Journals Presenting Original Research and Reviews—cont'd

Title	Publisher/URL[a,b]
Journal of Agricultural and Food Chemistry	American Chemical Society *www.pubs.acs.org/journal/jafcau*
Journal of the Academy of Nutrition and Dietetics	Academy of Nutrition and Dietetics *www.andjrnl.org*
JAMA (Journal of the American Medical Association)	American Medical Association www.jama.jamanetwork.com/journal.aspx
JAMA Internal Medicine	American Medical Association *www.archinte.ama-assn.org*
Journal of Biological Chemistry	American Society of Biological Chemists *www.jbc.org*
Journal of Clinical Biochemistry and Nutrition	Institute of Applied Biochemistry *www.jstage.jst.go.jp/browse/jcbn*
Journal of Food Composition and Analysis	Elsevier *www.journals.elsevier.com/journal-of-food-composition-and-analysis*
Journal of Immunology	American Association of Immunologists *www.jimmunol.org*
Journal of Lipid Research	American Society for Biochemistry and Molecular Biology *www.jlr.org*
Journal of Nutrition	American Society for Nutrition *www.jn.nutrition.org*
Journal of Nutritional Biochemistry	Elsevier *www.jnutbio.com*
Journal of Nutritional Sciences and Vitaminology	The Vitamin Society of Japan and Japanese Society of Nutrition and Food Science *www.jsnfs.or.jp/english/english_jnsv.html*
Journal of Parenteral and Enteral Nutrition	American Society of Parenteral and Enteral Nutrition *www.pen.sagepub.com*
Journal of Pediatric Gastroenterology and Nutrition	Lippincott Williams & Wilkens *www.journals.lww.com/jpgn/pages.default.aspx*
Lipids	American Oil Chemists Society *www.link.springer.com/journal/11745*
New England Journal of Medicine	Massachusetts Medical Society *http://www.nejm.org*
Nutrition Abstracts and Reviews Series A (human, experimental)	CABI *www.cabi.org/publishing-products/online-information-resources/nutrition-abstracts-and-reviews-series-ahuman-and-experimental*
Nutrition Abstracts and Reviews Series B (feeds, feeding)	CABI *www.cabi.org/publishing-products/online-information-resources/nutrition-abstracts-and-reviews-series-b-livestock-feeds-and-feeding*
Nutrition and Food Science	MICS Publishing Group *www.omicsonline.org/nutrition-food-sciences.php*
Nutrition in Clinical Care	Wiley *www.onlinelibrary.wiley.com/journal/10.1111/(ISSN)1523-5408*
Nutrition	Elsevier *www.journals.elsevier.com/nutrition/*
Nutrition Journal	Cell & Bioscience *www.nutritionj.biomedcentral.com*

Continued

TABLE C-1 Journals Presenting Original Research and Reviews—cont'd

Title	Publisher/URL[a,b]
Nutrition Research	Elsevier *www.journals.elsevier.com/nutrition-research*
Nutrition Research Reviews	The Nutrition Society *www.journals.cambridge.org/action/displayJournal?jid=NRR*
Nutrition Reviews	Wiley-Blackwell *www.onlinelibrary.wiley.com/journal/10.1111/(ISSN)1753-4887*
Nutrition Today	Lippincott Williams & Wilkins *www.journals.lww.com/nutritiontodayonline/pages/default.aspx*
Obesity	The Obesity Society *www.obesity.org/publications/obesity-journal*
PNAS (Proceedings of the National Academy of Sciences)	National Academy of Sciences (US) *www.pnas.org*
Proceedings of the Nutrition Society	Nutrition Society *www.journals.cambridge.org/action/displayJournal?jid=PNS*
Proceedings of the Society for Experimental Biology and Medicine	Society for Experimental Biology and Medicine *www.sebm.org/journal*

[a]*URL, uniform resource locator.*
[b]*Sites accessed June 21, 2016.*

TABLE C-2 Some Useful Websites

Programs/Information	URL[a,b]
United Nations	
Food and Agricultural Organization (FAO)	*www.fao.org*
Agriculture and Consumer Protection	*www.fao.org/ag/portal/ag-home/en*
Codex Alimentarius[c]	*www.fao.org/fao-who-codexalimentarius/en*
Committee of World Food Security	*www.fao.org/cfs/en*
Food Composition—INFOODS[d] project	*www.fao.org/infoods/infoods/en*
Hunger	*www.fao.org/hunger/en*
Nutritional Assessment	*www.fao.org/nutrition/assessment/en*
Nutrition Country Profiles	*www.fao.org/ag/agn/nutrition/profiles_en.stm*
Nutrition Education and Consumer Awareness	*www.fao.org/ag/humannutrition/nutritioneducation/en*
Nutrition Requirements	*www.fao.org/nutrition/requirements/en*
Statistics	*www.fao.org/statistics/en*
Sustainable Development Goals	*http://www.fao.org/sustainable-development-goals/home/en*
World Food Situation	*www.fao.org/worldfoodsituation/en*
UN University	*www.unu.edu*
World Health Organization (WHO)	*www.who.int/en*
Child Growth Standards	*www.who.int/childgrowth/en*
Global Database on Child Growth and Malnutrition	*www.who.int/nutgrowthdb/en*
Global Health Library	*www.globalhealthlibrary.net/php/index.php*

TABLE C-2 Some Useful Websites—cont'd

Programs/Information	URL[a,b]
Growth Reference Database	*www.who.int/healthinfo/indicators/2015/en*
Health Data and Statistics	*www.who.int/healthinfo/statistics/en*
Nutrition for Health and Development	*www.who.int/nmh/about/nhd/en*
Vitamin and Mineral Information Systems (VMNIS)	*www.who.int/vmnis/en*
World Health Statistics	*www.who.int/gho/publications/world_health_statistics/2014/en*
United States Government	
Let's Move	*www.letsmove.gov*
Nutrition.gov	*www.nutrition.gov*
Department of Agriculture (USDA)	*www.usda.gov/wps/portal/usdahome*
Ag. Res. Service National Program in Human Nutrition	*www.ars.usda.gov/research/programs/programs.htm?NP_CODE=107*
Center for Nutrition Policy and Promotion	*www.cnpp.usda.gov*
Child Nutrition Programs	*www.fns.usda.gov/school-meals/child-nutrition-programs*
ChooseMyPlate.gov	*www.choosemyplate.gov*
Dietary Assessment Tools	*www.choosemyplate.gov/tools-supertracker*
Dietary Guidelines for Americans	*www.cnpp.usda.gov/dietary-guidelines*
Food Availability (per capita) Data System	*www.ers.usda.gov/data-products/food-availability-(per-capita)-data-system.aspx*
Food and Nutrition Information Center	*www.fnic.nal.usda.gov*
National Agricultural Library	*www.nalusda.gov*
National Institute for Food and Agriculture (NIFA)	*www.nifa.usda.gov*
National Nutrient Database for Standard Reference	*www.ndb.nal.usda.gov*
Department of Defense	
U.S. Army Research Institute of Environmental Medicine	www.usariem.army.mil/index.cfm/about
Department of Health and Human Services	*www.hhs.gov*
Center for Disease Control and Prevention	*www.cdc.gov*
National Center for Health Statistics	*www.cdc.gov/nchs*
National Health and Nutrition Examination Survey (NHANES)	*www.cdc.gov/nchs/nhanes*
Food and Drug Administration (FDA)	*www.fda.gov*
Center for Food Safety and Applied Nutrition (CFSAN)	*www.fda.gov/AboutFDA/CentersOffices/OfficeofFoods/CFSAN*
Dietary Supplements	*www.fda.gov/Food/DietarySupplements*
National Institutes of Health (NIH)	*www.nih.gov*
Eunice Kennedy Shriver National Institute of Child Health and Human Development	*www.nichd.nih.gov/Pages/index.aspx*
National Cancer Institute	*www.cancer.gov*
National Human Genome Research Institute	*www.genome.gov*
National Heart, Lung and Blood Institute	*www.nhlbi.nih.gov*
National Institute of Diabetes and Digestive and Kidney Diseases	*www.niddk.nih.gov/Pages/default.aspx*

Continued

TABLE C-2 Some Useful Websites—cont'd

Programs/Information	URL[a,b]
National Institute on Aging	*www.nia.nih.gov*
National Library of Medicine	*www.nlm.nih.gov*
Office of Dietary Supplements	*www.ods.od.nih.gov*
Department of State	
Agency for International Development Global Health Initiative	*www.usaid.gov/what-we-do/global-health/cross-cutting-areas/global-health-initiative*
Professional Societies	
Academy of Nutrition and Dietetics	*www.eatright.org*
American Society for Nutrition	*www.nutrition.org*
University On-Line Resources	
Cornell University: "Cornell NutritionWorks"	*www.nutritionworks.cornell.edu/home*
Harvard University: "The Nutrition Source"	*www.hsph.harvard.edu/nutritionsource*
Johns Hopkins Bloomberg School of Public Health:"Johns Hopkins Public Health"	*www.magazine.jhsph.edu*
Tufts University Friedman School: "Nutrition & Health Newsletter"	*www.nutritionletter.tufts.edu*
University of California—Berkeley: "Berkeley Wellness"	*www.berkeleywellness.com*

[a]*URL, uniform resource locator.*
[b]*Sites accessed June 21, 2016.*
[c]*Joint program of FAO and WHO.*
[d]*International Network of Food Data Systems.*

TABLE C-3 A Book Shelf of Useful References

Bales, C.W., Locher, J.L., Saltzman, E., eds. 2015. "Handbook of Clinical Nutrition and Aging", 3rd Edition, Springer, New York, pp. 442.

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TABLE C-3 A Book Shelf of Useful References—cont'd

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Appendix D

Vitamin Contents of Foods (units per 100 g Edible Portion)

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Cereals												
Barley, pearled, cooked	7	0	0.01	0.08	0	0.083	0.062	2.063	0.115	0.135	16	0
Buckwheat groats, RSTD, cooked	0	0	0.09	1.9	0	0.04	0.039	0.94	0.077	0.359	14	0
Bulgur, cooked	2	0	0.01	0.5	0	0.057	0.028	1	0.083	0.344	18	0
Corn flour, whole grain, yellow	214	0	0.42	0.3	0	0.246	0.08	1.9	0.37	0.658	25	0
Cornmeal, whole grain, yellow	214	0	0.42	0.3	0	0.385	0.201	3.632	0.304	0.425	25	0
Couscous, cooked	0	0	0.013	0.1	0	0.063	0.027	0.983	0.051	0.371	15	0
Hominy, canned, white	1	0	0.05	0.2	0	0.003	0.006	0.033	0.005	0.154	1	0
Hominy, canned, yellow	110	0	NV	NV	0	0.003	0.006	0.033	0.005	0.154	1	0
Millet, cooked	3	0	0.02	0.3	0	0.106	0.082	1.33	0.108	0.171	19	0
Noodles, Chinese, chow mein	0	0	2.3	1.4	0	0.578	0.421	5.95	0.11	0.533	22	0
Noodles, egg, CKD, ENR	21	4	0.17	0	0	0.289	0.136	2.097	0.046	0.263	7	0.09
Noodles, egg, spinach, cooked, ENR	103	4	0.55	101	0	0.245	0.123	1.474	0.114	0.233	21	0.14
Noodles, Japanese, soba, CKD	0	0	NV	NV	0	0.094	0.026	0.51	0.04	0.235	7	0
Noodles, Japanese, somen, CKD	0	0	NV	NV	0	0.02	0.033	0.097	0.013	0.172	2	0
Oat bran, cooked	0	0	NV	NV	0	0.16	0.034	0.144	0.025	0.217	6	0
Oats	0	0	NV	NV	0	0.763	0.139	0.961	0.119	1.349	56	0
Rice, brown, long grain, CKD	0	0	0.17	0.2	0	0.178	0.069	2.561	0.123	0.38	9	0
Rice, white, glutinous, CKD	0	0	0.04	0	0	0.02	0.013	0.29	0.026	0.215	1	0
Rice, white, long grain, parboiled, CKD, ENR	0	0	0.01	0	0	0.212	0.019	2.309	0.156	0.323	3	0

