Laboratory Evaluations
for Integrative and Functional Medicine

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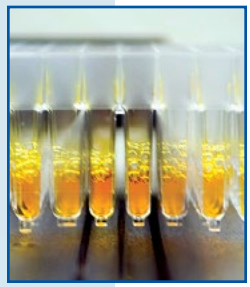

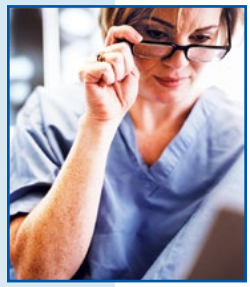

Revised 2nd Edition

Richard S. Lord
J. Alexander Bralley
Editors

METAMETRIX INSTITUTE ❧ DULUTH, GEORGIA

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Preface

The first edition, ***Laboratory Evaluations in Molecular Medicine***, has been well received by those interested in clinical laboratory tests for identifying nutrients, toxicants and other factors underlying patient health and disease. It was the first of its kind, pulling together much of the scientific literature's foundation for these tests and explaining their interpretation and clinical application in a systematic manner. This second edition incorporates the most recent advances in this field along with improved graphics, case illustrations and summary charts.

The title is also changed to more precisely reflect emerging terminology trends. "Molecular" in the original title referred to metabolic and biochemical functions that underlie health and disease. While molecular medicine is fundamentally based upon these functions (as are many areas of medicine), the term has come to be more closely identified with the genomic sciences. Molecular laboratory tests identify a disease or the predisposition for a disease by analyzing the DNA or RNA. As such, it is too narrow a term to represent the scope of this book. The terms "integrative" and "functional" are emerging as widely used descriptors for the clinical practice of medicine that takes a patient-centered, holistic approach to understanding and treating disease conditions. Clinicians using this approach seek to understand the various factors underlying patient symptoms. Therapy is based upon treating those factors rather than their symptomatic expression alone. The tests described in this book are tools clinicians use for this purpose. Hence, the title shift for the second edition better describes how these tests are used in clinical practice.

Integrative medicine takes account of the whole person (body, mind, and spirit), including all aspects of lifestyle. It emphasizes the therapeutic relationship between clinician and patient, restores focus on the body's own internal healing systems, and makes use of all appropriate therapies from both conventional medicine and evidence-based components of complementary and alternative medicine. Integrative medicine is a term used frequently by the National Center for Complementary and Alternative Medicine (nccam.nih.gov) at the United States National Institutes of Health, the Consortium of Academic Health Centers for Integrative Medicine (www.imconsortium.org) and various universities, hospitals, and clinics worldwide. Integrative medicine programs are increasingly being added to the curricula at many medical schools.

Functional medicine is grounded in the same basic principles, but its distinction lies in the conceptual system it provides for understanding the components of human health and various treatment modalities, from conventional to complementary and alternative. Its comprehensive and systematic model to describe the disparate science-based approaches and therapies that comprise integrative medicine makes it suitable for implementation into medical school curricula. The ***Textbook of Functional Medicine*** (functionalmedicine.org) presents the history, principles, concepts, and clinical application of functional medicine. It describes how environmental inputs into the patient's unique set of genetic predispositions, attitudes, and beliefs give rise to eight core clinical imbalances that are expressed as malfunctions within the body's physiological system. These imbalances are precursors to the signs and symptoms by which organ system disease is detected and diagnosed. Improving balance, the precursor to restoring health, involves much more than treating the symptoms. With its science-based emphasis, functional medicine relies heavily on laboratory tests for identifying nutritional, biochemical and metabolic imbalances underlying patient symptoms.

Our goal in presenting ***Laboratory Evaluations for Integrative and Functional Medicine*** is to organize and explain the various tests that are available so as to make their study and use more applicable to medical education and clinical practice. Since discussion of nutrient function, physiological roles and deficiency signs is widely available elsewhere, these topics are touched only briefly here. The primary focus of this book is on the laboratory tests themselves and how the test results are used clinically. The editors hope that the integration of descriptive text, summary tables, full color illustrations and over 3,800 citations to the medical and scientific literature will help clinicians make broader use of these tests and concomitant therapies to improve patient outcomes. ❖

PHYSICIAN PERSPECTIVES

Finding Pieces of the Puzzle

I began my medical practice in 1989, fresh out of medical residency, in a quintessential country practice. In fact, I took over the medical practice of Dr. Donald Campbell in Stockbridge, Massachusetts, pictured in Norman Rockwell's painting, *"Before the Shot,"* with a little boy looking intently at his doctor's diploma, with his bottom half exposed about to get a shot. It soon became evident, however, that clinical practice was much different from the idyllic Rockwell painting, and I felt adrift in the turbulent sea of treating real patients with multiple chronic medical problems. I became increasingly frustrated prescribing medication after medication to suppress symptoms without being able to get to biochemical imbalances underlying chronic symptoms. In the early 1990's I was fortunate to attend a seminar by Jeffrey Bland, PhD, with colleagues of mine from Canyon Ranch Health Spa in Lenox, MA where I was working part time as I grew my private practice. At this seminar my eyes were opened to the clinical application of biochemistry. I saw how the Krebs cycle, intercellular and intracellular communication, cellular membrane integrity, diet and nutrition were all interconnected. The Systems Biology approach that Functional Medicine has as its foundation has allowed me to have joy again and help patients regain health and vitality. The cutting edge laboratory testing described in this book has been invaluable in shining light on the biochemistry and nutritional status of patients that mainstream laboratory testing simply does not do. Time after time, tests for organic acids, fatty acids, amino acids, vitamins, minerals, and dysbiosis and GI markers have allowed me to put together the pieces of a patient's clinical puzzle, which I would not have been able to do with mainstream laboratory tests.

Each patient I encounter is genetically unique and has his or her own biochemical fingerprint showing patterns of health and ill-health. The ***Laboratory Evaluations for Integrative and Functional Medicine*** manual functions like a map to help show me the way when I encounter a complex patient with multiple complaints such as chronic fatigue, unexplained pain, chemical sensitivities, digestive issues, brain fog, etc. Even as I would not embark on a trip without a map, I would not try to figure out what is biochemically and nutritionally imbalanced in a complex patient without first getting advanced Functional Medical testing. I often will say to a patient, *"If you don't look, how do you really know what is wrong?"* I think it is all too easy for a doctor to give a patient a label, aka "an ICD-9 diagnostic code" to comply with insurance reimbursement regulations. Yet, "Diabetes," for example, is a label doctors stick onto a patient without realizing there are probably a dozen different ways to tip the scale towards promoting the development of type II diabetes, ranging from fatty acid deficiency, chromium deficiency, oxidative stress, mitochondrial dysfunction, etc. The difficult part in clinical medicine is making the *correct* diagnosis in a patient. This entails connecting the dots to see the full picture, much like stepping back from a Monet painting to see the beauty, not the apparent random array of dots when viewed close up. The correct diagnosis should be backed up by science, and the tests discussed in this book can allow clinicians to truly personalize diagnosis and treatment. ❖

— Todd LePine MD
Board certified in Internal Medicine
Private Practice

A New Standard of Care

During my years in medical school and residency training, I was taught a 'predestination' model of illness and a pharmaco-therapeutic approach to clinical practice. The clinical algorithm communicated through didactic lectures and hospital rotations was based on the fundamental assumption that disease was generally the result of inherited imperfections and that drugs and surgery were the appropriate solution to overcoming inherent genomic weakness. Recent science in molecular medicine, however, has challenged this fatalistic approach to disease management. The emerging evidence in epigenetic and molecular research has ushered in a new discourse in the understanding of health and disease. Rather than predestination, the truism of a 'predisposition' model of health and infirmity has emerged in the scientific and medical literature.

The predisposition model recognizes that we all inherit genetic vulnerability, but it incorporates unfolding evidence that environmental and nutritional factors interact with our genome to produce a unique phenotype

and the eventual clinical outcome. In fact, the Centers for Disease Control recently made the claim that "virtually all human diseases result from the interaction of genetic susceptibility and modifiable environmental factors."

Through my participation in clinical research and medical writing, I have come to realize that it is only in understanding and addressing the etiology of personal affliction that health professionals are able to facilitate sustained solutions to health difficulties. Infirmity commences because of a cause, infirmity continues because the cause continues, and infirmity can only be resolved when the cause is resolved. In order to identify the causative determinants that must be addressed in clinical situations, an objective mechanism is required to explore those nutritional and toxicological factors that are contributing on a molecular basis to health outcomes. Accordingly, laboratory testing to evaluate biochemical, toxicological and nutritional status in individual patients is an absolute prerequisite in modern health care to adequately investigate and manage health problems, particularly in patients with complex challenges.

I was first introduced to the work of Dr. J.A. Bralley and Dr. R.S. Lord when I attended a medical conference in 2005. Upon the recommendation of a respected colleague, I read the first edition of their volume on laboratory investigations entitled 'Laboratory Evaluations in Molecular Medicine.' This book provided what I was looking for to help me better care for patients – a readable tome detailing the latest knowledge and laboratory techniques to explore underlying biochemical problems contributing to illness and suffering in afflicted individuals. The book also chronicled available testing that could be undertaken on healthy individuals to identify modifiable abnormalities that might be corrected in order to prevent health problems. This important publication has since served as a basis for my understanding of nutrient evaluation; the laboratory evaluations discussed in this book have become an integral part of my everyday clinical practice. As this field continues to rapidly expand with the emergence of new testing and the evolving understanding of cellular mechanisms, it is necessary to continually update and revisit contemporary approaches to laboratory evaluation.

Accordingly, I am excited to recommend the new volume entitled ***Laboratory Evaluations for Integrative and Functional Medicine***. Rather than being an esoteric biochemistry text, it provides intelligent and comprehensive discussion of invaluable clinical information and provides direct guidance to investigate the source of patient problems. The information is well organized, it is interesting, and it serves both as a reference book as well as an instructional guide for state-of-the-art laboratory testing. The new publication is suitable for those interested in learning the basics of clinically relevant laboratory investigation but also serves as a comprehensive reference volume for those already trained in advanced molecular medical evaluation. I anticipate that this book will be recognized and honored as a standard reference volume in the expanding field of laboratory investigation. Most importantly, it will continue to provide very concrete and practical information for modern health practitioners who wish to explore the underlying causes of patient affliction. The information gleaned from the laboratory evaluations serve to direct clinical interventions in order to achieve optimal health restoration in individual patients.

Finally, it has been noted throughout medical history that it often takes a generation for new medical ideas to translate into widespread knowledge and to be incorporated into common clinical practice. Although much of the information presented in this volume is new and cutting edge, it is my sincere hope that dissemination of the knowledge within this publication will diffuse rapidly through the health care community to effect improved clinical care for patients. It is also my desire that the molecular based approach to laboratory investigations as discussed in this book will quickly become the standard of care for health practitioners. ❖

— Stephen J. Genuis
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The Editors

Richard S. Lord, PhD, director of the Department of Science and Education at Metametrix Clinical Laboratory, received his doctorate in Biochemistry from the University of Texas at Austin in 1970 with studies of methods for protein tertiary structure measurements. From 1970 to 1973 he was an NIH postdoctoral fellow at the University of Arizona, conducting studies of the mechanism of insulin self association. From there he moved to a staff fellowship at NIH in Bethesda, MD to work in the Institute of Arthritis and Metabolic Disease. Dr. Lord served as Professor and Chairman of Biochemistry at Life College for 10 years where he concurrently taught Advanced Clinical Nutrition and worked as part of the team that put in place a new undergraduate degree program in nutrition. He joined Metametrix Clinical Laboratory in 1989, where he has previously served as vice president of research development and laboratory technical director. His publications include articles in professional journals as well as books and laboratory manuals for college curricula. He has served as Clinical Laboratory Director at various facilities and has developed methods for analysis of trace elements, fatty acids, and organic acids. ❖

J. Alexander Bralley, PhD, CEO of Metametrix Clinical Laboratory, received his B.S. in biology from the University of Illinois in 1975 and his Ph.D. in medical sciences, specializing in neuroscience, from the University of Florida College of Medicine in 1980. He has worked and published in the area of nutritional biochemistry since then. For the past 24 years, he has been CEO and Laboratory Director of Metametrix Clinical Laboratory in Atlanta, Georgia, an internationally recognized clinical laboratory specializing in nutritional and metabolic testing. He has been instrumental in developing testing and interpretation guidelines for innovative nutritional and metabolic analyses. Dr. Bralley has extensive experience in the use of nutritional therapies to treat chronic illnesses through consulting with physicians, reviewing the medical literature, and attending and speaking at conferences, where he is widely sought after to lecture on the use of laboratory testing to define nutrient needs for individuals. He is a member of several professional organizations for nutrition and clinical laboratory science. He is on the editorial review boards for Alternative Medicine Review, the Journal of Applied and Nutrition, and Integrative Medicine: A Clinicians Journal. Dr. Bralley's ongoing research interests lie in examining the relationship between nutritional, metabolic, and toxic factors, how they affect chronic disease processes and influence health. ❖

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Metametrix Institute "appreciates the vision, dedication and outstanding contributions of the above-named individuals, without whom this book would not have been possible." ❖

Contents in Brief

A comprehensive Table of Contents is provided at the beginning of each chapter.

Chapter 1

Basic Concepts

J. Alexander Bralley and Richard S. Lord

CONTENTS

Notes:

A New Role for Clinical Laboratories

One lesson in medicine we have learned over the last century is that our successes often give rise to new challenges. Tremendous strides in reducing mortality from acute illness and extending life expectancy have yielded an increase in chronic, degenerative diseases among the aging population.[1] The power of antibiotics in saving lives has encouraged the hope of a "magic bullet" for treating symptoms, a model that has, to a large extent, failed to produce the same success with diseases that are multifactorial in origin and chronic in nature. Additionally, the very use of these drugs has created "super bugs" resistant to antibiotics.[2] Advances in food production and distribution have resulted in an abundance of processed foods altered from their original form and that have low nutrient-to-calorie ratios. Technological progress has created literally thousands of new chemicals that challenge the environment and our own metabolic processes.[3]

As more of the world's population experiences the benefits of our successes, overcoming the new challenges we have created requires a broader, more holistic approach that takes into account the web-like interconnectedness of human metabolism and its relation to the environment. The human body has great capacity to adapt to change, heal, and express its genetic potential if it is provided the raw materials—the basic macronutrients, micronutrients, and conditionally essential nutrients-that it requires for healthy metabolic function. What should become routine application of these principles to general patient care is already being done by some practitioners. In the U.S., they tend to be catagorized as practicing integrative or functional medicine.

Nutrition may be the single most influential component of health maintenance, since diet is a determining factor in many diseases, including obesity, cancer, diabetes, hypertension, heart disease, stroke, cirrhosis of the liver, childhood developmental and behavioral disorders, and celiac disease.[4,5] Yet optimal nutrition, the level of nutrient intake that maintains the best possible health, is highly variable from person to person.[6] The concepts of biochemical individuality and the genetotrophic theory of disease, as first described by Roger Williams, the discoverer of pantothenic acid (vitamin B_5), continue to exert a major influence on this emergent model of human health and the development of integrative functional medicine.

Biochemical individuality influences not only the individual need for nutrients, but also the expression of nutrient insufficiency.[7] Although this has been recognized for centuries, the concept has been virtually ignored by the current medical system. Diseases such as beriberi, pellagra, and scurvy, all single-nutrient deficiency diseases, are often thought of as specific sets of symptoms and disease progressions. This, however, is not the case. Biochemical individuality plays a key role in the expression of functional deficits as they develop, even in a single-nutrient deficiency disease. An excerpt from an account by the chaplain aboard Commodore Anson's British sailing ship HMS *Centurion*, which voyaged around the world from 1740 to 1744, and where 626 of 961 men died of scurvy, illustrates that even centuries ago this was apparent:

> This disease, so frequently attending all long voyages, and so particularly destructive to us, is surely the most singular and unaccountable of any that affects the human body. For its symptoms are inconstant and innumerable, and its progress and effects extremely irregular: for scarcely two persons have the same complaints, and where there is found some conformity in symptoms, the order of their appearance has been totally different. However, though it frequently puts on the form of many other diseases, and is therefore not to be described by any exclusive and infallible criterions (Chaplain Richard Walter, *Anson's Voyages Around the World in the Years 1740–1744*).

Because most essential nutrients play such fundamental roles in cellular metabolism, as insufficiencies develop, the effects can be system wide. Variation in disease expression is exponentially compounded when multiple nutrient deficiencies are involved, which is often the case in the chronic degenerative diseases. Any and every aspect of body function can potentially become compromised, depending on the patient's own unique biochemical expression.

The corollary of this situation is that nutritional and metabolic evaluations of patients with chronic illnesses also exhibit this variation, making it difficult to define a disease by any set of laboratory markers. That is, different illnesses may have very similar nutrient deficits. Conventional wisdom in diagnostic medicine requires that each diagnosed disease have its own unique set of

signs, symptoms, and laboratory indicators. Diabetics exhibit elevated blood glucose, prostate cancer patients have elevated serum PSA, and elevated white blood cells can indicate an infection. This same thinking has been applied to the diagnosis of various chronic disorders. Is there a metabolic or nutritional deficit pattern to fibromyalgia that would distinguish it from chronic fatigue or autistic spectrum disorders?