Rice, white, long grain, REG, CKD	0	0	0.04	0	0	0.02	0.013	0.4	0.093	0.39	3	0
Rye flour, medium	0	0	1.43	5.9	0	0.287	0.114	1.727	0.268	0.492	19	0
Semolina, enriched	0	0	0.26	NV	0	0.811	0.571	5.99	0.103	0.58	72	0
Sorghum	0	0	0.5	NV	0	0.332	0.096	3.688	0.443	0.367	20	0
Pasta, CKD, ENR	0	0	0.06	0	0	0.274	0.136	1.689	0.049	0.112	7	0
Spaghetti, spinach, CKD	152	0	NV	NV	0	0.097	0.103	1.53	0.096	0.183	12	0
Pasta, whole wheat, CKD	4	0	0.23	0.6	0	0.156	0.099	3.126	0.093	0.268	21	0
Tapioca, pearl, dry	0	0	0	0	0	0.004	0	0	0.008	0.135	4	0
Wheat bran, crude	9	0	1.49	1.9	0	0.523	0.577	13.58	1.303	2.181	79	0
Wheat flour, whole grain	9	0	0.71	1.9	0	0.502	0.165	4.957	0.407	0.603	44	0
Wheat flour, white, all purpose, ENR, bleached	0	0	0.06	0.3	0	0.785	0.494	5.904	0.044	0.438	26	0
Wheat germ, crude	0	0	NV	NV	0	1.882	0.499	6.813	1.3	2.257	281	0
Wild rice, cooked	3	0	0.24	0.5	0	0.052	0.087	1.287	0.135	0.154	26	0
Breads, Cakes, and Pastries												
Bagels, plain, ENR	0	0	7.46	1.2	0	0.568	0.344	4.515	0.07	0.407	24	0
Biscuits, plain/ buttermilk	2	0	1.32	4.1	0	0.427	0.292	3.352	0.047	0.3	12	0.14
Bread, cornbread, w/ 2% milk	277	NV	NV	NV	0.3	0.291	0.294	2.254	0.113	0.339	19	0.15
Bread, cracked wheat	0	NV	NV	NV	0	0.358	0.24	3.671	0.304	0.512	39	0.03
Bread, French/Vienna/ sourdough	1	0	0.2	0.7	0	0.71	0.427	4.817	0.107	0.455	56	0
Bread, Irish soda	194	NV	NV	NV	0.8	0.298	0.269	2.405	0.083	0.25	10	0.05
Bread, Italian	1	0	0.29	1.2	0	0.473	0.292	4.381	0.048	0.378	30	0
Bread, mixed grain	0	0	0.37	1.4	0.1	0.279	0.131	4.042	0.263	0.336	75	0
Bread, oat bran	5	0	0.44	1.2	0	0.504	0.346	4.831	0.073	0.581	25	0
Bread, oatmeal	16	0	0.48	1.5	0	0.399	0.24	3.136	0.068	0.341	27	0.02
Bread, pita, white, enriched	0	0	0	0.2	0	0.599	0.327	4.632	0.034	0.397	24	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Bread, pita, whole wheat	6	0	0.61	1.4	0	0.339	0.08	2.84	0.231	0.548	35	0
Bread, pumpernickel	0	0	0.42	0.8	0	0.327	0.305	3.091	0.126	0.404	34	0
Bread, raisin, enriched	0	0	0.28	1.7	0.1	0.339	0.398	3.466	0.069	0.387	34	0
Bread, rye	7	0	0.33	1.2	0.4	0.434	0.335	3.805	0.075	0.44	51	0
Bread, wheat bran	0	0	0.32	1.3	0	0.397	0.287	4.402	0.176	0.536	25	0
Bread, wheat germ	4	0	0.57	1.2	0.3	0.332	0.379	4.458	0.096	0.313	55	0.07
Bread, wheat	2	0	0.19	4.9	0.2	0.415	0.253	5.62	0.111	0.436	65	0
Bread, white	1	0	0.24	3.4	0	0.415	0.337	3.926	0.063	0.278	23	0.02
Bread, whole wheat	4	0	0.63	9	0	0.376	0.284	5.732	0.237	0.722	52	0
Cake, angel food	0	NV	NV	NV	0	0.102	0.491	0.883	0.031	0.198	3	0.06
Cake, Boston cream pie	82	5	0.15	3.1	0.2	0.408	0.27	0.191	0.026	0.301	8	0.16
Cake, fruitcake	22	0	0.9	1.5	0.5	0.05	0.099	0.791	0.046	0.226	3	0.01
Cake, gingerbread	48	NV	NV	NV	0.1	0.19	0.162	1.738	0.19	0.375	8	0.06
Cake, pound	241	34	0.65	1.7	0	0.173	0.249	1.105	0.036	0.485	28	0.36
Cake, shortcake, biscuit type	72	NV	NV	NV	0.2	0.311	0.272	2.573	0.03	0.248	10	0.07
Cake, sponge	154	9	0.24	0.2	0	0.243	0.269	1.932	0.052	0.478	13	0.24
Cake, white, w/o frosting	52	NV	0.12	5.1	0.2	0.186	0.242	1.533	0.021	0.184	7	0.08
Cake, yellow, w/o frosting	139	NV	NV	NV	0.2	0.183	0.233	1.456	0.036	0.31	10	0.16
Cheesecake	547	18	0.56	4.4	0.4	0.028	0.193	0.195	0.052	0.571	15	0.17
Cookies, animal crackers	0	0	0.12	5.9	0	0.35	0.326	3.47	0.022	0.376	14	0.05
Cookies, brownies	69	0	0.15	6.5	0	0.255	0.21	1.721	0.035	0.547	12	0.07
Cookies, butter, ENR	673	16	0.58	1.7	0	0.37	0.335	3.19	0.036	0.488	6	0.36
Cookies, choc chip, low fat	1	0	1.47	4.1	0	0.263	0.185	1.982	0.022	0.165	12	0
Cookies, choc sandwich, w/ creme filling	5	0	1.58	11.2	0	0.095	0.204	1.373	0.044	0.178	7	0.05
Cookies, fig bars	33	0	0.65	5.8	0.3	0.158	0.217	1.874	0.075	0.364	10	0.09

Cookies, fortune	3	0	0.03	1.1	0	0.182	0.13	1.84	0.013	0.297	10	0.01
Cookies, gingersnaps	2	0	0.97	2.5	0	0.2	0.293	3.235	0.098	0.38	6	0
Cookies, graham crackers	0	0	1.51	14.3	0	0.265	0.317	4.439	0.001	0.005	19	0
Cookies, molasses	0	0	0.11	5.5	0	0.355	0.264	3.031	0.104	0.411	7	0
Cookies, oatmeal	5	0	0.26	8	0.5	0.267	0.23	2.227	0.066	0.386	7	0
Cookies, peanut butter	32	0	3.53	4.4	0	0.208	0.208	3.86	0.122	0.429	27	0.24
Cookies, raisin, soft type	5	0	2.24	3.9	0.4	0.216	0.206	1.967	0.052	0.244	9	0.03
Cookies, vanilla wafers	8	0	0.23	6	0	0.275	0.32	3.106	0.073	0.41	9	0.13
Crackers, cheese	156	1	2.19	9.4	0	0.562	0.338	6.113	0.17	0.472	25	0.34
Crackers, cheese, w/ peanut butter filling	2	0	2.37	12	0	0.552	0.294	5.831	0.151	0.485	25	0.28
Crackers, matzo	0	0	0.06	0.3	0	0.387	0.291	3.892	0.115	0.443	14	0
Crackers, melba toast	0	0	0.43	0.9	0	0.413	0.273	4.113	0.098	0.693	26	0
Crackers, rusk toast	41	NV	NV	NV	0	0.404	0.399	4.625	0.038	0.406	64	0.07
Crackers, rye, wafers	5	0	0.8	5.7	0.1	0.427	0.289	1.581	0.271	0.569	45	0
Crackers, rye	0	0	0.81	6	0	0.243	0.145	1.04	0.21	0.676	22	0
Crackers, saltiness	2	0	1.15	25.4	0	0.702	0.487	6.442	0.086	0.536	17	0.09
Crackers, wheat	0	0	1.55	14.2	0	0.284	0.146	4.022	0.244	0.577	36	0
Croissants, butter	206	0	0.84	1.8	0.2	0.388	0.241	2.188	0.058	0.861	28	1.6
Croutons, plain	0	NV	NV	NV	NV	0.623	0.272	5.439	0.026	0.429	22	0
Danish pastry, cheese	128	2	0.35	6.9	0.1	0.19	0.26	2	0.04	0.304	25	0.2
Danish pastry, fruit, ENR	51	0	0.34	5.3	3.9	0.263	0.22	1.992	0.043	0.634	16	0.09
Doughnuts, cake type, plain	16	0	2.02	7.9	1.3	0.163	0.125	1.59	0.024	0.126	28	0.1
Doughnuts, cake type, plain, sugared/glazed	10	NV	NV	NV	0.1	0.233	0.198	1.512	0.027	0.435	12	0.024
English muffins, plain, ENR	0	0	0.31	1.2	1.8	0.477	0.25	4.07	0.054	0.363	40	0.04
English muffins, wheat	1	0	0.45	0.8	0	0.431	0.292	3.356	0.09	0.444	39	0
French toast, w/ low-fat (2%) milk	503	NV	NV	NV	0.3	0.204	0.321	1.628	0.074	0.549	23	0.31

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Hush puppies	186	0	1.26	23.6	0.2	0.352	0.332	2.782	0.102	0.357	20	0.19
Muffins, blueberry	73	4	1.63	39.2	0.9	0.168	0.163	1.418	0.04	0.47	12	0.16
Muffins, corn	208	0	0.8	2.3	0	0.273	0.326	2.037	0.084	0.444	34	0.09
Muffins, plain, w/ low-fat (2%) milk	0	NV	NV	NV	0.3	0.284	0.301	2.308	0.042	0.351	13	0.15
Pancakes, blueberry	199	NV	NV	NV	2.2	0.195	0.272	1.524	0.049	0.395	12	0.2
Pancakes, plain	196	NV	0	NV	0.3	0.201	0.281	1.567	0.046	0.405	12	0.22
Pie, apple, ENR FLR	124	0	1.52	3.5	3.2	0.028	0.027	0.263	0.038	0.119	4	0.001
Pie, banana cream	237	32	0.4	6.3	1.6	0.139	0.207	1.054	0.133	0.388	11	0.25
Pie, blueberry	140	0	1.04	10.5	2.7	0.01	0.03	0.3	0.037	0.136	4	0.01
Pie, cherry	237	0	0.76	7.6	0.9	0.023	0.029	0.2	0.041	0.319	8	0.01
Pie, chocolate crème	166	3	1.09	9.5	0	0.087	0.14	0.605	0.02	0.232	6	0.12
Pie, coconut crème	90	6	0.15	5.3	0	0.05	0.08	0.2	0.068	0.24	0.12	0.19
Pie, lemon meringue	173	7	0	2.1	3.2	0.062	0.209	0.649	0.03	0.793	8	0.17
Pie, peach	140	0	0.94	3.3	0.9	0.061	0.033	0.2	0.023	0.114	4	0
Pie, pecan	175	3	0.8	15.5	0	0.204	0.078	1.31	0.038	0.391	17	0.12
Pie, pumpkin	3434	2	0.76	13.2	0	0.177	0.124	1.107	0.063	0.452	10	0.35
Rolls, dinner, plain	5	0	0.28	10.6	0.2	0.526	0.374	5.367	0.097	0.452	30	0.13
Rolls, dinner, wheat	0	0	0.361	2.7	0	0.433	0.273	4.072	0.076	0.364	15	0
Rolls, French	0	0	0.3	1.8	0	0.523	0.3	4.352	0.039	0.452	33	0
Rolls, hamburger/hot-dog, plain	107	0	0.27	4.8	1.3	0.543	0.297	4.18	0.063	0.555	41	0.2
Rolls, hard (including kaiser)	0	0	0.42	0.6	0	0.478	0.336	4.239	0.035	0.41	15	0
Strudel, apple	30	0	1.42	2.9	1.7	0.04	0.025	0.33	0.046	0.27	6	0.22
Sweet rolls, cinnamon w/ raisins	214	0	1.99	4.4	2	0.324	0.265	2.384	0.107	0.406	24	0.14
Taco shells, baked	17	0	0.69	8.6	0	0.226	0.08	1.867	0.203	NV	6	0
Waffles, plain	228	NV	NV	NV	0.4	0.263	0.347	2.073	0.056	0.485	15	0.25
Wonton wrappers	14	NV	NV	NV	0	0.519	0.378	5.424	0.03	0.025	17	0.02

Breakfast Cereals												
All bran	1747	170	1.19	5.2	20	2.27	2.71	14.8	12	1.06	41	18.1
Corn flakes	3591	286	0.02	0	65	4.83	1.74	21.03	1.907	0.099	NV	5.36
Corn grits, CKD w/ water	0	0	0.03	0	0	0.086	0.058	0.79	0.046	0.046	14	0
Cream of rice, CKD w/ water	0	NV	0.02	NV	0	0.071	0.18	1.039	0.027	0.076	3	0
Cream of wheat, CKD w/ water	793	NV	NV	0.1	0	0.132	0.21	3.093	0.309	0.082	6	0
Farina, CKD w/ water	0	NV	0.04	0	0	0.126	0.065	1.493	0.096	0.256	17	0
Granola (homemade)	19	0	11.1	5.3	1.2	0.548	0.354	2.739	0.37	0.752	84	0
Oat bran	100	0	NV	NV	0	0.97	0.3	0.8	0.11	0.85	38	0
Oatmeal, instant regular	0	NV	0.47	NV	0	0.73	0.14	0.78	0.12	NV	32	0
Puffed rice	0	NV	NV	NV	0	2.6	1.8	35.3	0.075	0.32	19	0
Puffed wheat	0	NV	NV	NV	0	2.6	1.8	35.3	0.17	0.518	32	0
Raisin bran	1271	68	0.54	1.9	0.9	0.6	0.7	8.5	0.8	0.206	19	2.5
Rice cereal, crispy style	1515	121	0	0	18.2	1.13	1.28	15.14	1.51	NV	4	4.55
Rice cereal, check style	1852	148	0.35	1	22.2	1.39	1.6	18.5	1.85	NV	4	5.59
Shredded wheat	0	0	0	NV	0	0.226	0.105	5.24	0.256	NV	51	0
Wheat flakes	2586	138	46.35	1.4	207	5.17	5.86	69	6.9	34.5	19	21
Wheat germ, toasted	103	0	15.99	4	6	1.67	0.82	5.59	0.978	1.387	352	0
Vegetables												
Alfalfa seeds, sprouted, raw	155	0	0.02	30.5	8.2	0.076	0.126	0.481	0.034	0.563	36	0
Amarnath leaves, BLD, DRND	2770	0	NV	NV	41.1	0.02	0.134	0.559	0.177	0.062	57	0
Artichokes, BLD, DRND	13	0	0.19	14.8	7.4	0.5	0.89	1	0.081	0.24	89	0
Asparagus, BLD, DRND	1006	0	1.5	50.6	7.7	0.162	0.139	1.084	0.079	0.225	149	0
Balsam-pear (bitter gourd), tips, BLD, DRND	2416	0	1.45	163	55.6	0.147	0.282	0.995	0.76	0.06	88	0
Bamboo shoots, BLD, DRND	0	0	NV	NV	0	0.02	0.05	0.3	0.098	0.066	2	0
Beans, navy, sprouted, BLD, DRND	4	0	NV	NV	17.3	0.381	0.235	1.263	0.198	0.854	106.3	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Beans, pinto, immature, FRZ, BLD, DRND	0	0	NV	NV	0.7	0.274	0.108	0.632	0.194	0.258	34	0
Beans, snap, green, BLD, DRND	633	0	0.46	47.9	9.7	0.074	0.097	0.614	0.056	0.074	33	0
Beans, snap, yellow, BLD, DRND	81	0	0.46	47.9	9.7	0.074	0.097	0.614	0.056	0.074	33	0
Beet greens, BLD, DRND	7654	0	1.81	484	24.9	0.117	0.289	0.499	0.132	0.329	14	0
Beets, BLD, DRND	35	0	0.04	0.2	3.6	0.027	0.04	0.331	0.067	0.145	80	0
Beets, pickled, CND, w/ liquid	49	0	0.06	0.3	2.3	0.01	0.048	0.251	0.05	0.137	27	0
Broadbeans, immature, BLD, DRND	270	0	NV	NV	19.8	0.128	0.09	1.2	0.029	0.066	58	0
Broccoli, BLD, DRND	1548	0	1.45	141	64.9	0.063	0.123	0.553	0.2	0.616	108	0
Broccoli, raw	623	0	0.78	102	89.2	0.071	0.117	0.639	0.175	0.573	63	0
Cabbage, Chinese (bak-choi), BLD, DRND	4249	0	0.09	34	26	0.032	0.063	0.428	0.166	0.079	41	0
Cabbage, BLD, DRND	80	0	0.14	109	37.5	0.061	0.038	0.248	0.112	0.174	30	0
Cabbage, raw	98	0	0.15	76	36.6	0.061	0.04	0.234	0.124	0.212	43	0
Cabbage, red, BLD, DRND	33	0	0.12	47.6	34.4	0.071	0.06	0.382	0.225	0.154	24	0
Cabbage, savoy, BLD, DRND	889	0	NV	NV	17	0.051	0.02	0.024	0.152	0.159	46	0
Carrots, baby, raw	13,796	0	NV	9.4	2.6	0.03	0.036	0.556	0.105	0.401	27	0
Carrots, BLD, DRND	17,033	0	0	13.7	3.6	0.066	0.036	0.645	0.153	0.232	14	0
Carrots, FRZ, BLD, DRND	16,928	0	1.01	13.6	2.3	0.03	0.037	0.416	0.084	0.174	11	0
Carrots, raw	16,706	0	0.66	13.2	5.9	0.066	0.058	0.983	0.138	0.273	19	0
Cassava, raw	13	0	0.19	1.9	20.6	0.087	0.048	0.854	0.088	0.107	27	0
Catsup	527	0	1.46	3	4.1	0.011	0.166	1.434	0.158	0.047	9	0
Cauliflower, BLD, DRND	12	0	0.07	13.8	44.3	0.042	0.052	0.41	0.173	0.508	44	0
Cauliflower, raw	0	0	0	15.5	48.2	0.05	0.06	0.507	0.184	0.667	57	0
Celery, raw	449	0	0.27	29.3	3.1	0.02	0.057	0.32	0.074	0.246	36	0

Chard, Swiss, BLD, DRND	6124	0	1.89	327	18	0.034	0.086	0.36	0.085	0.163	9	0
Chives, raw	4353	0	0.21	213	58.1	0.078	0.115	0.647	0.138	0.324	105	0
Collards, BLD, DRND	7600	0	0	407	18.2	0.04	0.106	0.575	0.128	0.218	16	0
Coriander, raw~	6748	0	0	310	27	0.067	0.162	1.114	0.149	0.57	62	0
Corn, sweet, yellow, BLD, DRND	263	0	0	0.4	5.5	0.093	0.057	1.683	0.139	0.792	23	0
Corn, sweet, yellow, raw	187	0	0	0.3	6.8	0.155	0.055	1.77	0.093	0.717	42	0
Corn, sweet, yellow, CND, w/ liquids	34	0	0	0	2.6	0.015	0.015	0.884	0.037	0.522	38	0
Cowpeas (blackeyes), BLD, DRND	75	0	0	36.8	2.6	0.26	0.064	0.728	0.095	0.213	141	0
Cucumber, w/ peel, raw	105	0	0	16.4	2.8	0.027	0.033	0.098	0.04	0.259	7	0
Dandelion greens, raw	10,161	0	3.44	778	35	0.19	0.26	0.806	0.251	0.084	27	0
Eggplant, BLD, DRND	37	0	0.41	2.9	1.3	0.076	0.02	0.6	0.086	0.075	14	0
Endive, raw	2167	0	0.44	231	6.5	0.08	0.075	0.4	0.02	0.9	142	0
Garlic, raw	9	0	0.08	1.7	31.2	0.2	0.11	0.7	1.235	0.596	3	0
Ginger root, raw	0	0	0.26	0.1	5	0.025	0.034	0.75	0.16	0.203	11	0
Gourd, calabash, BLD, DRND	0	0	NV	NV	8.5	0.029	0.022	0.39	0.038	0.144	4	0
Hearts of palm, canned	0	0	NV	NV	7.9	0.011	0.057	0.437	0.022	0.126	39	0
Kale, BLD, DRND	13,621	0	0.85	817	41	0.053	0.07	0.5	0.138	0.049	13	0
Kohlrabi, BLD, DRND	35	0	0	0.1	54	0.04	0.02	0.39	0.154	0.16	12	0
Leeks, BLD, DRND	812	0	0.5	25.4	4.2	0.026	0.02	0.2	0.113	0.072	24	0
Lemon grass (Citronella), raw	6	0	NV	NV	2.6	0.065	0.135	1.101	0.08	0.05	75	0
Lettuce, butterhead, raw	3312	0	0	102	3.7	0.057	0.062	0.357	0.082	0.15	73	0
Lettuce, cos/romaine, raw	8710	0	0.13	103	4	0.072	0.067	0.313	0.074	0.142	136	0
Lettuce, iceberg, raw	502	0	0	24.1	2.8	0.041	0.025	0.123	0.042	0.091	29	0
Lima beans, BLD, DRND	303	0	0.14	6.2	10.1	0.14	0.096	1.04	0.193	0.257	26	0
Lotus root, BLD, DRND	0	0	0.01	0.1	27.4	0.127	0.01	0.3	0.218	0.302	8	0
Mung beans, sprouted, stir-fried	31	0	NV	NV	16	0.14	0.18	1.2	0.13	0.559	70	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Mushroom, cloud fungus, dried	0	0	NV	NV	0	0.015	0.844	6.267	0.112	0.481	38	0
Mushroom, oyster, raw	48	29	0	0	0	0.125	0.349	4.956	0.11	1.294	38	0
Mushrooms, CND, DRND	0	8	0.01	0	0	0.085	0.021	1.593	0.061	0.811	12	0
Mushrooms, raw	NV	18	NV	NV	NV	0.015	0.217	3.877	0.293	1.5	13	NV
Mushrooms, shitake, dried	0	154	0	0	3.5	0.3	1.27	14.1	0.965	21.879	163	0
Mushrooms, straw, CND, DRND	0	NV	NV	NV	0	0.013	0.07	0.224	0.014	0.412	38	0
Mustard greens, BLD, DRND	12,370	0	0	593	25.3	0.041	0.063	0.433	0.098	0.12	9	0
New zealand spinach, BLD, DRND	4400	0	1.42	337	16	0.03	0.107	0.39	0.237	0.256	8.3	0
Okra, BLD, DRND	575	0	0.69		30	0.04	0.13	0.5	0.304	0.312	15	0
Onions, BLD, DRND	283	0	0	40	16.3	0.132	0.055	0.871	0.187	0.213	46	0
Onions, raw	2	0	0	0.4	7.4	0.046	0.027	0.116	0.12	0.123	19	0
Parsley, raw	8424	0	0	1640	133	0.086	0.098	1.313	0.09	0.4	152	0
Parsnips, BLD, DRND	0	0	1	1	13	0.083	0.051	0.724	0.093	0.588	58	0
Peas, edible-pod type, BLD, DRND	1311	0	0.47	30.2	22	0.064	0.119	0.563	0.174	0.857	35	0
Peas, edible-pod type, raw	1087	0	0.39	25	60	0.15	0.08	0.6	0.16	0.75	42	0
Peas, green, raw	765	0	0.13	24.8	40	0.266	0.132	2.09	0.169	0.104	65	0
Peas, green, BLD, DRND	801	0	0.14	25.9	14.2	0.259	0.149	2.021	0.216	0.153	63	0
Pepper, banana, raw	340	0	0.69	9.5	82.7	0.081	0.054	1.242	0.357	0.265	29	0
Peppers, chili, green, CND	126	0	NV	NV	34.2	0.01	0.03	0.627	0.12	0.084	54	0
Peppers, Hungarian, raw	816	0	0.48	9.9	92.9	0.079	0.055	1.092	0.517	0.205	53	0
Peppers, Jalapeno, raw	1078	0	0	18.5	118.6	0.04	0.07	1.28	0.419	0.315	27	0

Peppers, sweet, green, raw	370	0	0.37	7.4	80.4	0.057	0.028	0.48	0.224	0.099	10	0
Peppers, sweet, red, raw	3131	0	0	4.9	127.7	0.054	0.085	0.979	0.291	0.317	46	0
Pickles, cucumber, sweet	764	0	0	47.1	0.7	0.025	0.03	0.115	0.024	0.051	1	0
Pickles, cucumber, dill	125	0	0	17.3	2.3	0.045	0.057	0.19	0.035	0.201	8	0
Pigeonpeas, BLD, DRND	3	0	NV	NV	0	0.146	0.059	0.781	0.05	0.319	111	0
Pimento, canned	2655	0	0.69	8.3	84.9	0.017	0.06	0.615	0.215	0.01	6	0
Potatoes, au gratin, w/ butter	264	0	NV	NV	9.9	0.064	0.116	0.993	0.174	0.387	8	0
Potatoes, BKD, flesh	0	0	0	0.3	12.8	0.105	0.021	1.395	0.301	0.555	9	0
Potatoes, CND, DRND	0	0	NV	NV	5.1	0.068	0.013	0.915	0.188	0.354	6	0
Potatoes, French fries, FRZ, oven heated	5	0	0.16	2.7	14.2	0.129	0.034	2.28	0.192	0.554	33	0
Potatoes, hashed brown	5	0	0	3.7	13	0.172	0.033	2.302	0.472	0.893	16	0
Potatoes, mashed, w/whole milk and margarine	187	7	0.42	6	10.5	0.092	0.42	1.174	0.247	0.476	9	0
Potatoes, microwaved in skin, flesh	0	0	NV	NV	15.1	0.129	0.025	1.625	0.319	0.597	12	0
Potatoes, scalloped, w/ butter	135	0	NV	NV	10.6	0.069	0.092	1.053	0.178	0.514	9	0
Pumpkin, CND	15,563	0	1.06	16	4.2	0.024	0.054	0.367	0.056	0.4	12	0
Radishes, raw	7	0	0	1.3	14.8	0.012	0.039	0.254	0.071	NA	25	0
Rutabagas, BLD, DRND	2	0	0.24	0.2	18.8	0.082	0.041	0.715	0.102	0.155	15	0
Sauerkraut, CND, w/ liquid	18	0	0	13	14.7	0.021	0.022	0.143	0.13	0.093	24	0
Shallots, raw	4	0	0.04	0.8	8	0.06	0.02	0.2	0.345	0.29	34	0
Soybeans, green, BLD, DRND	156	0	NV	NV	17	0.26	0.155	1.25	0.06	0.128	111	0
Spinach, BLD, DRND	10,481	0	2.08	494	9.8	0.095	0.236	0.49	0.242	0.145	146	0
Spinach, raw	9377	0	2.03	483	28.1	0.078	0.189	0.724	0.195	0.065	194	0
Squash, acorn, BKD	428	0	NV	NV	10.8	0.167	0.013	0.881	0.194	0.504	19	0
Squash, butternut, BKD	11,155	0	1.29	1	15.1	0.072	0.017	0.969	0.124	0.359	19	0
Squash, hubbard, BKD	6705	0	0.2	1.6	9.5	0.074	0.047	0.558	0.172	0.447	16	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Squash, spaghetti, BLD, DRND/BKD	110	0	0.12	0.8	3.5	0.038	0.022	0.81	0.099	0.355	8	0
Squash, summer, BLD, DRND	85	0	0.12	3.5	10.8	0.051	0.025	0.464	0.085	0.079	21	0
Squash, zucchini, BLD, DRND	1117	0	0	4.2	12.9	0.035	0.024	0.51	0.08	0.288	28	0
Succotash (corn and lima beans), BLD, DRND	294	0	NV	NV	8.2	0.168	0.096	1.327	0.116	0.567	33	0
Sweetpotato leaves, STMD	2939	0	0.96	109	1.5	0.112	0.267	1.003	0.16	0.2	49	0
Sweetpotato, BKD in skin, flesh	19,218	0	0.71	2.3	19.6	0.107	0.106	1.487	0.286	0.884	6	0
Taro leaves, STMD	4238	0	NV	NV	35.5	0.139	0.38	1.267	0.072	0.044	48	0
Taro shoots, CKD	51	0	NV	NV	18.9	0.038	0.053	0.81	0.112	0.076	3	0
Taro, COOKED	84	0	2.93	1.2	5	0.107	0.028	0.51	0.331	0.336	19	0
Tomato juice, CND	450	0	0.32	2.3	70.1	0.1	0.078	0.673	0.07	NV	20	0
Tomato paste, CND	1525	0	0	11.4	21.9	0.06	0.153	3.076	0.216	0.142	12	0
Tomato sauce, CND	435	0	1.44	2.8	7	0.024	0.065	0.991	0.098	0.309	9	0
Tomatoes, green, raw	642	0	0.38	10.1	23.4	0.06	0.04	0.5	0.081	0.5	9	0
Tomatoes, red, ripe, CND, STWD	172	0	0.83	2.4	7.9	0.046	0.035	0.714	0.017	0.114	5	0
Tomatoes, red, ripe, raw	833	0	0.54	7.9	13.7	0.037	0.019	0.594	0.08	0.089	15	0
Turnip greens, BLD, DRND	8612	0	2.13	415	18.2	0.05	0.065	0.486	0.067	0.083	33	0
Turnips, BLD, DRND	0	0	0.03	0.06	11.6	0.027	0.023	0.299	0.067	0.142	9	0
Waterchestnuts, Chinese, CND	0	0	0.5	0.2	1.3	0.011	0.024	0.36	0.159	0.221	6	0
Watercress, raw	3191	0	1	250	43	0.09	0.12	0.2	0.129	0.31	9	0
Winged beans, BLD, DRND	0	0	NV	NV	0	0.295	0.129	0.83	0.047	0.156	10	0
Yam, BLD, DRND, BKD	122	0	0.34	2.3	12.1	0.095	0.028	0.552	0.228	0.311	16	0
Yardlong bean, BLD, DRND	450	0	NV	NV	16.2	0.085	0.099	0.63	0.024	0.051	45	0