Since nutrient insufficiencies affect multiple organ systems in ways dependent on the history and genetic makeup of each patient, one would expect varying outcomes for similar patterns of nutrient depletions, rather than clear definitive patterns of nutrient status with each chronic illness. Decades of experience with nutritional and metabolic profiling calls into question the validity of categorizing and labeling chronic illness with a diagnosis, since there does not appear to be a unique set of diagnostic laboratory criteria matching a specific disease diagnosis. A specific chronic illness is not diagnostically defined by a unique set of laboratory values (e.g., elevated glucose is diagnostic of diabetes). Consequently, the clinician is not really treating, for example, fibromyalgia, chronic fatigue, and inflammatory bowel, but rather the individual nutritional and metabolic imbalances and toxicities that prevent normal function. These are expressed in each person as a different set of symptoms, perhaps the weakest links in a person's genetic makeup. Toxic lead exposure, for example, may be expressed in some individuals as inflammatory disorders, in others as immune disorders, and still others as neurological disorders.[8,9] Disease labels are conveniences that allow reductionism so our minds can categorize treatments; yet this labeling has inhibited our ability to effectively treat nutrient-related disorders. Patients are better served by approaching chronic disease as a manifestation of underlying nutritional and metabolic imbalances and/or toxicities. Correction of the imbalances yields elimination of the disease, just as the various expressions of vitamin C deficiency in scurvy are reversed once the patient is replete in the vitamin. This concept expresses the fundamental assumption of this book: that underlying all diseases are nutrient and/or toxicity factors that either cause or complicate the disease process.

In this book the terms "deficiency" and "insufficiency" are often used interchangeably. In general, however, "deficiency" relates to frank dietary deficiency disease conditions (i.e., pellagra, scurvy, beriberi), whereas "insufficiency" is used to express non-optimal nutrient status that can adversely affect function. In most cases the clinicians in developed countries are dealing with nutrient insufficiency and not deficiency disease. Although the classic nutrient deficiency diseases are not usually seen in developed countries, the need for additional nutrient supplementation for optimal function can be clearly demonstrated for many conditions.[6] Homocysteinemia, for example, is treated with high doses of vitamin B_6, folate, and vitamin B_{12}. In this case, there is an insufficiency of these nutrients to provide optimal homocysteine metabolism. This example illustrates the need for nutrients in sufficient amounts, usually higher than RDA levels, to maintain optimal function. When a broader definition of "deficiency" as any state in which dietary intake has failed to meet demand is used, both patients with vitamin B_{12}-responsive homocysteinemia and those with anemia due to poor vitamin B_{12} intake are deficient in vitamin B_{12}.[6]

Once the concept of nutrient insufficiencies manifesting as the etiology of chronic illness is understood, the relevant clinical question is how best to detect early states of insufficiency to restore and maintain function and wellness. We have attempted to compare clinical laboratory evaluations with regard to the sensitivity for detecting early states of nutrient depletion. Biochemical pathways involving large flux of metabolites are generally the first stage at which nutrient effects are seen. There is a progression from this stage to the end organ failure

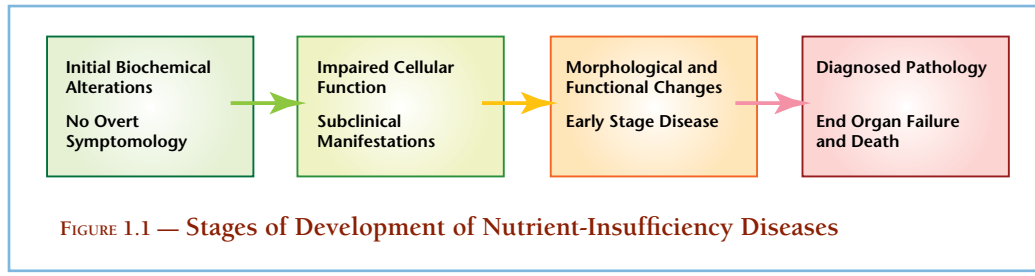

FIGURE 1.1 — Stages of Development of Nutrient-Insufficiency Diseases

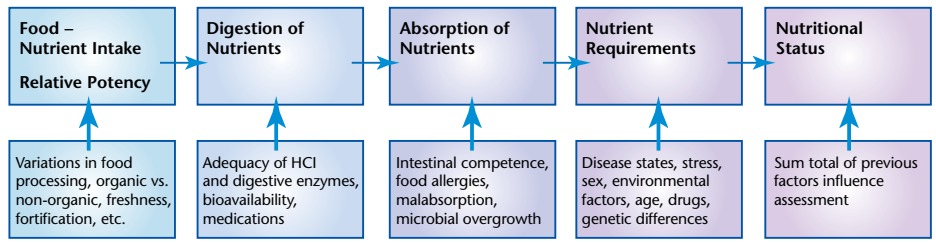

Figure 1.2 — Components Affecting Nutritional Status

that is usually observed when a classic diagnosis is made (Figure 1.1).

Initial conditions of insufficiency are seen at a biochemical level often without any overt symptom expression. This is why asymptomatic and symptomatic individuals may have similar laboratory markers for nutrient insufficiencies and metabolic imbalances. As the duration of the insufficiency increases, additional changes appear in cellular function that can be seen as subclinical manifestations such as behavioral and mental/emotional instabilities. As the insufficiency progresses into later stages, morphological and functional changes occur that can be defined as early stage disease. Finally, diagnosed pathology is expressed with end organ failure and ultimately death.

Many factors influence nutrient status in the individual (Figure 1.2), such as diet, digestion, absorption, disease states, age, medications, stress, toxic exposures, activity levels, genetics, and biological differences. Laboratory tests can provide valuable indicators of many for these factors.

Issues in Assessment

Not all tests for any particular analyte are equivalent, so it is important for the clinician to be aware of four variables involved in testing: the analyte, the specimen, patient preparation, and instrumentation. The analyte can be a nutrient, toxicant, or metabolic control molecule. The choice of specimen, such as blood, urine, stool, hair, or other tissue, is critical to the interpretation and clinical utility. Preparation of the patient is critical. Is the patient fasting? Was a challenge compound used? Has the patient been taking a nutrient that is being measured? And, finally, the analytical method itself can affect the sensitivity and specificity of the measurement. For example, homocysteine measured using a post-column ninhydrin derivatization liquid chromatographic method is much less sensitive than other pre-column derivatization methods.[10] Both methods produce a result for the analyte, but clinically relevant values are less than the limits of detection for the former method. Further, improvements in analytical instrumentation, such as tandem mass spectrometry, will allow measurement of analytes at very low concentrations that were not possible previously.[11] This is opening up a whole new area of environmental chemical toxicology and body burden assessment.

Static Measurements

As is the case with every laboratory measure, the physiology of the body needs to be taken into account when interpreting a laboratory value. A blood glucose level, for example, is interpreted differently depending on the time of the last meal. In addition, because nutrient insufficiency is initially expressed at the metabolic level, blood levels are often normal in early insufficiency stages, so measurements can be misleading for certain nutrients. Magnesium, for example, is found primarily intracellular with only 1% of body magnesium found in the serum, and its level is tightly regulated. Consequently, serum levels of magnesium, which change only after there is a significant depletion in the tissues, is not the best measurement when the purpose is to evaluate magnesium insufficiency in a patient.[12] This is often the case when the nutrient is primarily intracellular. Serum vitamin E, on the other hand, provides a useful measure of nutrient status, since a considerable portion of total body vitamin E resides in serum because

Notes:

it protects blood lipids from oxidation. Other macronutrient sources, such as amino acids and fatty acids, are dynamically metabolized in the body. Fasting plasma measurements of these nutrients reach a level of homeostatic stability that allows for consistent interpretation of data in repeat testing. Toxic metals and other chemicals in serum can be useful as indicators of an ongoing exposure, but are not necessarily reflective of the total body burden of the toxicant.[13–15]

Clinical interpretation of hormonal assessments is particularly problematic. Although this book does not exhaustively address this area, many clinicians using nutritional therapies regularly assess various hormonal issues that can affect function. Hormone levels that vary over time need to be assessed appropriately. Serum levels taken at different points in time may be a better indicator of proper function than a one-time measure. The use of 24-hour urine obviates the need for multiple blood draws, since it is used to measure total daily output. Salivary hormone levels may be valid for certain hormones and not others. Again, timing is an issue. Consideration of the physiological functioning of the hormone being assessed should always be a factor in determining useful and reliable laboratory testing procedures.

Methods of Assessment

The following discussion of possible strengths and limitations of various standard laboratory approaches to assessment provides the methodological background for more detailed chapters that follow.

Enzyme Stimulation Assays

Intracellular enzyme stimulation assays were developed to provide a more reliable, functional indication of nutrient needs. However, usually only one enzyme is assayed for a particular vitamin cofactor, such as erythrocyte transketolase for thiamin insufficiency or glutathione reductase for riboflavin insufficiency.[16] One drawback to this type of assessment is that not all enzymes that require the cofactor are measured. Other enzymes that use the same cofactor may need more or less of the essential nutrient. Genetic or toxicant effects will not be detected by measuring only one enzyme activity. Multiple metabolite screening increases the probability of detecting point weaknesses. The concept of employing large metabolite profiles during initial screening as a clinical approach to optimizing individual patient function is central to the discussions in this book.

Nutrient Concentrations and Biochemical Markers

Direct measurement of nutrients in blood or urine is the most obvious way to assess patient nutrient status. Clinically useful information about essential amino acids and some vitamins and mineral elements is gained in this way. Blood levels, however, may be sustained during periods when tissue demands are not being met. Measuring biochemical markers that respond to cellular metabolic restriction owing to nutrient insufficiency can provide more sensitive criteria of status. Thus, serum vitamin B_{12} may be normal while serum or urinary methylmalonate is elevated, indicating insufficiency of vitamin B_{12} for cellular functions.

Measurements of intermediary metabolite elevations are functional assays, since they detect the failure of a nutrient-dependent metabolic pathway. As with the stimulation assays, these functional markers are assessing only one nutrient-dependent metabolic biochemical reaction and may not give a complete picture of nutrient need at other steps because of genetic polymorphic effects on individual enzyme structures. Better overall assessment of nutrient need may be obtained when multiple metabolites are assessed that use the same cofactors for their metabolism, as is the case in B vitamin insufficiency states and multiple ketoacid elevations. Different dehydrogenase enzymes metabolize various ketoacids. Elevation of several ketoacids is a strong indicator of a global B vitamin insufficiency state decreasing the function of multiple enzymes.

Loading Tests or Saturation Measures

Tests that measure the retention of a nutrient load may utilize the dry-sponge concept; that is, if a nutrient is deficient or needed by the tissues, more will be retained by the body and less excreted on loading than in the nutrient-replete condition. Magnesium loading, then, is more a functional need assessment tool, giving the clinician a better picture of the sufficiency status of the patient.[17]

Challenge Tests

Chelation mobilization tests for toxic metals are useful to assess the body burden for a toxic element. This is a way to evaluate the need for detoxification treatments for patients with chronic illnesses. When a water-soluble challenge compound is administered orally, it has primary impact on the liver and kidney. Thus, these tests reveal primarily hepatic and renal toxic metal status. The use of challenge compounds to assess

a specific metabolic pathway is particularly valuable, as it allows the clinician to directly assess how well a particular critical biochemical system function is operating. Caffeine clearance, acetaminophen, and salicylate challenges have proven valuable in assessing important detoxification pathways. Loading with methionine before homocysteine testing may enhance the sensitivity of detecting homocysteinemia.[18]

Other Procedures

The assessment of nutritional and toxicological factors in health care would not be complete without an evaluation of the digestive tract. Stool analysis does not fit handily into any of the above categories, yet information gained from the stool can be very useful clinically. Microscopic and chemical analysis of stool reflects the efficiency of the digestive tract, nutritional status, or an inflammatory condition. Microbiological assessment examines the balance of gut ecology, which can have an important impact on health. Toxic metabolites from certain microflora overgrowing in the gut can be measured in the urine.[19] Culture and sensitivity measures provide the clinician with the information to restore normal microflora balance. New gene-based identifications of intestinal microbes have replaced standard culture techniques.

Food itself can have a significant negative impact on health if a patient is allergic or intolerant to the food. Analysis of the immunologic responses to foods has been very useful in determining clinically significant allergies and intolerances. Serum antibody measures for both immediate and delayed sensitivity reactions and provide the clinician with useful data to guide diet and nutritional recommendations.

Another area of rapid recent growth has been in the field of genomics. Susceptibility for diseases is often genetic in nature and much research is now being aimed at defining how the human genome is involved. Testing for the single nucleotide polymorphisms (SNPs) in the chromosomes that define relative risks are an increasingly important part of nutritional and preventive medicine. Nutrition can play a major role in the modification of risk once the genetic tendencies are identified.[20–22]

Instrumentation

Many of the tests discussed in this book are referred to as high-complexity tests in the clinical laboratory industry. This means the assays are not performed as routine automated tests, as are most blood chemistry profiles. Complicated analytical instruments are often used to measure specific compounds. Antibody assays may be used, and many of the tests are developed in-house by the lab, as opposed to purchasing a manufactured kit to do an analysis. What follows is a brief description of principal instruments used to perform the simultaneous multiple analyte assessments discussed in this book.

Hyphenated Instrumentation — Instruments that perform multiple analyte analyses must first handle necessary separations to isolate a compound of interest and then allow reproducible measurement of the amount of substance present in the specimen. These systems are composed of two separate instrument types coupled together to take advantage of the strengths of combined separation and detection techniques. They are known as "hyphenated instruments." In the past, a gas chromatograph coupled to a mass spectrometer was abbreviated as GC-MS. More recently, the forward slash (/) instead of the hyphen is normally used, but the description as hyphenated is still used. Thus, GC-MS has become GC/MS, representing the method of gas chromatography with mass spectrometric detection. For our discussions, such abbreviations will also be used. Short descriptions of common instrument types, how they work, and their strengths and weaknesses in the analysis of biological samples are provided here.

GC/MS

Theory: A GC/MS is a gas chromatograph coupled to a mass spectrometer. The gas chromatograph works by separating volatile molecules by their boiling points and their varying rates of migration through a very-small-diameter capillary column. The columns are available with a wide variety of coatings that have characteristic affinities for the compounds being separated. Gas chromatography offers superior performance in separation of closely related molecules. This is largely due to the separation mechanism and the length of the column used for the separation; a typical GC column is 30 to 60 meters long.

The ability of GC to separate multiple compounds usually exceeds all other types of separation techniques. A mass spectrometer (MS) functions as a detector for this instrument type. The GC introduces the molecules into the MS one at a time in small narrow bands called "peaks." These narrow bands of molecules are then ionized by the ion source of the mass spectrometer and pushed out of the source using an electric field. Ionic

fragments are then separated by their mass-to-charge ratio using an electric field or a magnetic field, depending on the type of instrument. Each compound generates a unique fragmentation pattern, and the mass spectrometer allows the analyst to "fingerprint" each compound for measurement and positive identification.

Uses: A GC/MS is used for the analysis of volatile compounds. Mixtures of long-chain fatty acid methyl esters with components that vary only by the positions of their double bonds can be fully separated by GC and measured with high sensitivity by the MS detector. Mixtures of industrial waste containing organic solvents and small organic compounds are analyzed by GC/MS analysis. Other uses range from positive identification for drugs of abuse to environmental monitoring and quick, positive identification of chemical weapons of mass destruction.

Advantages: A GC/MS has the greatest ability to resolve molecules of similar chemical and physical properties. The mass spectrometer is a universal detector that can detect any type of compound, and the fragment analysis allows the analyst to make positive identifications of compounds of interest as long as a pure standard is available for comparison.

Disadvantages: The biggest disadvantage of using a GC/MS is that the compounds being measured must be volatile in order for them to enter the GC. Compounds such as amino acids and most organic acids in human tissues decompose before they boil, so they cannot be directly analyzed. They may be made volatile by carrying the specimen through multiple steps of chemical reactions. This process introduces uncertainty into the analysis because operator error increases with the number of complex steps required. The reactions must be nearly 100% efficient and must be very reproducible. The chemical reactions change the fingerprint of the compound so that it may no longer have a unique fingerprint. Care must be taken to choose the appropriate mass values for measurement, as multiple mass spectral interferences can cause problems when reporting quantitative values.

The amount of time needed to run a sample through the instrument is also a problem when analyzing a complex sample mixture, because the instrument needs time to separate and measure all of the compounds of interest, as well as purge all of the other materials introduced with the sample. Biological samples contain hundreds of different compounds, drugs, metabolic waste products, and excess nutrients that the body does not store. All of these compounds require time to travel the length of the GC column and be purged from the instrument before the next sample can be analyzed. When a GC/MS method is developed, caution is taken that interfering compounds are not found in specimens. However, new drugs or special foods may introduce previously unseen compounds that exit the GC together with one of the analytes, causing false elevations.

Typical run time: 15 to 45 minutes per sample; tests looking for a small number of compounds can sometimes be run faster. Profiles of large numbers of compounds typically take 25 to 45 minutes for all of the compounds to be fully resolved by the GC.