Fruits and Fruit Juices												
Apple juice, CND/ BTLD, w/o added vitamin C	1	0	0.01	0	0.9	0.021	0.017	0.73	0.081	0.049	0	0
Apples, raw, w/skin	54	0	0.18	2.2	4.6	0.017	0.026	0.091	0.041	0.061	3	0
Applesauce, CND, w/o added vitamin C	29	0	0.16	0.5	21.2	0.026	0.03	0.084	0.027	0.041	3	0
Apricot nectar, CND, w/o added vitamin C	1316	0	0.13	1.2	0.6	0.009	0.014	0.26	0.022	0.096	1	0
Apricots, dehyd	12,669	0	NV	NV	9.5	0.043	0.148	3.58	0.52	1.067	4	0
Apricots, raw	1926	0	0	3.3	10	0.03	0.04	0.6	0.054	0.24	9	0
Avocados, raw	146	0	2.07	21	10	0.067	0.13	1.738	0.257	1.389	24	0
Bananas, raw	64	0	0	0.05	8.7	0.031	0.073	0.665	367	0.334	20	0
Blackberries, raw	214	0	0	19.8	21	0.02	0.026	0.646	0.03	0.276	25	0
Blueberries, raw	54	0	0.57	19.3	9.7	0.037	0.041	0.418	0.052	0.124	6	0
Cantaloupes, raw	3382	0	0.05	2.5	36.7	0.041	0.019	0.734	0.072	0.105	21	0
Casaba melons, raw	0	0	0.05	2.5	21.8	0.015	0.031	0.232	0.163	0.084	8	0
Cherries, sour, red, raw	1283	0	0.07	2.1	10	0.03	0.04	0.4	0.044	0.143	8	0
Cherries, sweet, raw	64	0	0.07	2.1	7	0.027	0.033	0.154	0.049	0.199	4	0
Crabapples, raw	40	NV	NV	NV	8	0.03	0.02	0.1	NV	NV	NV	0
Cranberries, raw	63	0	1.32	5	14	0.012	0.02	0.101	0.057	0.295	1	0
Cranberry sauce, CND	33	0	0.93	1.4	1	0.015	0.021	0.1	0.014	NV	1	0
Currants, European black, raw	230	NV	1	NV	181	0.05	0.05	0.3	0.066	0.398	NV	0
Custard apple (bullock's heart), raw	33	NV	NV	NV	19.2	0.08	0.1	0.5	0.221	0.135	NV	0
Elderberries, raw	600	NV	NV	NV	36	0.07	0.06	0.5	0.23	0.14	6	0
Figs, dried, uncooked	10	0	0.35	15.6	1.2	0.085	0.082	0.619	0.106	0.434	9	0
Figs, raw	142	0	0.11	4.7	2	0.06	0.05	0.4	0.113	0.3	6	0
Fruit cocktail, CND, water PK, w/liquids	250	0	0.4	2.6	2.1	0.016	0.011	0.363	0.052	0.062	3	0
Gooseberries, raw	290	NV	0.37	NV	27.7	0.04	0.03	0.3	0.08	NV	6	0
Grape juice, CND/ BTLD, w/o added vitamin C	8	0	0	0.4	0.1	0.017	0.015	0.133	0.032	0.048	0	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Grapefruit juice, CND~	35	0	0.04	0	28.3	0.042	0.02	0.231	0.02	0.13	10	0
Grapefruit, raw, pink/red/white	927	0	0.13	0	34.4	0.036	0.02	0.25	0.042	0.283	10	0
Grapes, adherent skin type, raw	100	0	0.19	14.6	4	0.092	0.057	0.3	0.11	0.024	4	0
Guavas, raw	624	0	0.73	2.6	228.3	0.067	0.04	1.084	0.11	0.451	49	0
Honeydew melons, raw	50	0	0.02	2.9	18	0.038	0.012	0.418	0.088	0.155	19	0
Jackfruit, raw	110	NV	0.34	NV	13.7	0.105	0.055	0.92	0.329	0.235	24	0
Kiwi fruit, raw	87	0	1.46	40.3	92.7	0.027	0.025	0.341	0.063	0.183	25	0
Kumquats, raw	290	0	0.15	0	43.9	0.037	0.09	0.429	0.036	0.208	17	0
Lemon juice, CND/BTLD	33	0	0.23	0.1	14.3	0.021	0.017	0.18	0.037	0.08	9	0
Lemons, raw, w/o peel	22	0	0.15	0	53	0.04	0.02	0.1	0.08	0.19	11	0
Lime juice, CND/BTLD	16	0	0.12	0.5	6.4	0.033	0.003	0.163	0.027	0.066	8	0
Litchis, raw	0	0	0.7	0.4	71.5	0.011	0.065	0.603	0.1	NV	14	0
Mangos, raw	1082	0	0.9	4.2	36.4	0.028	0.038	0.669	0.119	0.197	43	0
Nectarines, raw	332	0	0.77	2.2	5.4	0.034	0.027	1.125	0.025	0.158	5	0
Olives, ripe, CND	403	0	1.65	1.4	0.9	0.003	0	0.037	0.009	0.015	0	0
Orange juice, including from concentrate	42	0	0.2	0	33.6	0.046	0.039	0.28	0.076	0.195	19	0
Oranges, raw	247	0	0.15	0	59.1	0.068	0.051	0.425	0.079	0.261	34	0
Papayas, raw	950	0	0.3	2.6	60.9	0.023	0.027	0.357	0.038	0.191	37	0
Passion fruit, purple, raw	717	NV	0.01	NV	29.8	0	0.131	1.46	0.05	NV	7	0
Peaches, CND, water PK, w/liquids	532	0	0.49	1.7	2.9	0.009	0.019	0.521	0.019	0.05	3	0
Peaches, raw	326	0	0.73	2.6	6.6	0.024	0.031	0.806	0.025	0.153	4	0
Pears, CND, water PK, w/liquids	0	0	0.08	0.3	1	0.008	0.01	0.054	0.014	0.022	1	0
Pears, raw	25	0	0.12	4.4	4.3	0.012	0.026	0.161	0.029	0.049	7	0
Pineapple, CND, water PK, w/liquids	38	0	0.1	0.3	7.7	0.093	0.026	0.298	0.074	0.1	5	0

Pineapple, raw	58	0	0.02	0.7	47.8	0.079	0.032	0.5	0.112	0.213	18	0
Plantains, CKD	909	0	0.13	0.7	10.9	0.046	0.052	0.756	0.24	0.233	26	0
Plums, raw	345	0	0	6.4	9.5	0.028	0.026	0.417	0.029	0.135	5	0
Pomegranates, raw	0	0	0.6	16.4	10.2	0.067	0.053	0.293	0.175	0.377	38	0
Prickly pears, raw	43	NV	NV	NV	14	0.014	0.06	0.46	0.06	NV	6	0
Prunes, CND, HVY syrup, w/liquids	797	0	NV	NV	2.8	0.034	0.122	0.866	0.203	0.1	0	0
Prunes, DEHYD, STWD	523	0	NV	NV	0	0.046	0.03	0.985	0.191	0.108	0	0
Prunes, DEHYD, UNCKD	1762	0	NV	NV	0	0.118	0.165	2.995	0.745	0.418	2	0
Quinces, raw	40	NV	NV	NV	15	0.02	0.03	0.2	0.04	0.081	3	0
Raisins, golden seedless	0	0	0.12	3.5	3.2	0.008	0.191	1.142	0.323	0.14	3	0
Raisins, seedless	0	0	0.12	3.5	2.3	0.106	0.125	0.766	0.174	0.095	5	0
Raspberries, raw	33	0	0.87	7.8	26.2	1.032	0.038	0.598	0.055	0.329	21	0
Rhubarb, FRZ, CKD	73	0	1.9	21.1	3.3	0.018	0.023	0.2	0.02	0.05	5	0
Strawberries, CND, heavy syrup, w/liquids	26	0	0.19	1.5	31.7	0.021	0.034	0.057	0.049	0.179	28	0
Strawberries, raw	12	0	0.29	2.2	58.8	0.024	0.022	0.386	0.047	0.125	24	0
Tangerines, raw	681	0	0.2	0	26.7	0.058	0.036	0.376	0.078	0.216	16	0
Watermelon, raw	569	0	0.05	0.1	8.1	0.033	0.021	0.178	0.045	0.221	3	0
Beans and Peas												
Black beans, BLD	6	0	0.87	3.3	0	0.244	0.059	0.505	0.069	0.242	149	0
Chickpeas, BLD	27	0	0.35	4	1.3	0.116	0.063	0.526	0.139	0.286	172	0
Cowpeas (blackeyes), BLD	791	0	0.22	26.6	2.2	0.101	0.148	1.403	0.065	0.154	127	0
Falafel	13	0	NV	NV	1.6	0.146	0.166	1.044	0.125	0.292	78	0
French beans, BLD	3	0	NV	NV	1.2	0.13	0.062	0.546	0.105	0.222	75	0
Great northern beans, BLD	1	0	NV	NV	1.3	0.158	0.059	0.681	0.117	0.266	102	0
Humus	30	0	NV	NV	0	0.18	0.064	0.502	0.2	0.132	83	0
Kidney beans, BLD	2	0	NV	NV	35.6	0.362	0.273	3.024	0.093	0.381	47	0
Lentils, BLD	8	0	0.11	1.7	1.5	0.169	0.073	1.06	0.178	0.638	181	0
Lima beans, BLD	0	0	0.18	2	0	0.161	0.055	0.421	0.161	0.422	83	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Lupins, BLD	7	0	NV	NV	1.1	0.134	0.053	0.495	0.009	0.188	59	0
Mung beans, BLD	24	0	0.51	2.7	1	0.164	0.061	0.577	0.067	0.41	159	0
Navy beans, BLD	0	0	0.01	0.6	0.9	0.237	0.066	0.649	0.138	NV	NV	0
Peanut butter, smooth style	0	0	9.1	0.3	0	0.15	0.192	13.11	0.441	1.137	87	0
Peanuts, BLD	0	0	4.1	0	0	0.259	0.063	5.259	0.152	0.825	75	0
Peanuts, dry-roasted	0	0	4.93	0	0	0.152	0.197	14.36	0.466	1.011	97	0
Peanuts, oil-roasted	0	0	6.94	0	0.8	0.085	0.089	13.83	0.461	1.202	120	0
Peas, split, BLD	7	0	0.03	5	0.4	0.19	0.056	0.89	0.048	0.595	65	0
Pigeon peas (red gram), BLD	3	0	NV	NV	0	0.146	0.059	0.781	0.05	0.319	111	0
Pinto beans, BLD	0	0	0.94	3.5	0.8	0.193	0.062	0.318	0.229	0.21	172	0
Refried beans, canned	NV	NV	0.09	NV	NV	0.04	0.015	0.37	0.107	0.175	NV	NV
Soy flour, full-fat, RSTD	122	0	1.98	71	0	0.412	0.941	3.286	0.351	1.209	227	0
Soybeans, RSTD	0	0	NV	NV	2.2	0.1	0.145	1.41	0.208	0.453	211	0
Tempeh	0	0	NV	NV	0	0.078	0.358	2.64	0.215	0.278	24	0.08
Tofu, raw	166	0	NV	NV	0.2	0.158	0.102	0.381	0.092	0.133	29	0
Winged beans, BLD	0	0	NV	NV	0	0.295	0.129	0.83	0.047	0.156	10	0
Yardlong beans, BLD	450	0	NV	NV	16.2	0.085	0.099	0.63	0.024	0.051	45	0
Nuts												
Acorns, dried	0	NV	NV	NV	0	0.149	0.154	2.406	0.695	0.94	115	0
Almonds, dry RSTD, unblanched	1	0	23.9	0	0	0.077	1.117	3.637	1.36.136	0.321	55	0
Brazilnuts, dried, unblanched	0	0	5.65	0	0.7	0.617	0.035	0.295	0.101	0.184	22	0
Butternuts, dried	124	0	NV	NV	3.2	0.383	0.148	1.045	0.56	0.633	66	0
Cashew nuts, dry RSTD	0	0	0.92	34.7	0	0.2	0.2	1.4	0.256	1.27	69	0
Chestnuts, European, RSTD	24	0	0.5	7.8	26	0.243	0.175	1.342	0.497	0.554	70	0
Coconut meat, dried, flaked	0	0	0	0	0	0.015	0.015	0.697	0.03	0.14	3	0
Coconut meat, raw	0	0	0.24	0.2	3.3	0.066	0.02	0.54	0.054	0.3	26	0