ICP/MS

Theory: ICP/MS is a technique for the simultaneous measurement of multiple chemical elements such as calcium, copper, and chromium. The instrument consists of an inductively coupled plasma (ICP) device as a source for a mass spectrometer. The heart of these instruments is the plasma—a state of matter not normally encountered on the earth. A powerful electromagnetic field oscillating at a specific frequency is generated to cause extreme excitation of the argon that is flowing as a carrier of the specimen. The temperature at the center of the oscillating field rises to many thousands of degrees Kelvin, so that all organic matter is immediately destroyed and the chemical elements are almost completely ionized. Otherwise, the idea behind the instrument is simple; you pump a liquid sample into a sample introduction system that sprays the liquid into the plasma. The mass spectrometer then simultaneously measures the amount of each element by counting ions of specific mass-to-charge ratio.

Uses: An ICP/MS can be used for the fast and accurate measurement of toxic metals in any sample matrix that can be dissolved and introduced into the instrument as a liquid. The instrument can also measure all of the essential elements. For example, chromium, zinc, cobalt, and selenium can easily be measured by ICP/MS under various conditions in many different sample types, such as blood, urine, waste water, soil, and tissue digestions.

Advantages: ICP/MS is a very fast technique for the measurement of many different elements. It is also very sensitive, so measuring very low levels of some elements is easily accomplished.

Disadvantages: Interferences at specific masses is the greatest disadvantage of using this technique. Many

of the elements of interest in a biological sample cannot be measured with great accuracy because of high-energy byproducts generated by the plasma. Adducts of the carrier gas, argon, cause the most problems. These interferences can cause some elements to appear artificially high owing to the huge amount of argon used in the ionization process through the plasma.

Typical run time: Once the specimen is prepared by proper dilutions and insertions of internal standards, instrument run times of 3 to 10 minutes are common, depending on the number of elements being measured and their relative amounts in the sample. Low-level analysis takes longer because the MS requires longer reading times. Rinse times between samples also add up to 5 minutes per sample, depending on the type of sample being analyzed and the time needed to completely purge the sample introduction system between samples.

DRC ICP/MS

A DRC ICP/MS is a new type of instrument that helps eliminate the problem of high-energy polyatomic interferences. The gas flow system contains a separate compartment called a dynamic reaction cell (DRC). The cell is filled with a controlled amount of an inert gas such as argon or methane. As the polyatomic ions enter this chamber, they collide with the atoms of inert gas, causing fragmentation. The masses of the fragmented polyatomic ions no longer match the masses of the elements of interest. For example, argon carbide (ArC) is a polyatomic ion that interferes with the measurement of chromium. They both share mass 52 and cannot be distinguished using normal bench-top mass spectrometers. Inside the collision cell, ArC collides with methane and is fragmented into Ar and C. Argon's mass is 40, and carbon's mass is 12, so Cr 52 can be measured at much lower levels.

LC/MS/MS

Theory: Liquid chromatography with tandem mass spectrometric detection (LC/MS/MS) is a relatively new technology that is just now entering wide use in clinical labs. The instrument consists of a high-performance liquid chromatograph (HPLC, or simply LC) coupled to a tandem mass spectrometer (MS/MS). The LC separates molecules based on their various physical properties. Method developers must choose the correct separation technology and use it correctly to gain the best separation possible for the desired compounds and interfering compounds. Many different types of separation technology exist, including ion exchange, phenyl character, chiral-specific, normal, and reverse-phase separation technology. Each has its benefits for specific separations. The MS/MS detector for these instruments is sometimes called a triple quad mass spectrometer because it actually contains three mass spectrometers in series. A tandem mass spectrometer contains two mass filters (called quadrupoles) with a collision cell between them. An ionized compound is directed by the first filter into the collision cell where it is fragmented by collision with an inert gas, usually argon. The fragmentation pattern is unique for each type of compound. The final mass spectrometer directs a chosen identifying ion to the detector. In practice, there are many factors, such as molecular interferences, peak shapes, and detector dwell times, to consider.

Solvents that fully volatilize in the sample introduction chamber may be introduced into the MS/MS detector and only those solvents may be used for HPLC method development. This consideration will heavily limit development because there are very limited choices for volatile solvent/buffer combinations to move the compounds through the chromatographic column. This restriction is due to the requirement for total removal of the solvent before the target compounds move into the detector.

The liquid stream that exits the chromatograph is nebulized into tiny droplets that become charged as they begin to evaporate in the high temperature and vacuum of the MS/MS introduction chamber. As the droplets become smaller, the charge on each droplet becomes larger, eventually causing the droplet to disintegrate. The charge is transferred to the compounds dissolved as they become part of the gas phase. The very high vacuum inside the MS/MS draws the ions through the sampling cone where they are routed through the instrument using electric fields.

Notes:

Further filtering of unwanted solvent is performed in the first quadrupole stage. It's at this point that the tandem mass spectrometer differs greatly from the mass spectrometer of the GC/MS and ICP/MS. In the tandem mass spectrometer, the first mass spectrometer removes interfering solvent molecules and generates parent ions of compounds of interest, and the second one fragments the parent ions into daughter ions that are directed to the photomultiplier. The tandem selection of parent and daughter ions allows great confidence regarding the identities of the compounds being measured. The tandem mass spectrometer has the ability to rapidly and accurately measure a single compound in the presence of many others. Because of the speed of electronic tuning of the mass spectrometers, the tandem mass spectrometer allows measurement of multiple compounds that may be present in each peak that may emerge in the LC liquid stream.

Uses: LC/MS/MS instruments have many uses. They allow for the accurate measurement of very small amounts of specific compounds in the presence of large concentrations of contaminants. They are routinely used in clinical laboratories for screening of inborn errors of metabolism and therapeutic drug monitoring. They are applied for detection of macromolecules and biological screening for discovery of new drugs.

Advantages: The shorter run times offer the largest advantage over other analytical methods for multiple compound analyses. The transfer of compound-resolving power to the electronic speeds of the mass spectrometer allows for quick measurement of multiple compounds with great accuracy and reliability.

Disadvantages: Like all mass spectrometers the LC/MS/MS can also suffer from mass spectral interference. Even with the great specificity of the MS/MS, isomers may be present in physiological specimens that fragment in the same manner as compounds of interest. Such interfering compounds must be separated chromatographically to ensure accurate results.

Typical run time: A specimen may be analyzed in 2 to 15 minutes, depending on the number of interferences for each analyte, as well as the number of isomers being measured that must be separated chromatographically. A typical run time for a small number of analytes with no interfering contaminants is less than 5 minutes.

HPLC: FLD, DAD

Theory: High performance liquid chromatography is the oldest and most common separation and detection technology. The instrumentation consists of an HPLC coupled to a fluorescence detector (FLD) or diode array detector (DAD). This instrumentation has been in routine use for several decades and is well characterized in hundreds of published applications.

The liquid chromatographic systems operate at high pressures generated by pumps. Specimens are injected onto a column filled with silica beads with coatings chosen to match the chemical properties of the compounds being analyzed. The beads are called the stationary phase. The solvent or mobile phase consists of water with various salts and organic solvents. Compounds in the specimen adhere to the coating as they migrate down the length of the column.

Compounds with lower affinity for the coating reach the detector first. As the effluent stream passes through a chamber illuminated by an intense light source, the detector measures light that is emitted (FLD) or absorbed (DAD) by the compounds. The DAD is the most versatile detector of the two because it is able to simultaneously measure multiple wavelengths in the ultraviolet and visible spectrum. For FLD detection, compounds may need to be chemically modified with a fluorescent "tag." The instrument then detects the presence of this tag.

Uses: Any compound that can be dissolved in a solvent that does not destroy the system can probably be measured by HPLC with a properly chosen detector system. In addition to clinical laboratories, applications for this highly proven and mature technology are found in food processing, drug analysis, fine chemicals, refining, industrial purification, and quality-control processes in manufacturing.

Advantages: Simplicity and easy maintenance are the greatest advantages. These qualities must be ascribed to innumerable refinements by instrument manufacturers. Method development is relatively inexpensive.

Disadvantages: Many applications are too slow for high-volume clinical laboratories. Analytical limitations include lack of sensitivity or detection for some types of compounds that neither absorb nor fluoresce in the visible or near UV spectrum. Robust and reliable method development requires a scientist who is well versed in the science (and art) of chromatography. Typical run time is ten minutes to 2 hours or more.

Reliability of Test Results

A clinically useful test must provide reliable information that would not normally be derived from symptoms alone. Reliability means that a test must be adequately sensitive and highly reproducible. In the field of clinical chemistry, reliability is assessed in a number of ways.

For a test to be reliable, not only must it be reproducible over time, it must also vary with real change in the individual, either owing to treatment or some other factor, not just random variation of the assay. Reliability of a test is determined by both analytical and non-analytical factors.

Analytical Factors: Accuracy, Precision, Sensitivity, and Specificity

Accuracy — A test is accurate if it reflects the true value or detects the presence of the substance being measured. In analytical runs of multiple specimens, quality control (QC) samples of known values are included to check for the accuracy of the run. These results can then be charted to give a history of the assay to show if the accuracy is drifting or shifting. These charts allow the technologists to assess the accuracy of a test used in daily production. Proficiency testing programs that are discussed later in this chapter provide independent, external monitoring of accuracy.

Precision — Precision refers to the measure of variance of the assay. Imagine a target in a shooting range. If all of the hits form a tight cluster on the target, the shooter is very precise. If the hits cluster around the center, the shooter is also very accurate. Similarly, a test that is run on the same sample many times should yield results that are clustered in a small range. The variability around the number is calculated as the coefficient of variation, or CV, for that test. Typically, CVs should be no higher than 10 to 20% for high-complexity tests. A test can have a high degree of precision, yet be highly inaccurate. So accuracy and precision go hand in hand in determining a good laboratory test.

Sensitivity and Specificity — These terms refer to a diagnostic test's ability to reliably predict whether a person does or does not have a particular disease. Expressed mathematically, these values are expressions of the test's false-positive and false-negative rates. A test with high sensitivity correctly identifies a high percentage of patients who actually have a particular condition. A test with low sensitivity will produce a large number of false negatives, and it will miss many individuals who actually have the condition.

A test with high specificity correctly identifies a high percentage of subjects who do not have a particular condition. A test with poor specificity will have a high degree of false positives. It will inaccurately denote many individuals as having a condition when in reality they do not. It is easy to see that problems with accuracy and precision of the test can also affect the sensitivity and specificity of the test.

Laboratories must provide data demonstrating a test's accuracy and precision to licensing agencies when they are inspected. More detail on the inspection process is given later in this chapter. Many other analytical issues such as purity and consistency of reagents and matrix matching for biological specimens are checked because they can be critical for maintaining high quality standards.[26]

Non-Analytical Factors

Factors that do not involve analytical sensitivity and accuracy may be classified as pre-analytical factors and issues of biological variation. Pre-analytical factors are actions that can affect results before the sample is analyzed. These include variations in patient preparation (e.g., fasting vs. non-fasting), sample collection, sample temperature and exposure to light during transport, and equipment maintenance. A special patient-preparation issue concerns challenge testing where the nature and timing of the challenge may be critical. To determine whether a specimen is acceptable when it is received at the laboratory, stability studies should be run under various storage conditions.

Physiological variation is an important factor to understand when considering metabolic tests. If a test is to be used to design therapeutic regimens for patients and monitor progress, then the test must have general physiological stability over appropriate time spans, and a result must change because of an intervention rather than random physiological variation. This characteristic is widely appreciated in traditional blood chemistry measures. Serum cholesterol, for example, has fairly good physiological stability from day to day unless there is a significant change owing to drug usage or acute pathological issues.

Reference Intervals

95% Reference Interval

In the field of clinical chemistry, a reference interval is designed to allow the clinician to make a decision regarding the patient's level of health. Traditionally, the test result would indicate either the presence or absence of a disease regarding its own accuracy, precision, sensitivity, and specificity issues, which were discussed earlier. In integrative and functional medicine, this becomes a more difficult decision to make, since many of the chronic illnesses treated in this type of medicine are not clearly defined diseases, for reasons previously discussed. Rather, the analysis of nutrient and metabolic compounds indicates levels of insufficiency or imbalance that can relate to health issues.

Typically, an analyte in clinical chemistry is referenced by looking at population distributions of the test result, preferably in healthy individuals. For tests developed within a laboratory, the lab will usually draw from its database of tests that it has run over a period of time. The normal range for this group of test results is defined as that population within two standard deviations of the mean. In a normally distributed population, this would be 95% of the population, or the 95% reference or confidence interval. By definition, therefore, 5% of people tested would be abnormal.

Clearly, this is not the most useful way of evaluating whether a person may have marginal nutritional deficiencies. Take the example of magnesium: A recent government study showed 68% of Americans consumed less than the recommended daily allowance (RDA) for magnesium, and 19% consumed less than 50% of the RDA.[23] This would indicate that a large percentage of Americans are magnesium deficient. What, then, would be a useful reference interval for a clinician who wants to determine if their patient is magnesium deficient? Certainly, the typical two standard deviations from the mean would be relatively useless, since far more than 5% of the population should be magnesium insufficient.

Quintile Ranking

A more reasonable approach is percentile ranking of the result. This has been particularly useful in assessing the measures of risk factors for certain disease states. High-sensitivity C-reactive protein (hs-CRP) measures have been developed in this way to relate levels to risk of cardiovascular disease. By dividing the populations tested into quintile ranks, researchers were able to correlate risk of cardiovascular events with these rankings.[24] Those individuals falling in the highest or fifth quintile demonstrated significantly higher risk of cardiovascular events than those in the lowest quintile. In this way, hs-CRP has been shown to be a strong predictor of risk.

A similar approach can be taken with other analytes, whether nutrients, metabolites, or toxic compounds. By arranging the test data by quintile, the clinician can easily determine where a patient falls relative to a population from which the reference data were obtained. This allows the clinician to more easily assess the significance of the finding, particularly as he gains experience using the test with patients.

Most of the case illustrations in this book are taken from the actual laboratory reports where a combination of charts that show quintile positions and 95% percentile reference limits used to aid the interpretation of results. Figure 1.3 shows the display of results from a report on serum vitamins.

Quality Assurance

Advances in the medical sciences over the past 50 years have caused an exponential growth in numbers and types of laboratory tests and clinical laboratories. As in any production environment, concerns over quality of the clinical laboratory's product—test results—have been a driving force behind increased regulation and continual improvement in quality management systems (QMS). These include comprehensive policies, processes, and procedures to assure that all the various components affecting test results are designed, implemented, and monitored to assure highest quality.

Although laboratories are continually monitoring quality internally, external monitoring through a formalized process known as a laboratory inspection is also important. Every clinical laboratory in the United States is required by law to have a quality assurance program that is monitored by some accredited, unbiased, external agency. This includes an onsite inspection by an external agency that can take several days. During these visits, inspectors examine various aspects of the laboratory operation, as outlined below.

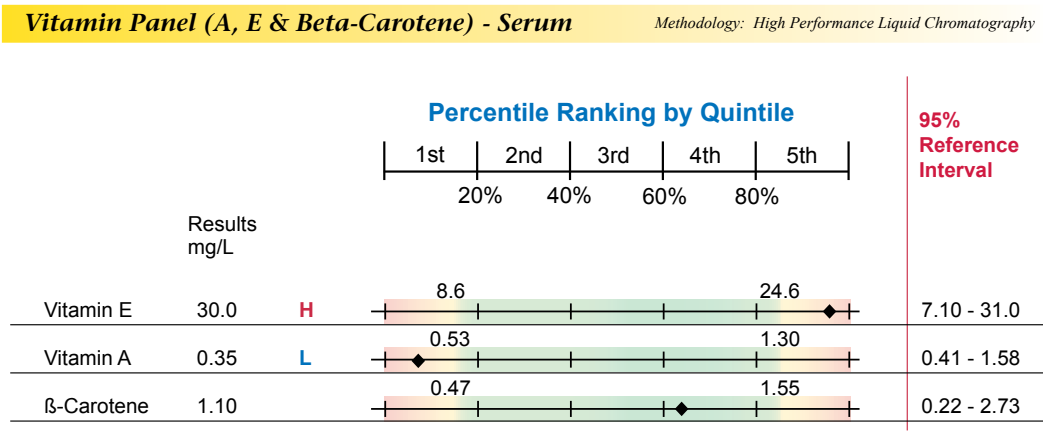

Figure 1.3 — An Example of Quintile and 95% Reference Interval Layout For Serum Vitamins

Each result is printed with units displayed above the column and abnormality flags (H or L) are printed to the right of the quantitative value. Charts show the position of the result relative to quintiles (hash marks) that are further sub-divided into deciles of the reference population. Abnormality is indicated when results fall below the 1st or above the 9th decile. To the far right the broader 95% reference interval is displayed for examination of more extreme abnormalities.