Coconut milk, CND	0	0	NV	NV	1	0.022	0	0.637	0.028	0.153	14	0
Coconut water	0	0	0	0	2.4	0.03	0.057	0.08	0.032	0.043	3	0
Filberts (hazelnuts), dry RSTD	61	0	15.28	NV	3.8	0.338	0.123	2.05	0.62	0.923	88	0
Pecans, dry RSTD	140	0	1.3	NV	0.7	0.45	0.107	1.167	0.187	0.703	16	0
Pine nuts, pinyon, dried	1	0	NV	NV	2	1.243	0.223	4.37	0.111	0.21	58	0
Pistachio nuts, dry RSTD	266	0	2.17	13.2	3	0.695	0.234	1.373	1.122	0.513	51	0
Pumpkin and squash seeds, WHL, RSTD	62	0	NV	NV	0.3	0.034	0.052	0.286	0.037	0.056	9	0
Sunflower kernels, dried	50	0	35.17	0	1.4	1.48	0.355	8.335	1.345	1.13	227	0
Tahini, from RSTD and TSTD sesame kernels	67	0	0.25	0	0	1.22	0.473	5.45	0.149	0.693	98	0
Walnuts, black, dried	40	0	2.08	2.7	1.7	0.057	0.13	0.47	0.583	1.66	31	0
Poultry												
Chicken, DK meat w/ skin, fried w/ batter	103	NV	NV	NV	0	0.117	0.218	5.607	0.25	0.953	9	0.27
Chicken, DK meat w/ skin, RSTD	201	NV	NV	NV	0	0.066	0.207	6.359	0.31	1.111	7	0.29
Chicken, giblets, simmered	9536	NV	NV	NV	5.5	0.09	1.047	4.971	0.41	2.971	367	9.48
Chicken, LT meat w/ skin, fried w/ batter	79	NV	NV	NV	0	0.113	0.147	9.156	0.39	0.794	6	0.28
Chicken, LT meat w/ skin, RSTD	110	NV	NV	NV	0	0.06	0.118	11.13	0.52	0.926	3	0.32
Duck, meat only, RSTD	77	4	0.7	3.8	0	0.26	0.47	5.1	0.25	1.5	10	0.4
Duck, meat w/ skin, RSTD	210	3	0.7	5.1	0	0.174	0.269	4.825	0.18	1.098	6	0.3
Goose, meat only, RSTD	40	NV	NV	NV	0	0.092	0.39	4.081	0.47	1.834	12	0.49
Turkey, breast w/ skin, RSTD	0	NV	NV	NV	0	0.057	0.131	6.365	0.48	0.634	6	0.36
Turkey, DK meat only, RSTD	18	10	0.07	0	0	0.06	0.375	6.685	0.438	1.015	9	1.65
Turkey, meat only, RSTD	14	10	0.06	0	0	0.047	0.28	9.5	0.643	NV	NV	0.94
Turkey, meat w/ skin, RSTD	39	15	0.07	0	0	0.045	0.281	9.573	0.616	0.948	9	1.02

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Beef												
Brisket, 1/8″ fat, BRSD	0	16	0.19	1.9	0	0.06	0.2	3.27	0.26	0.31	7	2.4
Chuck (arm pot roast), 1/8″ fat, BRSD	0	13	0.53	1.9	0	0.061	0.178	4.273	0.295	0.594	9	2.22
Corned beef, CND	0	10	0.15	1.6	0	0.02	0.147	2.43	0.13	0.626	9	1.62
Dried beef, cured	0	1	0.38	1.3	0	0.065	0.163	5.164	0.386	0.566	10	1.59
Flank, BRSD	37	11	0.93	NV	0	0.022	0.085	3.41	0.118	0.129	NV	1.38
Ground, lean, BKD-MED	9	2	0.12	2.9	0	0.051	0.171	4.026	0.311	0.512	7	2.49
Ground, REG, BKD-MED	9	2	0.12	1.5	0	0.032	0.171	5.648	0.36	0.666	6	2.94
Ribs (10–12), 0″ fat, BRLD	0	NV	0.41	1.5	0	0.077	0.148	8.521	0.601	0.567	10	1.8
Round (eye of round), 0″ fat, RSTD	14	1	0.3	1.3	0	0.08	0.232	8.464	0.811	NV	NV	2.32
Round, full cut, 1/4″ fat, BRLD	0	5	0.14	1.5	0	0.1	0.22	4.26	0.4	0.41	10	3.17
Round, top round, 1/8″ fat, BRLD	0	6	0.43	1.5	0	0.07	0.163	5.36	0.403	0.605	10	1.71
Short loin (porterhouse steak), 0″ fat, BRLD	0	NV	0.18	NV	0	0.099	0.228	4.21	0.365	0.314	7	2.18
Short loin (top loin), 1/8″ fat, BRLD	0	NV	NV	NV	0	0.08	0.18	4.77	0.38	0.33	7	1.94
Tenderloin, 1/8″ fat, BRLD	0	NV	0.4	1.4	0	0.083	0.154	8.494	0.642	0.573	10	1.39
Top sirloin, 1/8″ fat, BRLD	0	9	0.44	1.6	0	0.073	0.13	7.176	0.564	0.531	8	1.59
Pork												
Bacon, Canadian style, pan fried	0	9	0.41	0.2	0	0.669	0.185	9.988	0.28	0.72	4	0.43
Bacon, BRLD/pan fried/ RSTD	37	1	0.31	0.1	0	0.404	0.264	11.1	0.349	1.171	2	1.23
Cured ham, boneless, ex lean (5% fat), RSTD	0	32	0.25	0	0	0.754	0.202	4.023	0.4	0.403	3	0.65
Cured ham, boneless, REG (11% fat), RSTD	0	32	0.31	0	0	0.73	0.33	6.15	0.31	0.72	3	0.7

Cured ham, ex lean (4% fat),CND	0	93	0.17	0	0	0.836	0.23	5.302	0.45	0.492	6	0.82
Cured ham, REG (13% fat), CND	0	NV	NV	NV	14	0.82	0.26	5.3	0.3	0.73	5	1.06
Leg (ham), lean, RSTD	9	36	0.26	0	0.4	0.69	0.349	4.935	0.45	0.67	12	0.72
Loin, blade (chops), bone-in, BRSD	13	39	0.2	0	0	0.486	0.316	7.381	0.488	0.944	0	0.62
Loin (tenderloin), RSTD	0	10	0.08	0	0	0.95	0.387	7.432	0.739	1.012	0	0.57
Loin, top loin (chop), boneless, BRSD	11	38	0.24	0	0	0.526	0.238	9.905	0.537	1.03	0	0.67
Loin, top loin (roast), boneless, w/ fat, RSTD	4	20	0.11	0	0	0.547	0.232	7.104	0.692	0.674	0	0.58
Loin, lean, BRLD	7	37	0.27	0	0.7	0.923	0.338	5.243	0.492	0.729	6	0.72
Shoulder (arm picnic), lean, RSTD	0	35	0.26	0	0	0.727	0.226	4.798	0.37	0.654	4	1.11
Shoulder, lean, RSTD	0	34	0.26	0	0	0.68	0.254	5.02	0.47	0.498	4	0.7
Spareribs, w/ fat, BRSD	10	104	0.34	0	0	0.408	0.382	5.475	0.35	0.75	4	1.08
Sausages and Luncheon Meats												
Bologna, beef	90	28	0.56	2.4	15.2	0.03	0.065	2.321	0.157	0.385	3	1.19
Bologna, pork	0	56	0.26	0.3	0	0.523	0.157	3.9	0.27	0.72	5	0.93
Bologna, Turkey	32	26	0.45	0.3	13.3	0.049	0.095	2.607	0.243	0.466	9	0.23
Bratwurst, pork, CKD	6	44	0.26	3.4	0	0.459	0.307	4.795	0.327	0.666	3	0.73
Frankfurter, beef	0	38	0.2	1.8	NV	0.017	0.05	1.827	0.193	NV	10	1.44
Frankfurter, chicken	0	21	0.22	0	0	0.057	0.257	4.687	0.323	1.06	4	0.54
Frankfurter, Turkey	0	23	0.62	0	0	0.036	0.181	3.68	0.143	0.56	7	0.82
Ham, chopped, CND	0	24	0.25	0	2	0.535	0.165	3.2	0.32	0.28	1	0.7
Ham, sliced, REG (11% fat)	0	29	0.08	0	4	0.626	0.178	2.904	0.329	0.435	7	0.42
Italian sausage, pork, CKD	16	41	0.25	3.4	0.1	0.623	0.233	4.165	0.33	NV	5	1.3
Kielbasa, grilled	32	35	0.38	0	14.7	0.15	0.212	3.727	0.097	NV	NV	0.72
Knockwurst, pork and beef	0	44	0.57	1.6	0	0.342	0.14	2.734	0.17	0.32	2	1.18
Olive loaf, pork	200	44	0.25	3.4	0	0.295	0.26	1.835	0.23	0.77	2	1.26
Pastrami, Turkey	12	10	0.22	0	8.1	0.055	0.25	3.527	0.27	0.58	5	0.24

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Polish sausage, pork	0	NV	NV	NV	1	0.502	0.148	3.443	0.19	0.45	2	0.98
Salami, beef	0	48	0.19	1.3	0	0.103	0.189	3.238	0.18	0.95	2	3.06
Salami, beef and pork	0	41	0.22	3.2	0	0.367	0.357	6.053	0.459	1.201	3	1.52
Smoked link sausage, pork	0	43	0.25	0	0	0.212	0.18	4.807	0.179	0.536	1	0.66
Smoked link sausage, pork and beef	74	44	0.13	0	0	0.192	0.106	2.94	0.163	0.525	2	0.58
Turkey breast meat	6	NV	NV	NV	0	0.058	0.115	5.2	0.48	0.621	7	0.42
Turkey ham	0	2	0.39	0	0	0.05	0.25	3.53	0.23	NV	6	0.26
Vienna sausage, beef and pork, CND	0	25	0.22	1.6	0	0.087	0.107	1.613	0.12	0.35	4	1.02
Fish and Seafood												
Abalone, fried~	5	NV	NV	NV	1.8	0.22	0.13	1.9	0.15	2.87	5	0.69
Anchovy, CND in oil, DRND	40	69	3.33	12.1	0	0.078	0.363	19.9	0.203	0.909	13	0.88
Carp, dry heat CKD	32	NV	NV	NV	1.6	0.14	0.07	2.1	0.219	NV	NV	1.47
Catfish, channel, breaded and fried	28	NV	NV	NV	0	0.073	0.133	2.282	0.19	0.73	17	1.9
Caviar, black/red	905	117	1.89	0.6	0	0.19	0.62	0.12	0.32	3.5	50	20
Clams, breaded and fried	302	NV	NV	NV	10	0.1	0.244	2.064	0.06	0.43	18	40.27
Cod, Atlantic, dry heat CKD	47	46	0.81	0.1	1	0.088	0.079	2.513	0.283	0.18	8	1.05
Cod, Atlantic, dried and salted	140	161	2.84	0.4	3.5	0.268	0.24	7.5	0.864	1.675	25	10
Crab, Alaska king, moist heat CKD	29	NV	NV	NV	7.6	0.053	0.055	1.34	0.18	0.4	51	11.5
Crab, blue, moist heat CKD	2	0	1.84	0.3	3.3	0.023	0.093	2.747	0.156	0.997	51	3.33
Crayfish, moist heat CKD	50	0	1.5	0.1	0.9	0.05	0.085	2.28	0.076	0.58	44	2.15
Eels, dry heat CKD	3787	NV	NV	NV	1.8	0.183	0.051	4.487	0.077	0.28	17	2.89
Flatfish (flounder/sole), dry heat CKD	37	139	0.77	0.1	0	0.026	0.025	1.278	0.115	0.227	6	1.31

Gefilte fish	89	NV	NV	NV	0.8	0.065	0.059	1	0.08	0.2	3	0.84
Haddock, dry heat CKD	62	23	0.55	0.1	0	0.023	0.069	4.119	0.327	0.494	13	2.13
Halibut, dry heat CKD	73	231	0.74	0	0	0.058	0.036	7.911	0.632	0.416	14	1.27
Herring, dry heat CKD	120	214	1.37	0.1	0.7	0.112	0.299	4.124	0.348	0.74	12	13.14
Herring, kippered	135	86	1.54	0.1	1	0.126	0.319	4.402	0.413	0.88	14	18.7
Herring, pickled	860	113	1.71	0.2	0	0.036	0.139	3.3	0.17	0.081	2	4.27
Lobster, Maine, moist heat CKD	4	1	1	0	0	0.023	0.017	1.83	0.119	1.667	11	1.43
Mackerel, dry heat CKD	180	NV	NV	NV	0.4	0.159	0.412	6.85	0.46	0.99	2	19
Mackerel, jack, CND, DRND	433	292	1.03	0.1	0.9	0.04	0.212	6.18	0.21	0.305	5	6.94
Mussel, blue, moist heat CKD	304	NV	NV	NV	13.6	0.3	0.42	3	0.1	0.95	76	24
Ocean perch, dry heat CKD	44	58	0.91	0.1	0	0.046	0.057	1.215	0.084	0.329	10	1.72
Oyster, breaded and fried	302	NV	NV	NV	3.8	0.15	0.202	1.65	0.064	0.27	14	15.63
Oyster, canned	300	1	0.85	0.1	5	0.15	0.166	1.244	0.095	0.18	9	19.13
Oyster, raw	270	NV	NV	NV	8	0.067	0.233	2.01	0.05	0.5	10	16
Perch, dry heat CKD	32	NV	NV	NV	1.7	0.08	0.12	1.9	0.14	0.87	6	2.2
Pike, northern, dry heat CKD	81	NV	NV	NV	3.8	0.067	0.077	2.8	0.135	0.87	17	2.3
Pollock, walleye, dry heat CKD	81	NV	NV	NV	0	0.312	0.195	2.801	0.138	0.865	17	2.31
Roe, mixed species, raw	299	484	7	0.2	16	0.24	0.74	1.8	0.16	1	80	10
Salmon, Chinook, smoked	87	685	1.35	0.1	0	0.023	0.101	4.72	0.278	0.87	2	3.26
Salmon, coho, wild, moist heat CKD	108	NV	NV	NV	1	0.115	0.159	7.779	0.556	0.834	9	4.48
Salmon, pink, CND, w/ bone and liquid	55	NV	NV	NV	0	0.023	0.186	6.536	0.3	0.55	15	4.4
Salmon, sockeye, dry heat CKD	193	670	0.99	0.1	0	0.157	0.246	10.12	0.827	1.274	7	4.47
Sardine, CND in oil, DRND	108	193	2.04	2.6	0	0.08	0.227	5.245	0.167	0.642	10	8.94
Scallops, breaded and fried	75	NV	NV	NV	2.3	0.042	0.11	1.505	0.14	0.2	18	1.32

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Sea bass, dry heat CKD	213	NV	NV	NV	0	0.13	0.15	1.9	0.46	0.87	6	0.3
Shark, battered and fried	180	NV	NV	NV	0	0.072	0.097	2.783	0.3	0.62	5	1.21
Shrimp, breaded and fried	189	5	1.3	1	1.5	0.129	0.136	3.07	0.098	0.35	23	1.87
Shrimp, moist heat CKD	301	4	2.2	0.4	0	0.032	0.024	2.678	0.242	0.519	24	1.66
Smelt, rainbow, dry heat CKD	58	NV	NV	NV	0	0.01	0.146	1.766	0.17	0.74	5	3.97
Snapper, dry heat CKD	115	NV	NV	NV	1.6	0.053	0.004	0.346	0.46	0.87	6	3.5
Squid, fried	35	NV	NV	NV	4.2	0.056	0.458	2.602	0.058	0.51	5	1.23
Surimi	67	NV	0.63	0.1	0	0.02	0.021	0.22	0.03	0.07	2	1.6
Swordfish, dry heat CKD	129	666	2.41	0.1	0	0.089	0.063	9.254	0.615	0.417	2	1.62
Trout, rainbow, dry heat CKD	50	NV	NV	NV	2	0.152	0.097	5.77	0.346	1.065	19	6.3
Tuna, bluefin, dry heat CKD	2520	NV	NV	NV	0	0.278	0.306	10.54	0.525	1.37	2	10.88
Tuna, light, CND in water, DRND	57	47	0.33	0.2	0	0.03	0.084	10.14	0.319	0.148	4	2.55
Whiting, dry heat CKD	100	57	0.3	0.1	0	0.056	0.046	1.3	0.156	0.326	13	2.3
Dairy Products												
Butter	2499	0	2.32	7	0	0.005	0.034	0.042	0.003	0.11	3	0.17
Cheese, blue	721	21	0.25	2.4	0	0.029	0.382	1.016	0.166	1.729	36	1.22
Cheese, Brie	592	20	0.24	2.3	0	0.07	0.52	0.38	0.235	0.69	65	1.65
Cheese, Camembert	820	0.4	0.21	2	0	0.028	0.488	0.63	0.227	1.364	62	1.3
Cheese, Cheddar	1242	24	0.71	2.4	0	0.029	0.428	0.059	0.066	0.41	27	1.1
Cheese, Colby	994	24	0.28	2.7	0	0.015	0.375	0.093	0.079	0.21	18	0.83
Cheese, cottage, 1% fat	41	0	0.01	0.1	0	0.021	0.165	0.128	0.068	0.215	12	0.63
Cheese, cottage, creamed	140	3	0.08	0	0	0.027	0.163	0.099	0.046	0.557	12	0.43
Cheese, cream	1111	0	0.86	2.1	0	0.023	0.23	0.091	0.056	0.517	9	0.22

Cheese, cream, fat free	8	0	0.01	0	0	0.023	0.226	0.044	0.016	0.446	9	0.46
Cheese, Edam	825	20	0.24	2.3	0	0.037	0.389	0.082	0.076	0.281	16	1.54
Cheese, feta	422	16	0.18	1.8	0	0.154	0.844	0.991	0.424	0.967	32	1.69
Cheese, Gouda	563	20	0.24	2.3	0	0.03	0.334	0.063	0.08	0.34	21	1.54
Cheese, Gruyere	984	24	0.28	2.7	0	0.06	0.279	0.106	0.081	0.562	10	1.6
Cheese, Monterey	769	22	0.26	2.5	0	0.015	0.39	0.093	0.079	0.21	18	0.83
Cheese, mozzarella, skim milk	481	12	0.14	1.6	0	0.018	0.303	0.105	0.07	0.079	9	0.82
Cheese, mozzarella, whole milk	676	16	0.19	2.3	0	0.03	0.283	0.104	0.037	0.141	7	2.28
Cheese, Muenster	1012	22	0.26	2.5	0	0.013	0.32	0.103	0.056	0.19	12	1.47
Cheese, Parmesan	974	21	0.53	1.7	0	0.026	0.358	0.08	0.081	0.45	6	1.4
Cheese, American	705	NV	NV	NV	0	0.03	0.446	0.074	0.014	0.977	5	1.28
Cheese, Provolone	880	20	0.23	2.2	0	0.019	0.321	0.156	0.073	0.476	10	1.46
Cheese, Ricotta, skim milk	384	6	0.07	0.7	0	0.021	0.185	0.078	0.02	0.242	13	0.29
Cheese, Ricotta, whole milk	445	10	0.11	1.1	0	0.013	0.195	0.104	0.043	0.213	12	0.34
Cheese, Swiss	1047	0	0.6	1.4	0	0.011	0.302	0.064	0.071	0.353	10	3.06
Cream, half and half	354	2	0.25	1.3	0.9	0.03	0.194	0.109	0.05	0.539	3	0.19
Cream, light, coffee/ table	656	44	0.12	1.7	0.8	0.023	0.19	0.09	0.044	0.44	2	0.14
Cream, sour	447	0	0.38	1.5	0.9	0.02	0.168	0.093	0.041	0.472	6	0.21
milk, buttermilk, low fat	47	1	0.05	0.1	1	0.034	0.154	0.058	0.034	NV	NV	0.22
Milk, CND, Evap, whole, w/o added vitamin A	239	6	0.16	0.6	1.9	0.047	0.316	0.194	0.05	0.638	8	0.16
Milk, dry, skim, w/o added vitamin A	22	0	0	0.1	6.8	0.415	1.55	0.951	0.361	3.568	50	4.03
Milk, goat	198	51	0.07	0.3	1.3	0.048	0.138	0.277	0.046	0.31	1	0.07
Milk, human	212	3	0.08	0.3	5	0.014	0.036	0.177	0.011	0.223	5	0.05
Milk, 1% fat, w/ added vitamin A	204	40	NV	NV	1	0.04	0.173	0.09	0.045	0.336	5	0.38
Milk, 2% fat, w/ added vitamin A	75	NV	NV	NV	1.1	0.045	0.194	0.101	0.046	0.339	5	0.39

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Milk, skim, w/ added vitamin A	204	47	0.01	0	0	0.045	0.182	0.094	0.037	0.357	5	0.5
Milk, whole (3.3% fat)	162	2	0.07	0.3	0	0.046	0.169	0.089	0.036	0.373	5	0.45
Yogurt, plain, whole milk	15	0	0.01	0	0	0.023	0.278	0.208	0.063	0.331	5	0.75
Eggs												
Egg, white, dried	0	0	0	0	0	0.005	2.53	0.865	0.036	0.775	18	0.18
Egg, white, raw	0	0	0	0	0	0.004	0.439	0.105	0.005	0.19	4	0.09
Egg, whole, hard-boiled	520	87	1.03	0.3	0	0.066	0.513	0.064	0.121	1.398	44	1.11
Egg, whole, fried	787	88	1.31	5.6	0	0.044	0.495	0.082	0.184	1.66	51	0.97
Egg, whole, raw	540	82	1.05	0.3	0	0.04	0.457	0.075	0.17	1.533	47	0.89
Egg, yolk, raw	1442	218	2.58	0.7	0	0.176	0.528	0.024	0.35	2.99	146	1.95
Fats and Oils												
Fat, chicken	0	191	2.7	0	0	0	0	0	0	0	0	0
Lard (pork fat)	0	102	0.6	0	0	0	0	0	0	0	0	0
Margarine, hard, corn/ soy/cottonseed (HYDR)	3577	NV	3.1	NV	0.2	0.01	0.037	0.023	0.009	0.084	1	0.1
Mayonnaise	0	0	11.79	24.7	0	0.01	0.06	0.01	0.01	NV	0	0
Oil, Canola	0	0	17.46	71.3	0	0	0	0	0	0	0	0
Oil, cocoa butter	0	NV	1.8	24.7	0	0	0	0	0	0	0	0
Oil, coconut	0	0	0.11	0.6	0	0	0	0	0	0	0	0
Oil, cod liver	100,000	10,000	NV	NV	0	0	0	0	0	0	0	0
Oil, corn	0	0	14.3	1.9	0	0	0	0	0	0	0	0
Oil, mustard	0	NV	NV	NV	0	0	0	0	0	0	0	0
Oil, olive	0	0	14.35	60.2	0	0	0	0	0	0	0	0
Oil, palm	0	NV	15.94	8	0	0	0	0	0	0	0	0
Oil, peanut	0	0	15.69	0.7	0	0	0	0	0	0	0	0
Oil, rice bran	0	NV	32.3	24.7	0	0	0	0	0	0	0	0
Oil, sesame	0	0	1.4	13.6	0	0	0	0	0	0	0	0
Oil, soybean	0	0	8.18	184	0	0	0	0	0	0	0	0