Quality Assurance Program

Since it is impossible for inspectors to directly view all the details of a laboratory's operation, they want to ascertain that the laboratory has clear, planned, and systematic policies and procedures for each component. They also check to see that these policies and procedures are, in fact, being followed by the laboratory personnel. A typical QMS quality assurance program describes how the laboratory:

- monitors and evaluates the overall quality of testing and other laboratory procedures;
- assures accurate, reliable, and prompt reporting of test results;
- assures the competency of their staff to perform their tasks;
- identifies and resolves problems; and
- monitors proficiency testing and other quality control data to substantiate that all tests conform to the laboratory's specified performance criteria.

Standard Operating Procedure Manual (SOPM)

The SOPM describes the methods, materials, and other information necessary to perform all the tests offered by the laboratory. Review of this document occupies a significant portion of the inspection process. The inspectors will then observe and talk with the laboratory staff to verify the procedures are being followed as set forth in the SOPM. The procedure manual describes the following:

- Test principle
- Clinical significance of the test
- Specimen requirements
- Required reagents
- Details of the test calibration
- Required quality control material
- Assay procedural steps
- Result calculations
- Reference intervals
- Interpretation of test results

Personnel Records

Inspectors will check to make sure the laboratory personnel performing the test procedures have the appropriate training, experience, educational background, continuing education, and responsibilities. The appropriate documentation on each laboratory employee performing tests must be kept in the employee's personnel records.

QUALITY CONTROL DATA

Quality control is a set of procedures designed to ensure that the test results regularly produced by the clinical laboratory are sufficiently reliable for use by the clinician. Each time laboratory technologists run an assay, they include a quality control sample that produces results that must fall within the laboratory's criteria of precision and accuracy. The inspectors review whether the laboratory has been running a sufficient number of QC samples and monitoring their results in a manner that can assure accuracy of results for patient specimens.

PROFICIENCY TESTING

Proficiency testing refers to a program of external quality assessment designed to check the accuracy of a test by comparing it with a known concentration in a sample. The sample is provided by a certified external agency and is shipped to all the laboratories participating in the survey. Each laboratory analyzes the sample using its instrumentation and methodology and reports its results back to the agency. The agency compiles the results from all the laboratories and sends an analysis of the results back to the laboratories. Historically, these programs developed from the clinical chemistry industry's need for determining test accuracy to allow interlaboratory comparisons. Initially, programs were developed using what were called "Olympian Labs." These were organizations whose quality was considered to be beyond question, and if they produced a test result, it must be correct. A system was set up to compare all other labs with the Olympian ones. Unfortunately, this approach did not last long, as it was quickly discovered that even the Olympians can make mistakes. It has become much more useful to define the "correct result" by consensus. All results are pooled and the deviation from the collective mean determines the individual laboratory's accuracy.

Proficiency reports may also require laboratory directors to correctly identify diseases indicated by the results, thus testing all aspects, including diagnosis of pathology. Proficiency testing programs have contributed greatly to the improved accuracy in laboratory testing. However, because of the expense of developing proficiency samples and administering the process, these programs are available only for tests that are commonly performed. If proficiency testing is not available, other methods may be used to check accuracy, such as split-sample comparison with other laboratories. Spiked recovery studies are also used, wherein a specimen is split into several parts and each is spiked with a different, known amount of the analyte to be measured. The specimens are analyzed and the results are compared to see if the method is yielding the known difference amongst the samples.

WORKFLOW ASSESSMENT

Weaknesses in a quality assurance program can be determined by performing a workflow assessment, where a single specimen and test order are monitored from the beginning (pre-analytic) to the end (post-analytic) of the entire process. The assessment may be divided into the following three segments:

1. *Pre-analytic* (Was the specimen collected, shipped, received, and processed properly, and was the test order entered into the computer system correctly?)
2. *Analytic* (Was the test performed according to procedures, was the instrument functioning properly, and were the results reviewed before reporting?)
3. *Post-analytic* (Were the results reported and archived appropriately, and was the specimen stored properly for potential retest if necessary?)

LICENSING AND CERTIFICATION

MONITORING BY EXTERNAL AGENCIES

Federal: In response to public furor about one or more deaths attributed to false-negative Pap smear readings, Congress passed the Clinical Laboratory Improvement Amendments (CLIA) in 1988, which expanded earlier regulations to include all laboratories, regardless of size or location (including physician office laboratories), that test human specimens collected in the United States and its territories. This means that all clinical laboratories, except for those that perform only waived tests (such as urine dipstick), are inspected for compliance with CLIA regulations by the Center for Medicare and Medicaid Services (CMS) or another agency recognized by CMS as having standards equal to or more rigorous than its own.

State: States have their own licensing agencies, and some of these require out-of-state laboratories to be licensed in their states.

Voluntary: There are also voluntary accrediting agencies, such as the College of American Pathologists (CAP); COLA; and the Joint Commission of Healthcare Organizations (JCAHO). Although these agencies were originally created to serve different types of clinical laboratories, today they share similar scopes and objectives.

The International Standardization Organization (ISO) and Clinical and Laboratory Standards Institute (CLSI) have issued standards and guidelines for clinical laboratories. These are used by licensing and accrediting agencies as they write their own guidelines, and by clinical laboratories as they develop their own operating procedures and quality assurance programs.

Standards and guidelines are readily available free or for purchase on the Internet. Free guidelines are available through CLIA, CAP, and CLEP Web sites. Others are more restricted and require membership (COLA, JCAHO). CLSI and ISO standards can be purchased through their Web sites:

- CLIA/CMS: www.cms.hhs.gov
- CAP: www.cap.org
- CLEP: www.wadsworth.org
- COLA: www.cola.org
- JCAHO: www.jcaho.org
- CLSI: www.clsi.org
- ISO: www.iso.org

Conclusions

Certification by any agency does not ensure that laboratory errors will not occur. For example, errors were detected at a CAP-accredited laboratory, resulting in a potential for releasing several hundred inaccurate HIV results. CAP quickly responded by making significant alterations in its inspection program to improve its own quality assurance. It now uses unannounced inspections and is improving its training of inspectors. Each agency responds to such quality issues by improving its own standards for monitoring.

Some voluntary accrediting agencies will accredit laboratories only where a significant percentage of the test menu falls within the agency's area of expertise. This makes the inspection and accreditation process substantially simpler and reduces liability for the accrediting institution. Their "area of expertise" includes tests that:

- use FDA-approved kits and/or instrumentation,
- have external proficiency testing programs, and
- are widely accepted in the medical community as having relevance to patient care.

Many of the tests offered in the integrative and functional medicine market are innovative and not widely offered in conventional medicine. Many of the newer tests do not use FDA-approved kits or have external proficiency testing programs available. They may not be widely accepted in the medical community as having relevance to patient care. However, the testing that currently meets the criteria for accreditation began in the innovative laboratory. The accrediting agencies realize the contribution innovative tests make to medicine, and they are working to find ways to assist these laboratories with their quality assurance programs, while not incurring excessive burden and liability.

Since biochemical imbalances created by nutritional insufficiencies precede clinical signs and symptoms, laboratory testing can provide early warnings of nutrient-related clinical problems. By its nature, the assignment of sensitivity and specificity values for this type of testing is very difficult, since there are no established ways to identify true positives and true negatives. Nutrition affects multiple metabolic processes, and laboratory evaluation of nutritional status can take many forms. The chapters that follow provide the clinician with insight regarding the scientific basis of tools to evaluate patient metabolic issues and to assist in developing safe, cost-effective, and reliable treatments for restoring normal function.

Notes:

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Notes:

Chapter 2

Vitamins

Richard S. Lord and Elizabeth H. Redmond

CONTENTS

Notes:

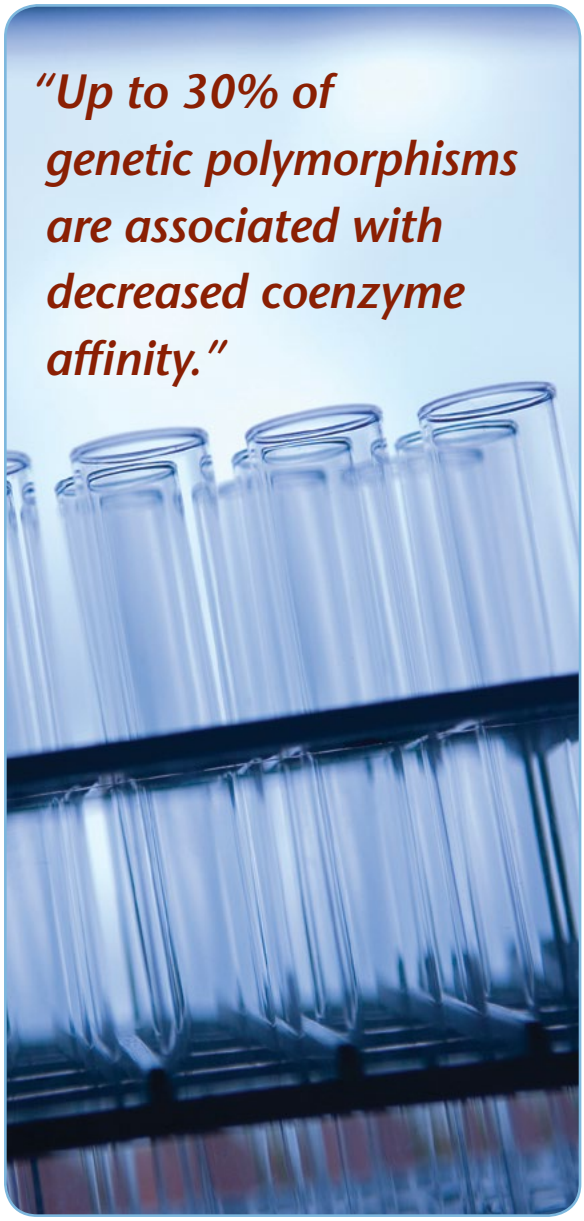
TABLE 2.1 — SUMMARY OF VITAMIN STATUS EVALUATIONS

Vitamin	Common Deficiency Tests	Result	Adult Repletion (/d)*
Vitamin A (Retinol)	Vitamin A – serum Beta-carotene – serum	Low Low	5,000–10,000 IU
Vitamin B_1 (Thiamin)	Alpha-keto acids – urine Erythrocyte Transketolase Index – red blood cell (RBC)	High High	50–300 mg
Vitamin B_2 (Riboflavin)	Alpha-keto acids – urine Ethylmalonate – urine EGR Activity Coefficient – RBC	High High High	50–200 mg
Vitamin B_3 (Niacin)	N-Methylnicotinamide – urine Lactate & pyruvate – urine	Low High	100–1000 mg
Vitamin B_5 (Pantothenate)	Alpha-keto acids – urine Pantothenic acid – urine	High Low	100–1,000 mg
Vitamin B_6 (Pyridoxine)	Xanthurenate – urine Kynurenate – urine Homocysteine – plasma EGOT Index – RBC	High High High High	50–200 mg
Vitamin B_{12} (Cyanocobalamin)	Methylmalonate – urine Homocysteine – plasma Vitamin B_{12} – serum	High High Low	100–1,000 µg
Tetrahydrobiopterin (BH_4), Dihydrobiopterin (BH_2)	BH_4 – plasma Phe or Phe/Tyr ratio – plasma BH_4 loading test – plasma	Low High Drop > 50%	2–20 mg/kg/d
Vitamin C (Ascorbate)	Vitamin C – serum Vitamin C – leukocyte Vitamin C – urine after 500 mg oral load	Low Low Low	1,000–5,000 mg
Vitamin K	PIVKAII – serum Undercarboxylated osteocalcin – serum Vitamin K – serum Prothrombin – plasma	Low High Low High	500–1,000 µg
Vitamin D	25-Hydroxyvitamin D – serum Bone-specific collagen fragments – urine	Low High	700–10,000 IU
Vitamin E (Tocopherol)	Tocopherol – serum Tocopherol/triglyceride – serum	Low Low	200–1,600 IU
Biotin	Beta-Hydroxyisovalerate – urine	High	500–5,000 µg
Folate	Homocysteine – plasma Folate – serum Folacin – RBC Macrocytic anemia – whole blood Neutrophil hypersegmentation – whole blood Formiminoglutamate (FIGLU) – urine	High Low Low Positive Positive High	200–800 µg
Carnitine	Adipate, suberate, ethylmalonate – urine	High	250–1,000 mg/TID
Coenzyme Q_{10}	Coenzyme Q_{10} – serum Succinate – urine Hydroxymethylglutarate – urine	Low High High	10–300 mg
Lipoic acid	Lactate and pyruvate – serum or urine	High	10–1,800 mg
Choline	Choline – plasma	Low	1–2 g

**Initial repletion should last 90 days. The client should then be retested and reevaluated.*

Introduction

Vitamin status may be assessed using a variety of direct and indirect measurements. Concentrations of vitamins can be measured in serum or blood cells. Vitamin metabolites may be measured in blood or urine. Changes in response to added vitamins may be measured as specific enzyme activities in blood or growth of leukocytes in cell culture. Finally, functional adequacy of a particular vitamin can be revealed by the urinary levels of specific metabolic intermediates controlled by the action of the vitamin.



Urinary organic acid analysis has traditionally been used for the detection of genetic diseases leading to frank inborn errors of metabolism.[1] Although up to 30% of genetic polymorphisms are associated with decreased coenzyme binding affinifty,[2] it is now widely accepted that genetic polymorphisms have a range of severity. One example is maple syrup urine disease (MSUD). The condition is primarily known to result from a faulty enzyme complex (branched-chain alpha-keto acid dehydrogenase). However there are numerous subunits and configurations that can be affected, so there is a range of possible downstream consequences. Research has identified patients diagnosed with MSUD who were found to be responsive to moderate to large doses of thiamin.[2] Similarly functional deficits of coenzyme vitamins are revealed in organic acid testing. It follows logically that there may be many yet undiagnosed milder forms of these genetic polymorphisms with symptoms not generally associated with the frank disease. These milder genetic variants may not appear until a patient encounters a specific stress, or life stage such as adolescence, or a nutrient deficiency that slows the enzyme reaction. For routine clinical purposes, the most useful assay gives a clear answer to the question of whether body pools are adequate to meet current demands. Table 2.1 shows a summary of the laboratory evaluations of vitamin status that are discussed in this chapter.

Methods of Vitamin Assessments

Vitamins encompass several classes of compounds. The challenge of determining adequacy of body stores is complicated by the diversity of structures, the presence of multiple forms and the availability of specimens reflecting the whole body vitamin stores. No single assay can give adequate information about all vitamins. Technological advances for detecting metabolic markers of vitamin deficiencies have made functional testing available. Concurrently, the knowledge of metabolic intermediates that can serve as markers of functional deficiencies has also grown.[3]

For various reasons specific to each vitamin, it is possible for an individual to have normal serum levels of a vitamin while exhibiting signs of insufficiency for that vitamin owing to a lack of adequate intracellular concentration to meet the metabolic demands of the cells. For example, serum vitamin B_{12} can be normal while the

metabolic intermediate methylmalonate is elevated owing to inadequate intracellular vitamin B_{12} to sustain the function of the enzyme methylmalonyl-CoA mutase.

An intermediate in a pathway that normally has high flux owing to macronutrient processing provides the greatest sensitivity of a metabolic marker for revealing early or mild vitamin insufficiency. The amino acid catabolic pathways offer this advantage. One example of this is the degradation of excess histidine to glutamic acid through the intermediate formiminoglutamic acid (FIGLU). The conversion of FIGLU to glutamic acid requires the participation of folic acid and mild insufficiencies of the vitamin produce elevations of FIGLU. The severity of FIGLU elevation may be related to the severity of folic acid deficiency, under standard dietary protein intake conditions. Using this type of relationship, it is therefore possible to specifically tailor nutrient supplementation according to the need for repletion to restore the metabolic capacity. Since many of the metabolic markers we will discuss are amino acid pathway intermediates, a high protein meal or challenge dose of amino acids prior to specimen collection may be employed to maximize the sensitivity of the test.

The first widely available tests of vitamin concentrations in serum were microbiological growth assays. Protozoan organisms whose growth is dependent on the presence of vitamins are grown in media that supply all but the vitamin being tested. The assays are very labor intensive and, because of the large number of variables affecting growth of whole cells, the reliability of results is frequently poor. These methods have been replaced by immunoassays with greater sensitivity and reproducibility and lower cost.

Vitamins A, E, beta-carotene and coenzyme Q_{10} are typically measured in serum using high performance liquid chromatography with various detectors. The fat-soluble components are extracted from the specimen and need no further preparation prior to injection into the instrument for chromatographic separation. Vitamin D, also in serum, can be measured by immunological assay or HPLC. The urinary organic acid markers of vitamin status, however, are more accurately measured by chromatographic separation (either gas or liquid) coupled with mass spectrometric detection. The newer technology of liquid chromatography with tandem mass spectrometric detection (LC/MS/MS) requires less sample preparation and therefore is more accurate than the older methods using gas chromatography (GC/MS). For further discussion of instrumentation, see Chapter 1, "Basic Concepts."

An enzyme stimulation assay works by adding saturating amounts of a vitamin to an enzyme whose activity is dependent on that vitamin to cause an increase in activity. The increase in enzyme activity corresponds to how poorly the enzyme was initially supplied with the vitamin (called cofactor saturation). Erythrocyte transketolase activity, for example, is dependent on thiamin pyrophosphate (TPP). The activity is measured without any added TPP and again after adding excess TPP. The increase in activity may be expressed as a ratio or percentage.[4] Abnormally high increases in activity indicate the degree of deficiency. An inherent limitation of the assay is that only one enzyme in erythrocytes is examined so total body functional status of the vitamin may not be revealed.