Oil, sunflower	0	NV	41.08	5.4	0	0	0	0	0	0	0	0
Oil, wheat germ	0	0	149.4	24.7	0	0	0	0	0	0	0	0
Salad dressing, 1000 island	213	0	4	69.1	0	1.445	0.058	0.418	0	0	0	0
Salad dressing, 1000 island, low fat	311	0	1	27.6	1.5	0.049	0.043	0.437	0	0	0	0
Salad dressing, French, low fat	541	0	1	17.8	4.8	0.024	0.052	0.467	0.055	0	2	0
Salad dressing, Italian, low fat	12	0	0	12.5	0	0.012	0.008	0.094	0.055	0	3	0
Salad dressing, Russian	577	0	3.32	53.7	6	0.029	0.046	0.594	0.097	0.4	5	0
Salad dressing, Russian, low fat	33	0	0.4	6.7	6	0.007	0.013	0.002	0.009	0.135	3	0.12
Shortening, soy bean and cottonseed oils (HYDR)	0	0	6.13	43	0	0	0	0	0	0	0	0
Tallow (beef fat)	0	28	2.7	0	0	0	0	0	0	0	0	0
Spices												
Allspice	540	0	NV	NV	39.2	0.101	0.063	2.86	0.21	NV	36	0
Anise seed	311	0	NV	NV	21	0.34	0.29	3.06	0.65	0.797	10	0
Basil	744	0	10.7	1714	0.8	0.08	1.2	4.9	1.34	0.838	310	0
Bay leaf	6185	0	NV	NV	46.5	0.009	0.421	2.005	1.74	NV	180	0
Caraway seed	363	0	2.5	0	21	0.383	0.379	3.606	0.36	NV	10	0
Cardamom	0	0	NV	NV	21	0.198	0.182	1.102	0.23	NV	NV	0
Celery seed	52	0	1.07	0	17.1	0.34	0.29	3.06	0.89	NV	10	0
Chili powder	29650	0	38.14	106	0.7	0.25	0.94	11.6	2.094	0.888	28	0
Cinnamon	295	0	2.32	31.2	3.8	0.022	0.041	1.332	0.158	0.358	6	0
Cloves	160	0	0	142	0.2	0.158	0.22	1.56	0.391	0.509	25	0
Coriander leaf, dried	5850	0	1.03	1359	566.7	1.252	1.5	10.71	0.61	NV	274	0
Coriander seed	0	0	NV	NV	21	0.239	0.29	2.13	NV	NV	0	0
Cumin seed	1270	0	3.33	5.4	7.7	0.628	0.327	4.579	0.435	NV	10	0
Curry powder	19	0	25.24	99.8	0.7	0.176	0.2	3.26	0.105	1.07	56	0
Dill seed	53	0	NV	NV	21	0.418	0.284	2.807	0.25	NV	10	0
Dill weed, dried	5850	0	NV	NV	50	0.418	0.284	2.807	1.71	NV	NV	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Fennel seed	135	0	NV	NV	21	0.408	0.353	6.05	0.47	NV	NV	0
Garlic powder	0	0	0.67	0.4	1.2	0.435	0.141	0.796	1.654	0.743	47	0
Ginger	30	0	0	0.8	0.7	0.046	0.17	9.62	0.626	0.477	13	0
Mace	800	0	NV	NV	21	0.312	0.448	1.35	0.16	NV	76	0
Marjoram, dried	8068	0	1.69	622	51.4	0.289	0.316	4.12	1.19	NV	274	0
Mustard seed, yellow	31	0	5.07	5.4	7.1	0.805	0.261	4.733	0.397	0.81	162	0
Nutmeg	102	0	0	0	3	0.346	0.057	1.299	0.16	NV	76	0
Oregano	1701	0	18.26	622	2.3	0.177	0.528	4.64	1.044	0.921	237	0
Paprika	42,954	0	29.1	80.3	0.9	0.33	1.23	10.06	2.141	2.51	49	0
Pepper, black	547	0	1.04	164	0	0.108	0.18	1.142	0.291	NV	NV	0
Pepper, red/cayenne	41,610	0	29.83	80.3	76.4	0.328	0.919	8.701	2.45	NV	106	0
Pepper, white	0	0	NV	NV	21	0.022	0.126	0.212	0.1	NV	10	0
Peppermint, fresh	4248	0	NV	NV	31.8	0.082	0.266	1.706	0.129	0.338	114	0
Poppy seed	0	0	1.77	0	1	0.854	0.1	0.896	0.247	0.324	82	0
Rosemary, dried	3128	0	NV	NV	61.2	0.514	0.428	1	1.74	NV	307	0
Saffron	530	0	NV	NV	80.8	0.115	0.267	1.46	1.01	NV	93	0
Sage	5900	0	7.48	17.1	32.4	0.754	0.336	5.72	2.69	NV	274	0
Savory	5130	0	NV	NV	50	0.366	NV	4.08	1.81	NV	NV	0
Spearmint, fresh	4054	0	NV	NV	13.3	0.078	0.175	0.948	0.158	0.25	105	0
Tarragon	4200	0	NV	NV	50	0.251	1.339	8.95	2.41	NV	274	0
Thyme	3800	0	7.48	1714	50	0.513	0.399	4.94	0.55	NV	274	0
Turmeric	0	0	4.43	13.4	0.7	0.058	0.15	1.35	0.107	0.542	20	0
Vanilla extract	0	0	0	0	0	0.011	0.095	0.425	0.026	0.035	0	0
Soups												
Bean w/ pork, CND	662	0	0.87	2.4	1.2	0.065	0.025	0.421	0.031	0.07	24	0.03
Black bean, CND	445	NV	0.36	1.8	0.2	0.042	0.039	0.41	0.07	0.16	66	0
Chicken broth, CND	0	0	0.01	0	0	0.006	0.046	2.23	0.02	0.04	4	0.2
Chicken gumbo, CND	98	NV	0.36	5.7	4	0.2	0.3	0.53	0.05	0.16	5	0.02
Chicken noodle, CND	410	0	0.05	0	0	0.079	0.07	1.222	0.042	0.107	5	0.12

Chicken w/ rice, CND	498	0	0.07	0.3	0	0.014	0.02	0.918	0.02	0.14	1	0.13
Clam chowder, Manhattan style, CND	762	NV	1.03	5.5	3.2	0.024	0.032	0.65	0.08	0.15	8	3.23
Clam chowder, New England style, CND	58	0	0.42	0.8	4.1	0.125	0.165	1.55	0.104	0.231	14	9.47
Cream of asparagus, CND	441	0	0.49	22	2.2	0.043	0.062	0.62	0.01	0.11	19	0.04
Cream of celery, CND	282	0	1.39	17.2	0.2	0.023	0.039	0.265	0.01	0.92	2	0.04
Cream of chicken, CND	182	0	0.54	4.1	0.1	0.013	0.046	0.392	0	0.192	2	0
Cream of mushroom, CND	8	9	0.5	19.6	0	0.012	0.016	0.345	0.015	0.148	2	0
Cream of onion, CND	113	0	0.43	2.2	1	0.04	0.06	0.4	0.02	0.24	6	0.04
Cream of potato, CND	68	0	0.07	1.1	0.2	0.028	0.029	0.43	0.03	0.7	2	0.04
Lentil w/ ham, CND	145	NV	NV	NV	1.7	0.07	0.045	0.545	0.09	0.14	20	0.12
Minestrone, CND	1708	0	0.46	7.8	0.9	0.044	0.036	0.77	0.08	0.28	13	0
Oyster stew, CND	58	NV	NV	NV	2.6	0.017	0.029	0.19	0.01	0.1	2	1.79
Pea, green, CND	25	0	0.18	0.4	1.3	0.082	0.052	0.943	0.04	0.1	1	0
Pea, split w/ ham, CND	331	NV	NV	NV	1.1	0.11	0.056	1.098	0.05	0.2	2	0.2
Tomato rice, CND	421	0	1.73	3	11.5	0.048	0.039	0.822	0.06	0.1	11	0
Tomato, CND	392	0	0.34	3.2	12.9	0.042	0.015	0.858	0.086	NV	0	0
Turkey noodle, CND	120	NV	NV	NV	0.1	0.03	0.026	0.572	0.015	0.07	1	0.06
Vegetable beef, CND	3104	0	0.48	5.6	1.9	0.029	0.039	0.823	0.06	0.28	8	0.25
Vegetarian vegetable, CND	2842	0	1.17	4.2	1.2	0.044	0.037	0.747	0.045	0.28	9	0
Beverages												
Beer, REG	0	0	0	0	0	0.005	0.025	0.513	0.046	0.041	6	0.02
Clam and tomato juice, CND	149	NV	0.11	NV	5	0.021	0.012	0.231	0.061	0.083	8	0.03
Cocoa mix, PDR	15	0	0.04	0.7	0	0.267	1.4	1.084	0.318	3.826	14	1.18
Coffee, brewed, espresso	0	0	0.01	0.1	0.2	0.001	0.177	5.207	0.002	0.028	1	0
Coffee, brewed, regular	0	NV	0	0	0	0	0.001	0.236	0	0.001	0	0
Distilled (Gin/rum/ vodka/whiskey), 80 proof	0	0	0	0	0	0.006	0.004	0.013	0.001	0	0	0
Sodas (ginger ale/grape/ orange)	0	0	0	0	0	0	0	0	0	0	0	0

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
Sodas, lemon–lime	0	0	0	0	0	0	0	0.015	0	0	0	0
Tea, brewed	0	0	0	0	0	0	0	0	0	0	0	0
Wine, table	0	0	0	0	0	0.005	0.023	0.166	0.054	0.037	1	0
Snack Foods and Desserts												
Banana chips	83	0	0.24	1.3	6.3	0.085	0.017	0.71	0.26	0.62	14	0
Candies, caramels	42	0	0.46	1.8	0.4	0.103	0.256	0.148	0.056	0.62	4	0.3
Candies, gumdrops	0	0	0	0	0	0.006	0.013	0.01	0.005	0.012	0	0
Candies, hard	0	0	0	0	0	0.004	0.003	0.007	0.003	0.008	0	0
Candies, jellybeans	0	0	0	0	0	0.004	0.011	0.008	0.004	0.009	0	0
Chocolates, milk	195	0	0.51	5.7	0	0.112	0.298	0.386	0.036	0.472	11	0.75
Chocolates, semisweet	0	0	0.26	5.6	0	0.055	0.09	0.427	0.035	0.105	13	0
Frosting, vanilla, creamy	0	0	1.53	13	0	0.01	0.302	0.22	0	0.055	8	0
Gelatins, PREPD w/ water	0	0	0	0	0	0	0.006	0.001	0	0.002	1	0
Jams and preserves	0	0	0.12	0	8.8	0.016	0.076	0.036	0.02	0.02	11	0
Jellies	5	0	0	0.3	0.9	0.001	0.026	0.036	0.02	0.197	2	0
Marmalade, orange	62	0	0.06	0	4.8	0.005	0.025	0.052	0.019	0.015	9	0
Marshmallows	0	0	0	0	0	0.001	0.001	0.078	0.003	0.005	1	0
Molasses	0	0	0	0	0	0.041	0.002	0.93	0.67	0.804	0	0
Popcorn, air-popped	196	0	0.29	1.2	0	0.104	0.083	2.308	0.157	0.51	31	0
Popcorn, cakes	72	0	0.29	1.1	0	0.075	0.178	6.006	0.181	0.434	18	0
Popcorn, caramel-coated, w/ peanuts	78	0	0.85	3.9	0	0.051	0.126	1.99	0.185	0.23	16	0
Popcorn, oil-popped	11	NV	NV	NV	0.3	0.134	0.136	1.55	0.209	0.305	17	0
Potato chips, plain	0	0	10.45	22.1	21.6	0.213	0.088	4.762	0.531	0.956	29	0
Potato chips, barbecue	393	0	4.42	16.1	62.4	0.221	0.119	4.957	0.375	0.85	64	0
Pretzels, hard	0	0	0.47	2.8	2.1	0.424	0.332	5.27	0.074	0.322	166	0
Pudding, chocolate	46	0	0.31	0.6	0.3	0.024	0.072	0.123	0.018	0.244	3	0.09
Pudding, lemon, PREPD w/ 2% fat milk	170	33	NV	NV	0.8	0.033	0.137	0.072	0.036	0.267	4	0.3

Pudding, rice, PREPD w/ whole milk	115	34	NV	NV	0.7	0.074	0.138	0.441	0.034	0.283	4	0.24
Pudding, tapioca, PREPD w/ whole milk	108	34	NV	NV	0.7	0.03	0.14	0.073	0.033	0.272	4	0.25
Rice cakes, brown rice, plain	0	0	1.24	1.9	0	0.061	0.165	7.806	0.15	1	21	0
Sugar, brown	0	0	0	0	0	0	0	0.11	0.041	0.132	1	0
Sugar, granulated	0	0	0	0	0	0	0.019	0	0	0	0	0
Syrup, choc, fudge-type	2	0	2.63	2.5	0.2	0.034	0.091	0.25	0.02	0.119	4	0.06
Syrup, corn, light	0	0	0	0	0	0.059	0	0	0	0	0	0
Syrup, maple	0	0	0	0	0	0.066	1.27	0.081	0.002	0.036	0	0
Syrup, sorghum~	0	0	0	0	0	0.1	0.155	0.1	0.67	0.804	0	0
Tortilla chips, plain	4	0	3.53	20.9	0	0.14	0.07	0.838	0.179	0.297	12	0.36
Tortilla chips, taco-flavored	905	NV	NV	NV	0.9	0.242	0.204	1.999	0.297	0.29	21	0
American Fast Foods												
Biscuit, w/ egg, cheese and bacon	294	21	1.02	4.2	0	0.269	0.247	2.35	0.113	0.772	8	0.86
Biscuit, w/ ham	118	13	1.23	5.7	0.1	0.45	0.28	3.08	0.12	0.36	7	0.03
Burrito, w/ beans and cheese	68	0	0.37	7.6	0.3	0.287	0.143	2.48	0.085	0.403	58	0.08
Burrito, w/ beans and meat	212	NV	0.64	6.8	0.8	0.393	0.236	2.889	0.09	0.008	17	NV
Cheeseburger, single patty, w/ condiments	254	2	0.52	4.2	0	0.293	0.363	5.217	0.228	0.545	42	0.76
Chicken fillet sandwich, w/ cheese	32	5	2.41	8.5	0.8	0.23	0.3	7.72	0.383	1.2	35	0.13
Chicken, boneless pieces, breaded and fried	16	7	1.12	7	0.6	0.092	0.204	5.985	0.148	1.182	6	0.33
Chili con carne	467	1	0.5	4.6	0.2	0.019	0.029	0.782	0.105	0.297	21	0.28
Croissant, w/ egg, cheese and ham	529	31	0.76	2.1	0.1	0.34	0.2	2.1	0.15	0.82	22	0.66
Enchilada, w/ cheese	195	NV	0.89	NV	NV	0.065	0.22	0.78	0.125	0.37	NV	0.72
English muffin, w/ egg, cheese and Canadian bacon	347	26	0.91	0.8	1.1	0.378	0.293	3.187	0.071	NV	20	0.81