Another type of stimulation assay involves whole cell responses. Leukocytes are removed from the patient sample and grown in media with increasing concentrations of the vitamin to be assayed. Vitamins must be added in their active cofactor forms. Changes in growth rate at increasing levels of added nutrients provide a measure of the initial state of nutrient adequacy. Cells from a vitamin-deficient patient will require more added vitamin to achieve maximum growth than those from a well-nourished patient. This approach has great potential because it measures the total cellular response to the nutrient. The limitation is that, as with protozoan growth assays, there are many variables controlling the growth of whole leukocytes. Current techniques present extreme challenges to control all possible variables in the growth media. Because the technique depends on the initial viability of the cells, specimen transport also adds a significant variable. It is available for most vitamins and other classes of essential nutrients.[5]

Notes:

Assessing Vitamin Status

Vitamin A (Retinol) and Beta-Carotene

Active forms: All-*trans*-retinoic acid (retinoic acid), 9-*cis*-retinoic acid, 13-*cis*-retinoic acid,[6] 11-*cis*-retinal

Biochemical role: Cell proliferation,[6] cell differentiation, vision

Examples: 11-*cis*-retinal + light ➔ all-*trans*-retinoic acid

Deficiency tests: Vitamin A-serum / beta-carotene-serum

Adult repletion: 5,000 to 10,000 IU/d

Physiological Function: Specific maintenance roles for vitamin A have been reported for vision, bone growth, skin and mucosal integrity, spermatogenesis, as well as protection against cataracts, atherosclerosis, macular degeneration and cancer.[6, 7] The effects of vitamin A are tissue-specific. Retinoic acid inhibits proliferation of tumors, including breast, prostate, non-small-cell lung and thyroid cancers, as well as myeloid leukemias.[6] Retinoic acid and 9-*cis*-retinoic acid bind to nuclear retinoic acid receptors (RAR), while 9-*cis*-retinoic acid can also bind to nuclear retinoid X receptors (RXR), thereby acting as hormones and affecting cell cycle.[8] In addition to the mechanism of retinoic acid in vision, vitamin A is also involved in sub-cellular membrane function and mucopolysaccharide synthesis.[9] Vitamin A in animal foods (e.g., liver, dairy products and fish) comes in the form of preformed vitamin A. Vitamin A from plants comes from provitamin A or carotenoids.[10] Preformed vitamin A is more active than provitamin A, thus a larger amount of provitamin A from carotenoids is needed to meet the vitamin A requirement.[10]

Deficiency: Deficiencies of vitamin A result from inadequate diets, excessive alcohol intake, gastrointestinal conditions with diarrhea or fat malabsorption, as well as insufficient protein, energy and zinc status leading to inadequate retinol binding synthesis.[11-13] Hypovitaminosis A is a major nutritional problem throughout the world, including in several North American populations, though more profound in developing countries.[14] In these populations, severe vitamin A deficiency leads to xerophthalmia, the most common cause of blindness in young children.[15] Hypovitaminosis A can also lead to anorexia, growth retardation, recurrent infections, obstruction and enlargement of hair follicles, keratinization of skin epithelial cells and microcytic anemia.[8] Increased mortality is associated with clinically evident hypovitaminosis A in children; however, children with hypovitaminosis A but without overt clinical signs are also at increased risk for mortality.[8]

Assessment of Status: Vitamin A status has no known impact on circulating enzyme activities or excretion of metabolic intermediates, so there is no biochemical measurement of functional vitamin A deficiency. Thus measurement of serum vitamin A concentration remains the best method to assess individual nutritional status of this vitamin. Serum concentrations of vitamin A fall during low dietary intake.[16] A dose-response relationship exists between oral supplementation and serum vitamin A in humans. Once normal tissue levels are obtained, daily intake of vitamin A has been maintained at levels of 10,000 IU for five years without significant long-term rise in serum retinol.[17] Serum retinol levels < 30 µg/dl indicate depleted liver stores of vitamin A, while levels between 30 and 60 reflect increasing liver stores. A patient with both a long-standing low intake of vitamin A and a low tissue reserve may have a serum concentration < 20 µg/dl. Adult men achieve a level of approximately 60 µg/dl at a retinol intake of 2,400 µg (8,000 IU) per day.[18, 19] Adult repletion can generally be achieved with supplementation of 5,000 to 10,000 IU a

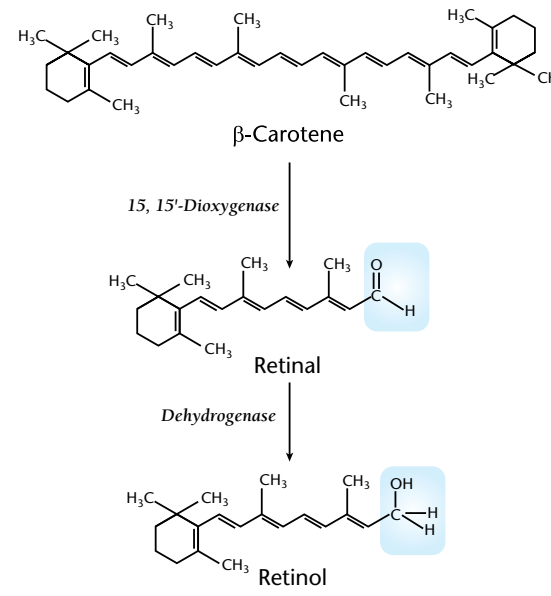


Figure 2.1 — Conversion of β-Carotene to Retinol

Beta-carotene, a carotenoid found in plants, is absorbed and converted to vitamin A (retinol) in the intestine in the presence of bile acids.

day for 90 days, though consideration should be made to the size of the person and any immune challenges, or losses of vitamin A, such as during lactation. Serum levels greater than 150 µg/dl indicate excessive vitamin A intake with potential toxic responses. Acute, chronic and teratogenic syndromes of vitamin A toxicity have been associated with very high doses. Danger of such toxic responses is especially high during early pregnancy.[19]

Carotenoids — Dark-colored fruits and vegetables are excellent sources of carotenoids.[20] Carotenoids are a family of closely related compounds. More than 600 carotenoids have been identified and many, but not all, can be converted into retinol.[8] Of all the carotenoids, beta-carotene is converted into retinol most efficiently.[21] The absorption of beta-carotene and its conversion to vitamin A varies among individuals. Research has found some individuals have an impaired absorption and conversion and are referred to as low responders.[22] Methods have been developed for the measurement of up to 50 different carotenoids in plasma.[23, 24] Although little useful clinical information is gained from such exhaustive analysis, the measurement of beta-carotene along with serum vitamin A is useful because beta-carotene is converted into vitamin A in the liver (Figure 2.1). Thus beta-carotene serves as a pool that may be converted into vitamin A. It also has antioxidant and other roles independent of vitamin A. Research has shown beta-carotene supplementation to result in significant dose-related decreases in serum cholesterol and triglyceride fractions, indicating anti-hyperlipidemic effects in spontaneously hypertensive rats.[25] Body pools of the two compounds are maintained independently so that, unless vitamin A levels are initially low, increased intake of beta-carotene may not affect higher levels of vitamin A.[26]

OF FURTHER INTEREST...

Epidemiologic research and clinical trials overall have found that increased dietary intakes and elevated serum levels of beta-carotene are associated with decreased rates of disease.[27] However, two large clinical trials of smokers and asbestos workers found those given high doses of synthetic beta-carotene supplements had higher rates of lung cancer and raised questions concerning the safety of supplements for high risk populations.[28-30] There are many possible reasons why these clinical trials did not show the protective benefits seen in other research and are discussed elsewhere.[31, 32]

Vitamin A deficiency inhibits thyroid function.[33] Conversely, thyroid hormone is involved in carotenoid metabolism and transport. Elevated beta-carotene and retinol concentrations in plasma are often associated with hypothyroidism.[34, 35] In the United States, almost 75% of average dietary retinol equivalents is supplied as preformed vitamin A and 25% is in the form of beta-carotene.[36]

REFER TO CASE ILLUSTRATION 2.1

Notes:

VITAMIN B_1 (THIAMIN)

Active form: Thiamin pyrophosphate (TPP)
Biochemical role: Decarboxylation
Example: Pyruvate ➔ Acetyl-CoA + CO_2
Deficiency tests: alpha-keto acids- urine; Erythrocyte Transketolase Index
Adult repletion: 50 to 300 mg/d

Physiological Function: The two primary functions of thiamin are alpha-keto acid decarboxylation and transketolation.[10] Decarboxylation reactions are an integral part of carbohydrate metabolism.[37] Thiamin is involved in the alpha-keto acid decarboxylation of pyruvate, alpha-ketoglutarate and the branched-chain alpha-keto acids (leucine, isoleucine and valine metabolites). Transketolation is involved in the pentose phosphate pathways and is an early marker of decreasing thiamin levels.[7, 38] Thiamin is converted to its active form—thiamin pyrophosphate—the primary coenzymatic form (Figure 2.2).[38]

Deficiency: Food processing, elevated pH and high temperatures result in significant losses of thiamin.[10] The ease with which thiamin is depleted by processing rice creates an increased risk of deficiency among populations where rice is the staple food. A cross-sectional survey of an adult Mediterranean population found 6.4% deficient in thiamin according to the erythrocyte transketolase assay (described below).[39] Increased vigilance to ensure thiamin supplementation during pregnancy is suggested because sub-clinical thiamin hypovitaminemia is prominent during pregnancy despite vitamin supplementation.[40] During the third trimester among Spanish women, 25% of subjects were found to have intakes below recommended levels and 14% showed signs of severe thiamin deficiency.[41] Because of the thiamin depleting effects of gestational diabetes, subclinical thiamin hypovitaminemia was also found in 19% of pregnant mothers despite vitamin supplementation and prescribed glycemic control. These observations have led to the hypothesis that macrosomic neonates from mothers with gestational diabetes might display a fetal form of wet beriberi with symptoms of pudgy face, plump body, cardiomegaly and pitting edema.[40]

Assessment of Status: Thiamin deficiency will occur when intake is insufficient to maintain tissue levels high enough to supply all enzymatic demands. The need for adequate functional thiamin to achieve optimal health for individuals is demonstrated in the growing list of known thiamin-responsive disorders of metabolism, which includes hyperkinetic cardiomyopathy, megaloblastic anemia and thiamin-responsive maple syrup urine disease.[2, 42] Genetic polymorphisms of thiamin-dependent enzymes can alter thiamin binding affinity and enzyme function. This can result in abnormally rapid urinary losses of thiamin. Tissue and serum levels that are in the "normal range" may be insufficient in most individuals. In these people, providing higher-than-normal doses of thiamin can increase enzyme activity and correct functional deficiencies. Biochemical markers of functional sufficiency are therefore the most useful measure of adequacy. For example, three family members with dysautonomic symptoms, comparable to those seen in classical beriberi, required high doses of thiamin tetrahydrofurfuryl disulfide to restore biochemical function as measured by transketolase activity.[43]

Transketolase — Erythrocytes heavily utilize the pentose phosphate pathway to produce reducing equivalents for maintaining reduced glutathione. This pathway involves two steps catalyzed by a transketolase enzyme that requires thiamin pyrophosphate (TPP). This enzyme may be used to perform a stimulation assay from a whole blood specimen. A "TPP stimulation" result greater than 14% demonstrates a significant risk

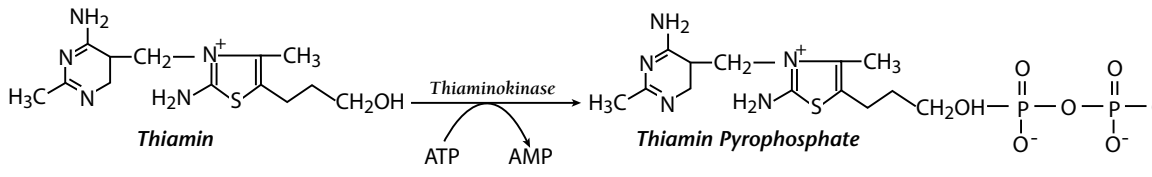


FIGURE 2.2 — **Conversion of Thiamin to Thiamin Pyrophosphate**
In the intestines, thiamin pyrophosphate is released from proteins and converted to thiamin. Thiamin is transported throughout the body and converted back to thiamin pyrophosphate.

TABLE 2.2 — MARKERS ELEVATED IN VITAMIN B_1 DEFICIENCY

Test
Isoleucine, plasma
Isoleucine, urine
α-Ketoisovalerate, urine
α-Ketoisocaproate, urine
α-Keto-β-methylvalerate, urine
Erythrocyte transketolase

of thiamin deficiency.[44] This approach is useful, but it is focused on a single enzyme in erythrocytes. Limits have been proposed for higher risk of deficiency (Table 2.3).

Urinary Functional Markers — A better test for revealing functional thiamin deficiency may be the detection of elevated branched-chain keto acids in urine. The most sensitive and definitive measure of thiamin adequacy is obtained from detection of increased levels of amino acids and their keto acid analogs that are excreted in the absence of adequate tissue levels of thiamin. Because the catabolism of amino acids is a heavily utilized pathway in the liver, the breakdown of branched-chain amino acids (BCAAs) affords an opportunity for detecting interruptions in the pathway caused by inadequacy of thiamin. Leucine, isoleucine and valine are deaminated to their corresponding keto acids prior to the first thiamin-dependent step.

Increased plasma or urine isoleucine, or the appearances of significant levels of branched-chain keto acids (BCKAs) in urine are markers of functional thiamin deficiency.[45] Thiamin deficiency also results in increased blood pyruvate, but this effect is not specific for thiamin.[46] The combination most useful for assessing an individual's need for thiamin is isoleucine, pyruvate, alpha-ketoisovalerate, alpha-ketoisocaproate and alpha-keto-beta-methylvalerate (Table 2.2). The latter three are the keto acids of valine, leucine and isoleucine, respectively. Further information may be found in Chapter 6, "Organic Acids."

Urinary Thiamin — A portion of a thiamin load administered orally or intramuscularly will appear in urine within a few hours if the tissues are replete with thiamin. Parenteral administration of 5 mg of thiamin should result in the appearance of at least 20 µg in urine over the next four hours.[47] The parenteral thiamin load test is primarily useful as an indicator of intake, rather than functional sufficiency of thiamin.

The test used most frequently for population surveys is a measurement of urinary thiamin concentration. Urinary excretion of thiamin decreases proportionately with thiamin intake. This fact has led to measurement of thiamin levels in urine as an indicator of adequacy in nutrition surveys. Both 24-hour and random urine tests have been used to assess thiamin intake. Random urine readings, expressed as mg per gm of creatinine to correct for dilution, are directly related to intake in the range of 0.2 to 0.8 mg thiamin/100 Cal/day.[48, 49] When results are expressed per gm creatinine, children have much higher excretion rates than adults, necessitating the use of a sliding scale for reference limits (Table 2.3).

REFER TO CASE ILLUSTRATION 2.2

TABLE 2.3 — URINARY EXCRETION OF THIAMIN

Test	Unit	Subjects	Risk of Deficiency		
			High	Medium	Low
Loading urine	µg/4 hr	Adults	< 20	20–79	> 80
Timed excretion	µg/24 hr	Adults	< 40	40–99	> 100
	µg/6 hr		< 10	10–24	> 25
Random urine	µg/gm creatinine	Adults	< 25	25–55	> 55
		13–15 yr	< 27	27–65	> 65
		10–12 yr	< 60	60–180	> 180
		7–9 yr	< 70	70–180	> 180
		4–6 yr	< 85	85–120	> 120
		1–3 yr	< 120	120–175	> 175

VITAMIN B_2 (RIBOFLAVIN)

Active forms: Flavin mononucleotide (FMN) Flavin adenine dinucleotide (FAD)

Biochemical role: Redox – Central energy pathways

Example: Succinate + FAD ➔ Fumarate + $FADH_2$

Deficiency tests: alpha-keto acids – urine, ethylmalonate – urine, EGR Activity Coefficient

Adult repletion: 50 to 200 mg/d

Physiological Function: Riboflavin is primarily involved in energy production in the respiratory chain and in oxidative-reduction reactions.[7, 38] Riboflavin is converted to its coenzyme forms, flavinmononucleotide (FMN) and flavin adenine dinucleotide (FAD), which accept and transfer electrons[50] (Figure 2.3). FMN and FAD-linked enzymes are involved in many essential reactions, including mitochondrial respiration, citric-acid cycle, ATP synthesis and fatty acid metabolism.[51]

Deficiency: National Health and Nutrition Examination Survey II (NHANES II) data reported that up to 27% of elderly participants had low riboflavin intakes.[52, 53] The United Kingdom's National Diet and Nutrition Survey of adults > 65 years old showed that 41% of free living elderly participants have low vitamin B_2 status, while < 10% of participants recorded a low intake.[54] A study of 2,000 young adults found that 95% of adolescent girls ages 15 to 18 had low riboflavin levels with equally low intakes.[53, 55] In another study, during their first year of institutionalization, almost half of French elderly subjects tested were at risk of riboflavin deficiency.[56] Riboflavin comes primarily from dairy products and meats; therefore, diets low in these foods increase the risk for riboflavin deficiency.

Assessment of Status: Vitamin B_2 may be evaluated by assays of urinary markers, erythrocyte enzyme activation or urinary vitamin output (Table 2.4). Excretion of ethylmalonate, beta-hydroxyisovalerate and several acyl glycine compounds is evidence of inadequate activity of the flavin-binding enzyme system, multiple acyl dehydrogenase (MAD).[49] Individuals who excrete high levels of these compounds and show symptoms of easy fatigability and exercise intolerance have been successfully treated with riboflavin.[57] Similarly, elevated urinary methylsuccinate and suberylglycine are associated with riboflavin-responsive mitochondrial oxidation impairment.[58] Treatments usually involve 100 mg riboflavin with other related nutrients such as biotin, niacin and carnitine.

Erythrocyte Glutathione Reductase — In erythrocytes, oxidized glutathione is recycled to the reduced form by the action of the enzyme, erythrocyte glutathione reductase (EGR). The stimulation of activity of this enzyme by the addition of the FAD coenzyme is a good

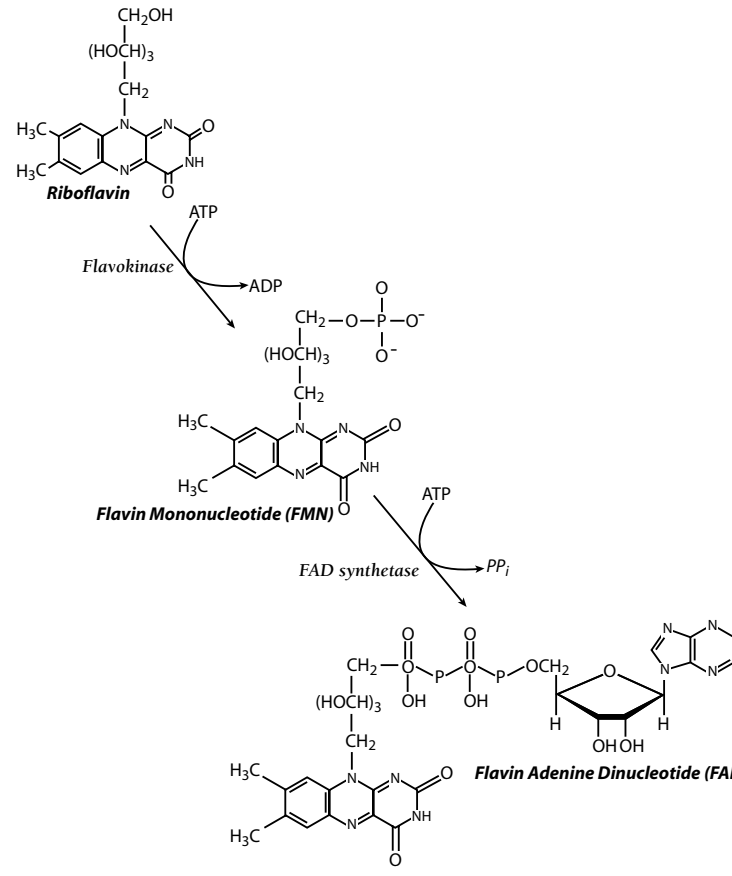


FIGURE 2.3 — Conversion of Riboflavin to Flavin-Adenine Dinucleotide (FAD)

Riboflavin is absorbed in the ileum and converted to FMN, FMN is converted to FAD. Both FMN and FAD can be hydrolyzed in the gut back to free riboflavin.

TABLE 2.4 — RIBOFLAVIN DEFICIENCY TESTS

Marker	Riboflavin Deficiency Indication
Methylsuccinate, ethylmalonate and suberylglycine	Levels above normal limits
EGR Activity Coefficient	> 1.2
Urinary riboflavin	< 80 nmol/g (Adult)
Erythocyte Flavin Concentrations (EFC)	< 270 nmol/L

functional measure of riboflavin adequacy. The value is expressed as the EGR Activity Coefficient, which is the ratio of activity with added FAD, divided by the activity without added FAD. This assay was used to demonstrate the presence of riboflavin deficiency in 5.3% of a random sample of 3,390 subjects from a cross-sectional survey of an adult Mediterranean population.[39]

EGR Activity Coefficient is a good indicator of riboflavin status, but not function, in a population with low (< 1.0 mg) dietary riboflavin intake.[59]

Riboflavin Excretion — Controlled human studies have established a correlation between dietary intake of riboflavin and urinary excretion of the vitamin. Individuals consuming less than the recommended amount of riboflavin excrete less than 100 µg of riboflavin per day. Urinary levels tend to reflect recent dietary intake and thus are prone to large variations. Children excrete more riboflavin per gram of creatinine than adults, so age-adjusted reference ranges are necessary. Erythocyte Flavin Concentrations (EFC) can be done to assess cellular conentrations.[59]

Notes:

VITAMIN B_3 (NIACIN)

Active forms: Nicotinamide adenine dinucleotide (NAD) Nicotinamide adenine dinucleotide phosphate (NADP)

Biochemical role: NAD^+: Redox – Central energy pathways $NADP^+$: Biosynthesis

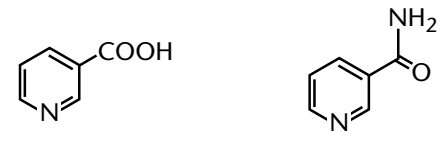
Example: Lactate + NAD ➔ pyruvate + NADH + H+

Deficiency tests: N-Methylnicotinamide, lactate & pyruvate – urine

Adult repletion: 100 to 1,000 mg/d

Physiological Function: The two main forms of vitamin B_3 are niacinamide and niacin (Figure 2.4). Its active coenzyme forms are nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Vitamin B_3 maintains cellular redox state via NAD and NADP, which function as coenzymes in oxidation and reduction reactions. NADP serves as a hydrogen donor and NAD serves as an electron acceptor.[7, 38] NAD and NADP cycle between their reduced forms, $NADH+H^+$ and $NADPH+H^+$[50]. Many oxido-reductase enzymes that utilize NADP and NAD function in catabolic pathways.[51]

Deficiency: In niacin-deficient animals, the biological responses to niacin, nicotinic acid and its amine form, niacinamide, are virtually equivalent (except at supraphysiological doses) because of the ease with which the liver produces the amine from the acid. Dietary analysis is complicated by the fact that nicotinamide is frequently bound in unavailable forms in foods, especially corn. The amino acid tryptophan can serve as a precursor to niacin in humans, but the conversion is dependent on adequate status of both vitamin B_6 and iron.[60] Although the optimum conversion of tryptophan to niacin yields only 1 mg from 60 mg of amino acid, daily L-tryptophan intake of several hundred milligrams can make a very significant contribution to total body niacin. For this reason, studies of niacin requirements are conducted by restricting dietary niacin and tryptophan.



Nicotinic Acid (Niacin) *Nicotinamide (Niacinamide)*

FIGURE 2.4 — Niacin and Niacinamide

Dietary niacin is amidated to form niacinamide, the precursor of the active coenzyme forms NAD and NADP.

The increased diversion of tryptophan into the serotonin pathway that occurs in carcinoid cancer patients puts them at increased risk of niacin deficiency.[61]

In addition to its enzyme cofactor role, NAD^+ is also the substrate for the enzyme that adds polyadenosyl chains to DNA as part of the gene repair mechanism. This reaction acts as a drain on the cofactor pool. Hyperoxia causes increased poly(ADP-ribose) formation to promote DNA repair and the ensuing niacin deficiency causes adaptive shifts in whole body NAD^+ metabolism.[62] Other experimentally induced cell injury effects are enhanced by niacin deficiency.[63]

Assessment of Status: Biochemical markers are especially helpful in assessment of niacin adequacy owing to the difficulty in establishing an accurate dietary intake and the wide range of symptoms related to niacin deficiency. Urinary nicotinic acid is relatively uninfluenced by dietary intake of niacin or tryptophan.

Testing based on excretion of catabolic products of niacin (i.e., N-methylnicotinamide) has been developed, but the test is not sensitive enough for use as a functional marker of mild deficiency states. The whole blood niacin number, a derived ratio of NAD/NADP x 100, has been used to demonstrate niacin deficiency (niacin number < 130) in carcinoid syndrome patients.[61] Erythrocyte NAD and polyadenosine diphosphate (ADP) ribosylation are promising markers of niacin depletion.[59] Urinary keto acid elevations provide potential biochemical markers of niacin deficiency. Since the keto acid dehydrogenase complex requires NAD^+ as a coenzyme, a niacin deficiency is also likely to produce keto acid elevations similar to that described above for thiamin.

OF FURTHER INTEREST...

When choosing forms of supplemental niacin, consider potential nicotinamide effects on DNA. The sirtuins are a family of proteins linked to lifespan determination. In lower organisms, increased expression of the NAD-dependent deacetylase Sir2 increases lifespan.[66] Genes for mitochondrial biogenesis are among those regulated by Sir2.[67] Nicotinamide is an inhibitor of human class III histone deacetylases such as SIRT1. Genetic expression of the hematopoietic malignancy-stimulating protein called B cell leukemia 11A protein (BCL11A) is inhibited (silenced) by SIRT1.[68] The overall effects may be a shortening of maximum lifespan along with increased rates of malignancy while experiencing short term benefits from regular, aggressive use of niacinamide instead of niacin, especially in the patient with relatively replete body stores of the vitamin. However, no adverse effects on aging have been demonstrated when nicotinamide is used for repletion of a niacin-deficient patient.

Increased fecal fat has been noted in niacin deficient rats,[64] possibly due to impairment of intestinal protein absorption. Animal research has also found the maintenance of intestinal epithelial cells to be highly dependent on niacin availability.[65]

Notes:

VITAMIN B_5 (PANTOTHENIC ACID)

Active form: Coenzyme A (CoA)
Biochemical role: Carrier for acyl groups
Examples: Acetyl-CoA, Fatty acyl-CoA
Deficiency tests: Alpha-keto acids – urine, Pantothenic acid – urine
Adult repletion: 100 to 1,000 mg/d

Physiological Function: By utilizing 3 ATPs and cysteine, pantothenic acid is converted into its active form, coenzyme-A in the liver (Figure 2.5). It functions as the acyl carrier in keto acid and fatty acid oxidation. All oxidation pathways for carbohydrate, fat and protein require coenzyme-A to carry acetate, keto-acids, or fatty acid intermediates during key oxidative steps.

Deficiency: Pantothenic acid or pantothenate derives its name from the observation that it is found in all life forms. It is found therefore, in all whole foods. Because of the centrality of coenzyme A in metabolic function, a mild pantothenic acid deficiency, or insufficiency, is one of the most difficult nutrient conditions to establish conclusively. Animals placed on diets without pantothenic acid display loss of appetite, slow growth, skin lesions, weakness and death.[50, 69] When body stores of coenzyme A are exhausted, all tissues experience massive decline in activity of energetic and biosynthetic pathways.[70] The pervasive nature of the effects on critical organs results in rapid, simultaneous decline of many functions, including the central nervous system.

Assessment of Status: When multiple intermediates from pathways that require the carrier function are found to be elevated, it is an indication that inadequate pantothenic acid is limiting optimum metabolism. Intermediates commonly reported in urinary organic acid analysis that can provide such clues are pyruvate, alpha-ketoisocaproate, alpha-ketoisovalerate, alpha-keto-β-methyl-valerate and alpha-ketoglutarate (see Chapter 6, "Organic Acids"). Urinary excretion of pantothenic acid less than 1.0 mg/day is also indicative of inadequate intake.[71]

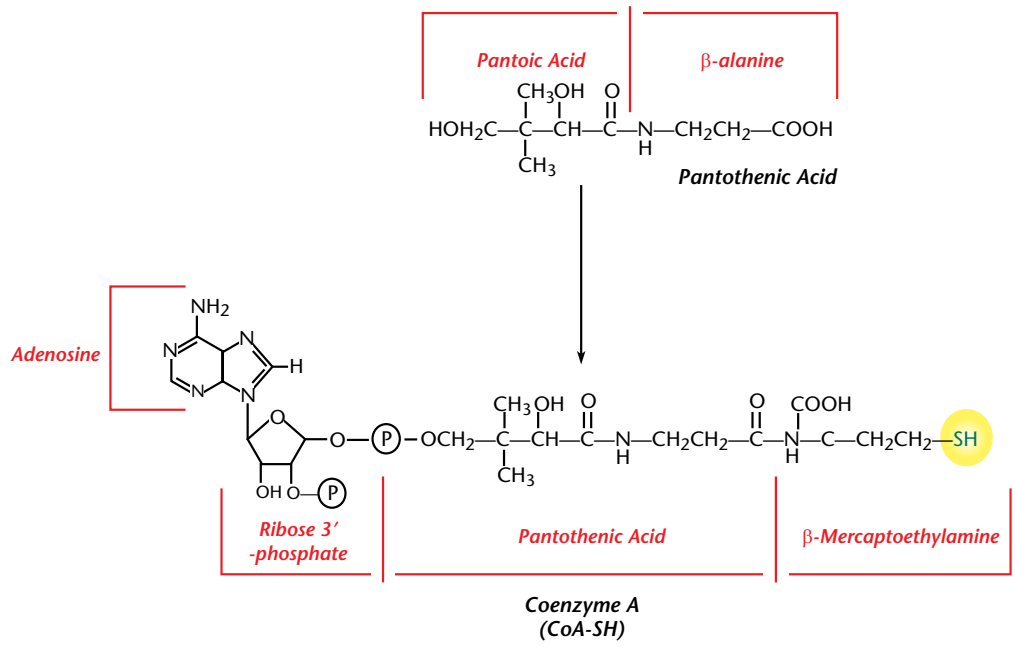


FIGURE 2.5 — **Conversion of Pantothenic Acid to Its Active Form, Coenzyme A**

Pantothenic acid is the dietary essential precursor required to form coenzyme A in tissues. Its conversion requires cysteine and ATP. Dietary forms include free pantothenic acid, coenzyme A, and acetyl-coenzyme A.

VITAMIN B_6 (PYRIDOXINE)

Active form: Pyridoxal-5-phosphate (P-5-P or PLP)
Biochemical role: Amino acid metabolism
Example: Homocysteine + Serine ➔ Cystathionine
Deficiency tests: Xanthurenate and Kynurenate – urine, Homocysteine – plasma, EGOT Index, EGPT Index, PLP- plasma
Adult repletion: 50 to 200 mg/d

Physiological Function: Multiple forms of vitamin B_6, called vitamers, are found in body fluids. Foods or dietary supplements may contain several forms. After ingestion, any of the following forms may appear in portal blood: pyridoxal-5'-phosphate (P-5-P or PLP), pyridoxal (PL), pyridoxine (PN) and 4-pyridoxic acid (4-PA) (Figure 2.6). This multiplicity of forms has been given the collective name, "Vitamin B_6." Erythrocytes have a high capacity for conversion of PL to the active form PLP and they can retro-convert the phosphorylated form to PL for export. Erythrocyte PLP content may help to maintain tissue cofactor levels by release of PL following a meal with high vitamer content.[72] The converting enzyme, pyridoxal kinase, has a gene expression regulation for local PLP requirements that is widely distributed. Thus, while supplemental PLP is more effective for a short term increase of plasma PLP, local conversions may be of more importance for governing tissue cofactor status.[73] For vitamin B_6 status assessment the requirement for local tissue conversions supports measuring functional biochemical markers over plasma PLP levels.

Supplementation with 40 mg of vitamin B_6 for three days in a randomized placebo-controlled study produced increases of 10-, 50- and 100-fold of PLP, 4-PA and PL, respectively. The concentrations remained at the same levels for the entire 84 days of supplementation.[73]

The active cofactor form is PLP, formed by phosphorylation of PL. Reports of plasma concentrations of vitamin B_6 have generally been based on HPLC or enzyme stimulation methods that measure PLP. There are approximately 60 enzymes involved in amino acid metabolism that require the active form PLP for their function; thus, vitamin B_6 deficiencies lead to decreases in B_6 dependent enzymes. Research has found that enzyme activities are not affected equally because of differing cofactor binding activities and variability in age.[74] Other PLP-requiring enzymes are needed for conversions of carbohydrates and fats. Sensitive tests for the detection of vitamin B_6 are necessary because of the many factors influencing its status (Table 2.5).