Continued

Food, by Major Food Group	Vitamin A (IU)	Vitamin D (IU)	Vitamin E (mg)	Vitamin K (mg)	Vitamin C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B6 (mg)	Pantothenic Acid (mg)	Folate (µg)	Vitamin B12 (mg)
French toast sticks	5	0	0.88	14.5	0	0.284	0.175	2.575	0.053	0.336	142	0
Hamburger, single patty, w/ condiments	57	2	0.07	4.9	0.3	0.349	0.186	4.061	0.108	0.37	17	1.2
Hotdog, beef, plain	0	38	0.2	1.8	NV	0.017	0.05	1.827	0.193	NV	10	1.44
Hotdog, w/ chili	51	NV	NV	NV	2.4	0.19	0.35	3.28	0.04	0.48	44	0.26
Hotdog, w/ corn flour coating (corn dog)	166	15	0.6	5.8	0.5	0.141	0.155	2.786	0.117	0.406	7	0.48
Hush puppies	1	0	1.06	3.4	0	0.17	0.07	2.13	0.11	NV	29	0
Nachos, w/ cheese	21	0	4.08	19.3	1.1	0.123	0.133	0.63	0.215	0.38	10	0.07
Onion rings, breaded and fried	0	0	NV	NV	4.6	0.1	0.079	0.693	0.103	0.24	19	0
Pizza w/ cheese~	358	0	0.83	6.7	1.4	0.39	0.195	3.825	0.08	NV	40	0.42
Pizza w/ cheese, meat and vegetables	339	0	1.13	8.2	3.4	0.216	0.233	2.379	0.149	0.332	0	0.62
Pizza w/ pepperoni~	365	0	0.87	6.4	0.9	0.43	0.22	4.14	0.08	NV	36	0.5
Potato salad	100	NV	NV	NV	1.1	0.07	0.11	0.27	0.15	0.37	25	0.12
Potato, mashed	205	2	0.29	2.4	0.1	0.085	0.088	1.02	0.109	0.405	6	0.22
Potatoes, hashed brown	NV	0	0.07	0.4	4.9	0.027	0.022	1.777	0.257	0.555	NV	0
Roast beef sandwich, plain	8	1	0.43	2.8	0	0.217	0.287	4.343	0.215	NV	39	0.96
Submarine sandwich, w/ cold cuts	170	7	0.41	4.5	7.5	0.343	0.267	4.323	0.203	0.555	9	0.22
Submarine sandwich, w/ roast beef	77	1	0.29	4.5	0	0.23	0.257	4.183	0.275	0.415	9	0.44
Submarine sandwich, w/ tuna salad	78	21	1.57	22.4	0	0.19	0.217	7.727	0.271	0.35	18	1.06
Taco	159	3	0.25	7.4	0.1	0.18	0.13	2.88	0.06	NV	16	0.84
Taco salad	297	NV	NV	NV	1.8	0.05	0.18	1.24	0.11	0.68	20	0.32

BKD, baked; *BLD*, boiled; *BTLD*, bottled; *BRSD*, braised; *CND*, canned; *CKD*, cooked; *DRND*, drained; *ENR*, enriched; *HYDR*, hydrogenated; *NV*, no value available; *PREPD*, prepared; *RSTD*, roasted; *STMD*, steamed; *STWD*, stewed.
Nutrient Data Laboratory, Agricultural Research Service, US Department of Agriculture, Release 28 (Database contains no biotin data.)

Appendix E

Vitamin Contents of Feedstuffs (units per kg)

Feedstuff	Vitamin E	Riboflavin	Niacin	Vitamin B6	Biotin	Pantothenic Acid	Folate	Vitamin B12	Choline
	IU	mg	mg	mg	mg	mg	mg	ug	mg
Alfalfa leaf meal, dehydrated	140	15	55	11	0.35	33	4	NV	1600
Alfalfa meal, dehydrated	120	13	46	10	0.33	27	3.5	NV	1600
Alfalfa meal, sun-cured	66	11	40	9	0.3	20	3.3	NV	1500
Bakery product, dehydrated	25	0.8	50	4.4	0.07	9	0.15	NV	660
Barley	36	2	57	2.9	15	6.6	0.5	NV	1100
Beans, field	1	1.8	24	0.3	0.11	3.1	1.3	NV	NV
Blood meal	0	4.2	29	0	NV	5.3	NV	NV	280
Brewers' dried grains	26	1.5	44	0.66	NV	8.8	9.7	NV	1600
Buckwheat	NV	11	18	NV	NV	5.9	NV	NV	13,000
Buttermilk, dried	6.3	30	9	2.4	0.3	30	0.4	20	1800
Casein, purified	NV	1.5	1.3	0.4	NV	2.6	0.4	NV	200
Citrus pulp, dried	NV	2.2	22	NV	NV	13	NV	NV	900
Coconut oil	35	0	0	0	0	0	0	0	0
Coconut oil meal (copra meal)	NV	3.5	24	4.4	NV	6.6	0.3	NV	1100
Corn and cob meal	20	1.1	20	5	0.05	5	0.3	NV	550
Corn germ meal	87	3.7	42	NV	3	3.3	0.7	NV	1540
Corn gluten feed	24	2.2	66	NV	0.3	0.5	0.2	NV	1100
Corn gluten meal	42	1.5	50	8	0.15	10	0.7	NV	330
Corn gluten meal, 60% protein	50	1.8	60	9.6	0.2	12	0.84	NV	400
Corn oil	280	0	0	0	0	0	0	0	0
Corn, dent, no. 2, yellow	22	1.3	22	7	0.06	5.7	0.36	NV	620
Cottonseed meal, dehulled	NV	5.7	51	7	0.1	15	1.1	NV	3300
Cottonseed meal, hydraulic/expeller	40	4	5	5.3	0.1	11	1	NV	2800
Cottonseed meal, solvent	15	5	44	6.4	0.1	13	1	NV	2900
Crab meal	NV	5.9	44	NV	NV	6.6	NV	330	2000
Distillers' dried grains (corn)	30	3.1	42	NV	0.7	5.9	NV	NV	1900
Distillers' dried grains w/sol's (corn)	40	8.6	66	NV	1.1	11	0.9	NV	2500
Distillers' dried solubles (corn)	55	17	115	10	1.5	22	2.2	NV	4800
Feathers, poultry, hydrolyzed	NV	2	24	NV	44	11	0.22	70	900

Fish meal, anchoveta	3.4	6.6	64	3.5	0.26	8.8	0.2	100	3700
Fish meal, herring	27	9	89	3.7	0.42	11	0.24	240	4000
Fish meal, menhaden	9	4.8	55	3.5	0.26	8.8	0.2	88	3500
Fish meal, pilchard	9	9.5	55	3.5	0.26	9	0.2	100	2200
Fish meal, redfish waste	6	NV	NV	3.3	0.08	NV	0.2	100	3500
Fish meal, whitefish waste	9	9	70	3.3	0.08	8.8	0.2	100	2200
Fish oils, stabilized	70	0	0	0	0	0	0	0	0
Fish solubles, dried	6	7.7	230	NV	0.26	45	NV	400	5300
Hominy feed, yellow	NV	2.2	44	11	0.13	7.7	0.28	NV	1000
Lard, stabilized	23	0	0	0	0	0	0	0	0
Liver and glandular meal	NV	40	160	5	0.8	105	4	440	10,500
Meat and bone meal, 45% protein	1	5.3	38	2.3	0.1	2.4	0.05	44	2000
Meat and bone meal, 50% protein	1	4.4	49	2.5	0.14	3.7	0.05	44	2200
Meat meal, 55% protein	1	5.3	57	3	0.26	4.8	0.05	44	2200
Milk, dried skim	NV	20	11	4.9	0.33	33	0.02	60	1400
Milo (grain sorghum)	12	1.2	40	4	0.18	11	0.24	NV	680
Molasses, beet	NV	0.4	40	5.4	88	66	0.2	NV	880
Molasses, cane	5	2.5	100	4.4	100	58	0.04	NV	880
Oat mill by-product	NV	1.5	10	NV	NV	3.3	NV	NV	440
Oatmeal feed	24	1.8	13	2.2	0.22	15	0.35	NV	1200
Oats, heavy	20	1.1	18	1.3	0.11	13	0.3	NV	1100
Olive oil	125	NV	NV	NV	NV	NV	NV	NV	NV
Peanut meal, dehulled, solvent	3	12	180	10	0.39	60	0.36	NV	2100
Peanut meal, solvent	3	11	170	10	0.39	53	0.36	NV	2000
Peanut oil	280	0	0	0	0	0	0	0	0
Peas, field dry	NV	1.8	37	1	0.18	10	0.36	NV	NV
Potato meal, white, dried	NV	0.7	33	14	0.1	20	0.6	NV	2600
Poultry by-product meal	2	11	40	NV	0.3	8.8	1	NV	6000
Poultry offal fat, stabilized	30	0	0	0	0	0	0	0	0
Rapeseed meal	19	3.7	155	NV	NV	9	NV	NV	6600
Rice bran oil	420	NV	NV	NV	NV	NV	NV	NV	NV
Rice bran	60	2.6	300	NV	0.42	23	NV	NV	1300

Continued

Feedstuff	Vitamin E	Riboflavin	Niacin	Vitamin B6	Biotin	Pantothenic Acid	Folate	Vitamin B12	Choline
	IU	mg	mg	mg	mg	mg	mg	ug	mg
Rice polishings	90	1.8	530	NV	0.62	57	NV	NV	1300
Rice, rough	14	0.5	37	NV	NV	3.3	0.25	NV	800
Rice, white, polished	3.6	0.6	14	0.4	NV	3.3	0.15	NV	900
Safflower meal	1	4	6	NV	1.4	4	0.44	NV	2600
Safflower oil	500	0	0	0	0	0	0	0	0
Sesame meal	NV	3.3	30	12.5	NV	6	NV	NV	1500
Sesame oil	250	0	0	0	0	0	0	0	0
Soybean meal	2	3.3	27	8	0.32	14.5	3.6	NV	2700
Soybean meal, dehulled	3.3	3.1	22	8	0.32	14.5	3.6	NV	2700
Soybean oil	280	0	0	0	0	0	0	0	0
Soybean, isolated protein	0	1.25	4.9	1.3	NV	0.63	NV	NV	NV
Soybeans, full-fat, processed	50	2.6	22	11	0.37	15	2.2	NV	2800
Sunflower oil	350	0	0	0	0	0	0	0	0
Sunflower seed meal, dehulled, solvent	20	7.2	106	16	NV	40	NV	NV	4200
Sunflower seed meal, solvent	11	6.4	91	16	NV	10	NV	NV	2900
Tallow, stabilized	13	0	0	0	0	0	0	0	0
Tomato pomace, dried	NV	6.2	NV	NV	NV	NV	NV	NV	NV
Wheat bran	17	3.1	200	10	0.48	29	0.78	NV	1000
Wheat germ meal	130	5	50	13	0.22	12	2.4	NV	3300
Wheat middlings	44	2	100	11	0.37	20	1.1	NV	1100
Wheat shorts	57	2	95	11	0.37	8	1.1	NV	930
Wheat, hard, northern US and Canada	11	1.1	60	4	0.11	13	0.4	NV	1000
Wheat, hard, south-central US	11	2	60	4	0.11	13	0.35	NV	1000
Wheat, soft	11	1.1	60	4	0.11	13	0.3	NV	1000
Whey product, dried	NV	40	15	3.2	0.28	60	0.8	40	260
Whey, dried	NV	30	11	2.5	0.25	47	0.58	0.3	200
Yeast, brewers', dried	0	35	450	3.3	1.3	110	12	NV	3900
Yeast, torula, dried	0	44	500	NV	2	83	21	NV	2900

NV, no value available. From Scott, M.L., Nesheim, M.C., Young, R.J., 1982. The Nutrition of the Chicken, third ed. Scott, M.L., & Assoc., Ithaca, NY, pp. 490–493; with permission.

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The Vitamins

Fifth Edition

Fundamental Aspects in Nutrition and Health

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James P. McClung, Westborough, Massachusetts, USA

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ISBN 978-0-12-802965-7

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