Plasma vitamin B_6 concentration may provide a useful measure of a patient's inflammatory status. Individuals with active inflammatory bowel disease were found to have lower median vitamin B_6 levels than normal controls. Furthermore, the frequency of low plasma vitamin B_6 in patients with active disease was found to be much higher than in patients without active disease. Low vitamin B_6 concentrations were also strongly correlated with elevated levels of C-reactive protein.[75]

Deficiency: Vitamin B_6 deficiencies affect the metabolism of polyunsaturated fatty acids. This can result in seborrheic dermatitis, cheilosis and glossitis, or cause a decrease in neurotransmitters leading to irritability, depression and confusion. Finally, vitamin B_6 deficiency can decrease hemoglobin synthesis leading to microcytic anemia.[38, 59] Patients whose plasma fatty acids profile shows an elevated ratio of alpha linolenic acid to docosahexaenoic acid may be deficient in vitamin B_6.[76] Smoking further increases the incidence of vitamin B_6 deficiency.[77]

TABLE 2.5 —FACTORS AFFECTING VITAMIN B_6 STATUS

Factor	Example
Decreased supply	Marked loss in food processing
Increased loss	Urinary loss due to diuretics
Increased demand	Increased needs with pregnancy
Medications that reduce absorption, increase PLP turnover or bind PLP	
Oral contraceptives	
Isoniazid and cycloserine (anti-tuberculosis)	
Hydralazine (hypertension)	
Alcohol	
Penicillamine (antirheumatic & lead poisoning therapeutic)	
Antimetabolite environmental chemicals	
Hydrazines from smoking	
Rocket fuels	
Maleic hydrazide (herbicide) Succinic acid-2,2-dimethylhydrazide (fruit-ripening agent) Tartrazine (FD&C yellow No. 5)	

Assessment of Status: Vitamin B_6 is the principal participant of the nutritional triad in the metabolism of homocysteine (Figure 2.7). The other nutrients, vitamin B_{12} and folate, are also discussed. The B_{12}-folate system allows the recovery of homocysteine as methionine, while the PLP pathway is necessary for the conver-

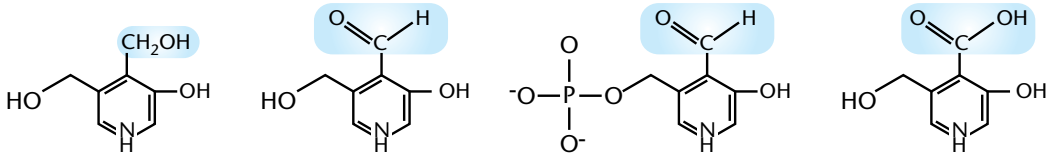


FIGURE 2.6 — **Multiple Forms of Vitamin B_6 (Pyridoxine)**

Vitamin B_6 has several forms. Proteolytic enzymes in the stomach and gut release various forms of vitamin B_6 from food. PL and PN are transported to tissues and converted to PLP. Vitamin supplements generally contain PN.

sion of homocysteine to cysteine.[78] Significant portions of the population in developed countries carry a genetic determinant that causes weaker cofactor binding to the enzymes, cystathionine alpha-synthase and 5,10-methylenetetrahydrofolate reductase (MTHFR). Individuals with defective enzymes require greater concentrations of the coenzyme to achieve necessary enzyme activity levels to protect from homocysteine accumulation.[79] A limitation of homocysteine as a marker of B_6 deficiency is its lack of specificity, owing to the concurrent involvement of B_{12}- and folate-dependent enzymes.

Specific biochemical markers of functional vitamin B_6 status are xanthurenate and kynurenate, which are organic acids produced from tryptophan. The breakdown of these three compounds depends on enzymes that function only when PLP is bound.[80] Inadequate PLP results in the accumulation of these markers and spilling into urine; thus their presence above normal limits in urine indicates a vitamin B_6 deficiency (Table 2.6).

Erythrocyte transaminase enzymes provide another method for assessing vitamin B_6 status. This sensitive and specific assay has been frequently used in investigations of vitamin B_6 status.[81] Erythrocyte glutamate-oxaloacetate transaminase (EGOT) and glutamate-pyruvate transminase (EGPT) activities decline with inadequate intake of vitamin B_6. Their activities can be restored in the laboratory by the addition of PLP and the ratio of the stimulated to unstimulated reaction rates provides a reliable measure of vitamin B_6 depletion (Table 2.6). One reason this assay has not found wide clinical application is its relative expense as a single nutrient marker. Plasma PLP has been found to reflect tissue stores, though it is slow to respond to changes in vitamin status and decreases with increasing protein intake and age.[59]

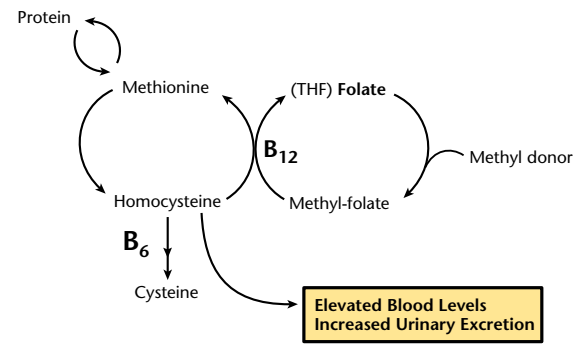


FIGURE 2.7 — **Nutrient Requirements for Homocysteine Metabolism**

Re-methylation of homocysteine to form methionine requires folic acid and vitamin B_{12}. Methyl donors include glycine, betaine and dimethylglycine. The alternative conversion of homocysteine to cysteine is vitamin B_6 dependent. Insufficiencies of any of these factors can cause elevated homocysteine in blood and urine.

TABLE 2.6 — TESTS FOR VITAMIN B_6 DEFICIENCY

Vitamin B_6 Test	Deficiency Indication
Plasma homocysteine (HCys)	> 15 nmol/mL
Urinary homocysteine	> 25 µg/mg creatinine
Urinary xanthurenate, kynurenate	Above reference limit
EGOT index	> 1.5
EGPT index	> 1.25
Plasma PLP	< 30 nmol/L

Notes:

VITAMIN B_{12} (COBALAMIN)

Active forms: Methylcobalamin, and 5′-Deoxyadenosylcobalamin (Coenzyme B_{12})

Biochemical role: Methyl group transfer

Examples: Methylmalonyl-CoA ➔ Succinyl-CoA (Coenzyme B_{12}) Homocysteine➔ Methionine (methylcobalamin)

Deficiency tests: Methlymalonate – urine Homocysteine – plasma, Vitamin B_{12} – plasma

Adult repletion: 100 to 1,000 µg/d

Physiological Function: Having the most complex structure of all vitamins, vitamin B_{12} uniquely contains an organometallic bond (Figure 2.8). All forms of vitamin B_{12} contain hexavalent cobalt as the central ion in the corrin ring that forms the core of the molecule. Four nitrogen atoms of the corrin ring and one from a side group surround the cobalt ion. The sixth valence may be occupied by a variety of ligands. During early purification work the cyanide group was picked up as the sixth ligand leading to the cyanocobalamin form being designated as "vitamin B_{12}." The cyanide form is easily metabolized to active forms, although tobacco smoking causes a displacement back to the inactive vitamin form owing to chronic inhalation of low levels of cyanide. The corrin ring is similar to the heme ring, but key structural alterations dictate the highly specific and stable binding of cobalt rather than iron.

Deficiency: Deficiencies of vitamin B_{12} result from inadequate dietary intake or impaired absorption from lack of intrinsic factor or insufficient gastric secretions. Approximately 60% of B_{12} deficiency in elderly adults was due to decreased absorption of protein bound cobalamin.[82] Because of the close relationship between the biochemical role of vitamin B_{12} and folic acid, the clinical and hematological results of deficiencies of the two nutrients are indistinguishable. The relative time scale for various manifestations of folate deficiency may be applied similarly to vitamin B_{12}.

TABLE 2.7 —TESTS FOR VITAMIN B_{12} DEFICIENCY

Vitamin B_{12} Test	Deficiency Indication
Methylmalonate, urine	> 3 µg/mg creatinine
Methymalonic acid, blood	> 0.4 µmol/mL
Homocysteine, urine	> 25 µg/mg creatinine
Homocysteine, plasma	> 9.8 nmol/mL
Serum vitamin B_{12}	< 150 pg/mL

The detection of vitamin B_{12} deficiency raises other questions regarding the metabolic impact and origin of the deficiency. Patients receiving low dose aspirin therapy are more likely to have vitamin B_{12} deficiency, probably owing to the toxic effects of aspirin on the gastric mucosa that cause decreased secretion of intrinsic factor.[83] In other patients, the accumulation of methylmalonic acid caused by vitamin B_{12} deficiency can cause a disruption of normal glucose and glutamic acid metabolism.[84]

Assessment of Status: Tests specific for vitamin B_{12} measure serum concentration and urinary methylmalonate (MMA) excretion. Urine MMA is a measure of functional adequacy, while serum concentration measures the amount available for use. The distinction is important because of the possibility that a "normal" serum concentration may be inadequate to saturate vitamin B_{12}-requiring enzymes to carry out essential metabolic functions in some individuals. Because of its specificity and sensitivity, the test for methylmalonate elevation has been called the gold standard for assessing tissue vitamin B_{12} status (Table 2.7).[85]

Neonatal screening for elevated urinary MMA levels reveals vitamin B_{12} deficiency in infants. Two cases of

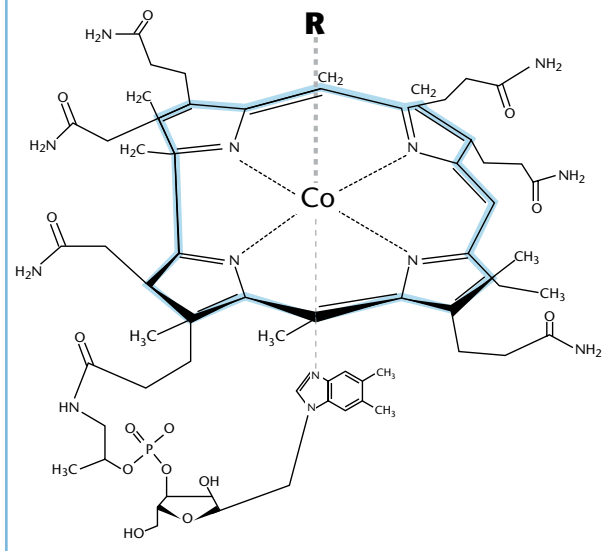


FIGURE 2.8 —Structure of Vitamin B_{12} (cobalamin)

Vitamin B_{12} contains hexavalent cobalt as the central ion in the corrin ring that forms the core of the molecule. Adenosyl, methyl or hydroxyl groups are represented by "R."

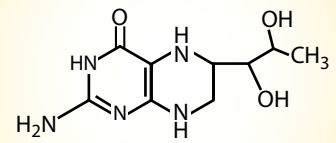
deficiency in otherwise non-symptomatic newborns have been reported, the mother being vegetarian in one case and having pernicious anemia in the other.[86] This is an example of the use of metabolic testing to detect preventable vitamin deficiency cases before the onset of symptoms that might otherwise persist undetected for many years.

Although the detection of elevated homocysteine in plasma or urine is not as specific as methylmalonic aciduria for vitamin B_{12} status, the increasing use of this test as a screen for nutrient-related cardiovascular risk will allow the discovery of many cases of vitamin B_{12} deficiency. Vitamin B_{12} and folate supplementation in these individuals may not only reduce cardiovascular risk but, equally important, will allow the early correction of other symptoms caused by vitamin B_{12} deficiency. The Schilling test, used to determine vitamin B_{12} deficiency owing to malabsorption, is described in Chapter 7, "GI Function."

REFER TO CASE ILLUSTRATION 2.3

Notes:

TETRAHYDROBIOPTERIN (BH_4)



Active forms: Tetrahydrobiopterin (BH_4), Dihydrobiopterin (BH_2)

Biochemical role: Hydroxylation and reduction

Examples: Phenylalanine + BH_4 + O_2 → Tyrosine + BH_2, Arginine + BH_4 → Citrulline + BH_2 + NO

Other role: Precursor to folate biosynthesis in plants where para-aminobenzoic acid (PABA) is added to complete the folic acid structure

Deficiency tests: BH_4 blood levels, urine 5HIAA, 5MTHF, HVA, blood levels of prolactin, pterins, Phe/Tyr ratio, Phe and a BH_4 loading test

Adult repletion: 2 to 20 mg/kg/d

Physiolgical Function: Tetrahydrobiopterin is not a classically essential nutrient because it can be synthesized in human tissues from guanosine triphosphate (GTP). BH_4 has four primary functions. It is a cofactor utilized by the rate-limiting enzymes responsible for the hydroxylation of aromatic amino acids, which include phenylalanine to tyrosine, tyrosine to DOPA and tryptophan to serotonin. It is also a cofactor in nitric oxide production from arginine (see Figure 2.9).[87,88]

The rate of BH_4 synthesis is important because BH_4 is so easily oxidized that its concentration may limit BH_4-dependent metabolic steps, thus producing the clinical state of conditional BH_4 insufficiency. All of the biochemical functions described below are affected when BH_4 is low and they can respond to BH_4 supplementation. Extensive work on the clinical use of BH_4 has been done to ameliorate the devastating effects of phenylketonuria.[89]

The first step in the synthesis of BH_4 is governed by the enzyme GTP cyclohydrolase (GTPCH), where GTP is converted to dihydroneopterin triphosphate (Figure 2.9). Since diversion of guanosine from polynucleotide synthesis must be closely regulated, levels of GTPCH are translationally controlled by many factors, including estrogen.[87, 90] GTPCH activity is also affected by a negative feedback response from BH_4 and positive feedback response from phenylalanine.[91] Dihydroneopterin triphosphate is then converted to 6-pyruvoyl-tetrahydropterin by the enzyme 6-pyruvoyl-tetrahydropterin synthase (PTPS).[3] In PTPS deficiency the conversion

cannot take place and the formation of the pterin intermediate, neopterin, increases. Neopterin is a marker of inflammation-induced cytokine production. It is simultaneously elevated with the quinolinic acid response to interferon gamma.[92, 93] 6-Pyruvoyl-tetrahydropterin is converted through several steps involving three different enzymes, sepiapterin reductase (SR), carbonyl reductase (CR), aldose reductase (AR), culminating in the formation of BH_4. BH_4 is converted to pterin-4a-carbinolamine during its cofactor functions. BH_4 is needed in four enzymatic reactions. It is a cofactor for phenylalanine-4-hydroxylase (PAH) in the conversion of phenylalanine to tyrosine, resulting in pterin-4a-carbinolamine. It is also essential in the enzymatic reactions of tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), nitric oxide synthase (NOS), which along with BH_4, convert tyrosine to DOPA, tryptophan to 5-OH-tryptophan and arginine to citrulline, respectively. BH_4 is then regenerated.[88] Pterin-4a-carbinolamine is then dehydrated by the enzyme pterin-4 carbinolamine dehydrase (PCD) to q-dihydrobiopterin. Though rare, mild and transient, a PCD deficiency leads to the accumulation of primapterin.

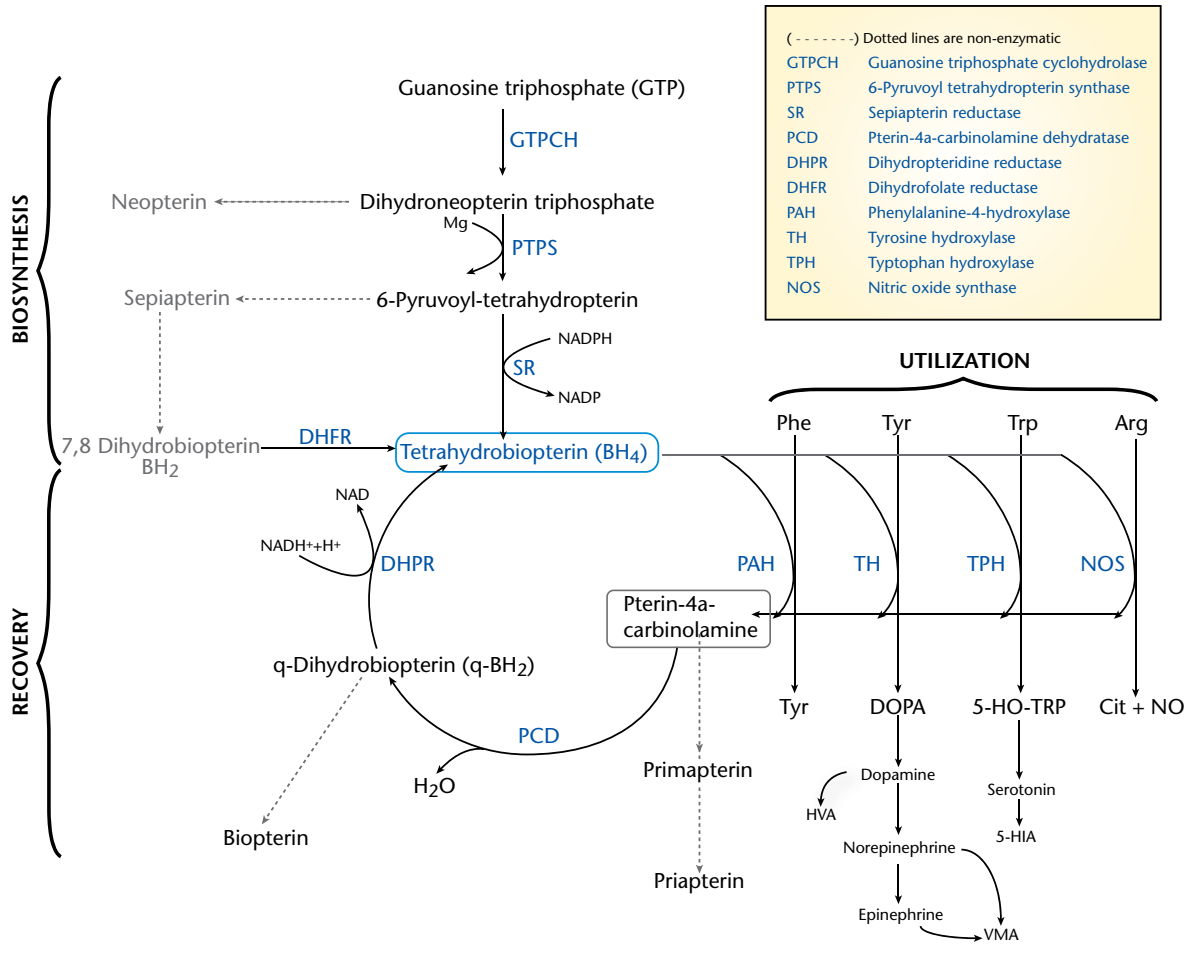


FIGURE 2.9 —**Tetrahydrobiopterin (BH_4) Synthesis, Utilization and Recycling**

Guanosine triphosphate is the substrate for the enzyme GTPCH that starts the sequence of reactions for BH_4 synthesis in human tissues. Because of genetic polymorphic effects or toxic interferences, the rate of BH_4 formation and recovery can be insufficient to meet demands in some individuals. In these people one or more of the functions of BH_4 (shown as parallel pathways at the lower left) may be significantly impaired.

q-Dihydrobiopterin is reduced by dihydropterine reductase (DHPR) to reform BH_4. In a DHPR deficiency, q-dihydrobiopterin is tautomized to biopterin.[88, 94]

BH_4 can also be produced from a salvage pathway. The salvage pathway starts at 6-pyruvoyl-tetrahydropterin and, with a nonezymatic reaction goes to sepiapterin, which turns into 7,8-dihydrobiopterin. BH_4 is then produced via the reaction of dihydrofolate reductase (DHFR). Thus folic acid supplementation has been shown to increase intracellular BH_4 levels by stimulation of DHFR.[95-97]

Deficiency: BH_4 deficiencies can range from mild to severe. In some instances enzymes may not form properly and increased amounts of BH_4 may be required. Mutations have been found in several of the required enzymes including PTPS, SR, GTPCH and DHPR, although alterations in PTPS are the most common.

BH_4 deficiency due to autosomal recessive mutations, causing the metabolic conversion of phenylalanine to tyrosine to be slowed with clinically significant accumulation of phenylalanine oxidation products, is recognized as a cause of hyperphenylalaninemia (HPA), including mild and severe forms of phenylketonuria (PKU).[98] Eighteen out of 40 infants with severe elevated phenylalanine (> 240 microM) were found to be BH_4 responsive. Treatment with BH_4 (20 mg/kg/day) over a period of 24 months significantly improved phenylalanine tolerance.[99] The incidence of BH_4 deficiency and PKU varies according to national origin. In the United States, BH_4 deficiency accounts for less than 2% of PKU cases, while in Saudia Arabia it is up to 66%.[94] More on each of these amino acid conversions can be found in Chapter 4, "Amino Acids."

Restricted BH_4 cofactor availability has also been suggested as an etiologic factor in neurological diseases, including DOPA-responsive dystonia, Alzheimer disease, Parkinson disease, autism and depression; as well as in other conditions such as insulin sensitivity and vascular disease.[100, 101] BH_4 is obligatory for the activity of all nitric oxide (NO) synthase isoforms and glyceryl-ether mono-oxygenase. By its regulation of neuronal NO synthase, BH_4 is a neuroprotective factor. BH_4 restriction limits proliferation of hematopoietic and other mammalian cell lines. Low BH_4 levels are also associated with impaired eNOS activity, leading to endothelial dysfunction.[102]

Assessment of Status: Several markers can help assess BH_4 status and may need to be determined to accurately identify the location of a specific defect. These markers include BH_4 blood levels, 5HIAA, 5MTHF, HVA, prolactine, pterins, Phe/Tyr ratio and Phe blood levels. A BH_4 loading test can aid in distinguishing BH_4 deficiency from classic PKU.[91]

Hyperphenylalaninemia arising from defects in BH_4 biosynthesis or some forms of phenylalanine hydroxylase deficiency can respond to BH_4 supplementation. The current recommended dosage to maintain conversion of phenylalanine to tyrosine is 2 to 10 mg/kg body weight.[101] BH_4 loading tests can be done to identify BH_4 supplement responders by measuring the fall of phenylalanine following BH_4 dosing. Phenylalanine levels will decrease to normal levels 4 to 8 hours after a BH_4 load in those with a BH_4 deficiency.[91] Various factors may affect the response, such as the type of hydroxylase deficiency, individual response, dose and quality of supplement, as well as the patient's phenylalanine level.[101] Loading tests will not differentiate between defects in biosynthesis of BH_4 and phenylalanine hydroxylase enzyme deficiency. Testing of urinary pterins may be done to narrow the possible causes of HPA (Table 2.8).[91, 101, 103] Biopterin levels were found to peak 1 to 4 hours following a 10 mg/kg load. Sublingual administration resulted in a 60% higher plasma increase compared to oral.[101]

TABLE 2.8 —BH_4 ENZYME DEFICIENCIES [88, 94]

Enzyme Deficiency	Urinary Pterins		Low Phe Diet	Therapy	Forms
	Neopterin	Biopterin			
GTPCH	Low	Low	No	BH_4, L-DOPA, 5-HTP	Severe
PTPS	High	Low	No	BH_4, L-DOPA, 5-HTP	Severe, Mild, Transient
DHPR	NL or slightly increased	High	Yes	L-DOPA, 5-HTP, Folinic Acid	Severe, Mild, Transient
PCD	High	Below NL			Transient

FOLIC ACID

Active forms: Tetrahydrofolic acid or Tetrahydrofolate (THF), Folinic acid (5-formylTHF), 5-MethylTHF, 5,10-MethyleneTHF, FormiminoTHF

Biochemical role: Single carbon (i.e. methyl group) transfer

Example: Homocysteine + MethylTHF = Methionine + THF

Deficiency tests: Homocysteine – plasma, Folate – serum, Folacin – RBC, CBC – Macrocytic anemia, Neutrophil hypersegmentation

Adult repletion: 200 to 800 µg/d

Physiological Function: Folate, also called pteroylglutamic acid (PGA), is categorized as a B vitamin. Folate is made up of three distinct parts, pterin, para-aminobenzoic acid (PABA) and glutamic acid (Figure 2.10). The pterin ring is conjugated to PABA and the carboxyl group of PABA is bound to the alpha-amino group of glutamate. Active folate has multiple glutamic residues attached. Tetrahydrofolate (THF) is the active form of folate and accepts single carbon atoms from various catabolic reactions in amino acid metabolism. Substituted folate can then donate the single carbon groups in biosynthetic reactions. Many functions depend on folate's single carbon transfer ability. The reduction of 5,10-methylene THF to 5-methyl THF produces the cofactor necessary for the conversion of homocysteine to methionine and ultimately for DNA production. THF regenerates 5,10-methylene THF in a reaction that also produces glycine from serine. THF can also convert to 5,10 methylene THF in other pathways as well (Figure 2.11).

Although we have all the components of the vitamin, humans do not synthesize folate. We do not have the enzyme necessary for coupling the pterin molecule to PABA to form pteroic acid. Humans must obtain folate from the diet. Folate in foods exists primarily as pteropolyglutamates with up to nine glutamates. The polyglutamate forms must be hydrolyzed to monoglutamate in order to be absorbed. Conjugase enzymes required for the hydrolysis are zinc-dependent. Alcohol and zinc deficiency impede folate absorption by inhibiting conjugase activity.

The dietary forms of folate are chemically reduced. They frequently have one-carbon substitutions in the pteridine ring and may have up to 10 additional glutamate residues linked to the proximal glutamic acid moiety. Approximately 50 to 95% of folate is destroyed during food processing such as canning.[104, 105]

Deficiency: Megaloblastic anemia resulting from folate deficiency is found in up to 5% of pregnant women in developed countries.[3] Since megaloblastic anemia is a sign that develops long after biochemical lesions (Figure 2.12), functional folic acid deficiency may be present in a larger than expected percentage of the population. This rationale is supported by the variety of causes of folic acid deficiency, including inadequate dietary intake, impaired absorption, excessive tissue demands and metabolic derangements and polymorphisims.[106] Folate deficiency is associated with conditions such as cancer, IBD and Down syndrome, and is known to respond to treatments.[107-110] Since 1998 the FDA has mandated that grains, flours and breads be fortified with folic acid. However, this raises concern that a higher folate level may mask a B_{12} deficiency, especially in older adults.

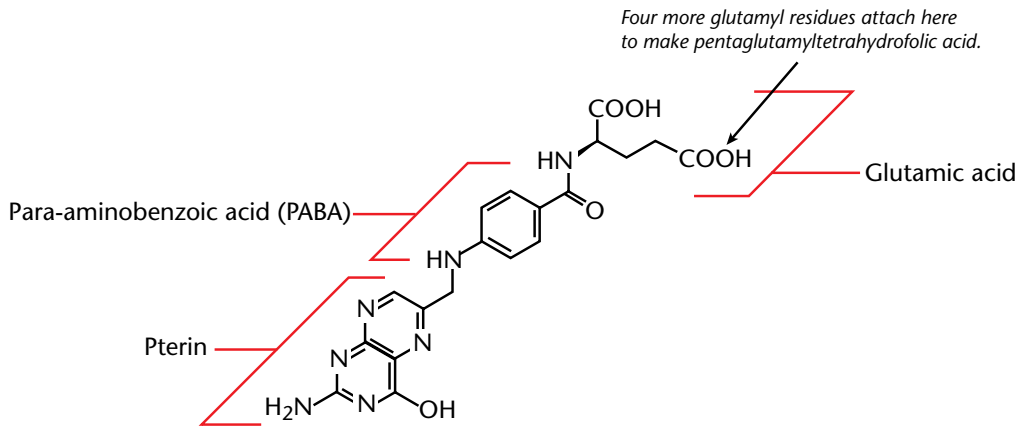


FIGURE 2.10 — **The Three Parts of Folic Acid: Pterin, Para-amino-benzoic Acid (PABA) and Glutamic Acid**

Folic acid is made from three distinct parts, pteridine, para-amino-benzoic acid (PABA) and glutamic acid. Folic acid is the simplest form of the vitamin. Folate refers to many compounds as shown in Figure 2.11.

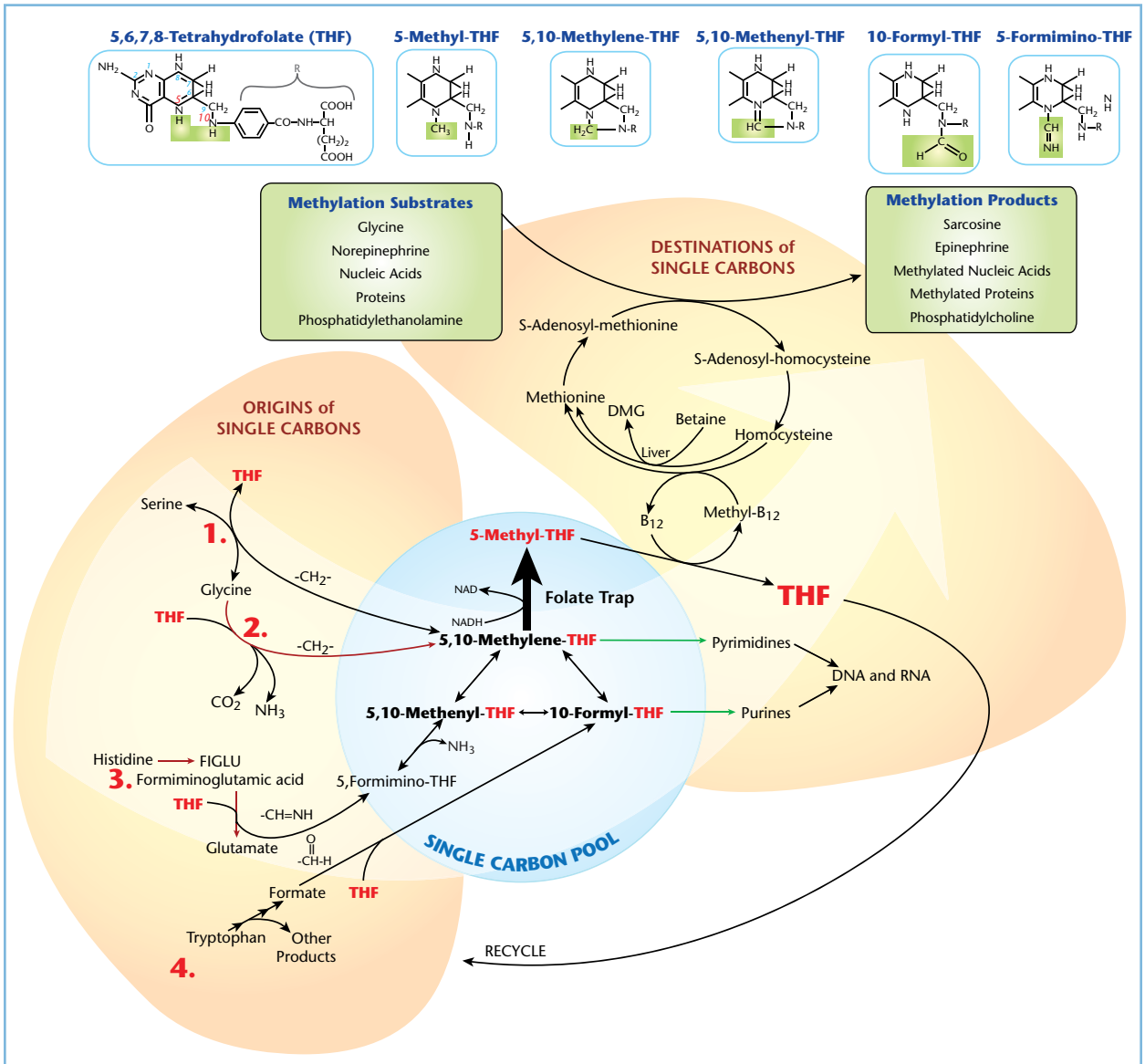


FIGURE 2.11 — **Folate and the Single Carbon Pool**

Only methionine can serve as a direct supplier of methyl groups to form SAMe. If betaine is supplied from choline intake, then the remethylation of homocysteine is facilitated in the liver. Catabolism of serine (1), glycine (2), histidine (3) and tryptophan (4), however, contributes the bulk of total methyl and other single carbon units. The central triangle shows how the various single carbon forms flow into an interchanging pool of methylene, methenyl and formyl units attached to folic acid (THF). The step in which 5,10-Methylene-THF is reduced to 5-methyl-THF, however, is not reversible. If vitamin B_{12} or any of the steps that utilize methyl-B_{12} are lacking, then folic acid can become trapped as 5-methyl-THF, constituting the "folate trap."

Genetic polymorphisims in the MTHFR (methylene THF reductase) gene may be a significant issue that has been associated with cardiovascular and cerebrovascular disease.[111, 112] In terms of intervention options, the clinical significance of identifying individuals with the current single nucleotide polymorphism testing is questionable since functional biomarkers like homocysteine lowering have not shown any greater response to methyl-THF than to THF in positive individuals.[113] Such results indicate that the rate of 5,10-methylene THF reduction is sufficient to sustain homocysteine methylation requirements, even in affected individuals.

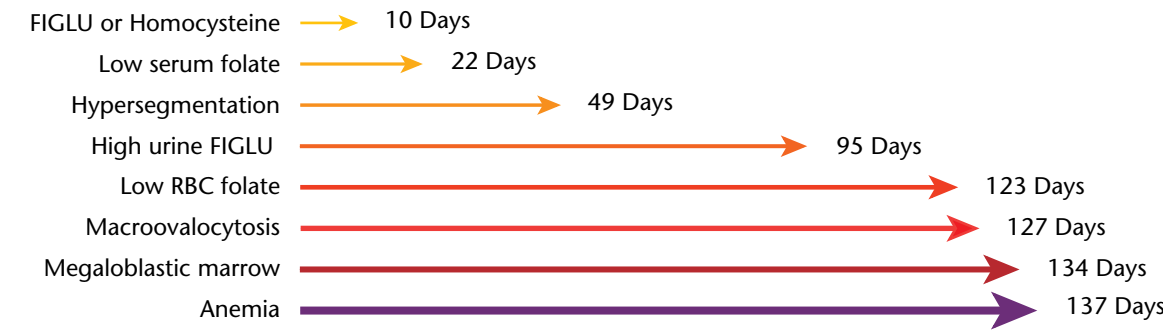


FIGURE 2.12 — **Serological Indicators of Folate Deficiency Over Time**

Assessment of Status: Homocysteine accumulation is a sensitive marker of folate inadequacy at the biochemical level because of the great flux of metabolites from methionine through homocysteine and cysteine to supply the constant demand for glutathione. Since elevated homocysteine is not necessarily owing to folate deficiency, measurement of serum folate or urinary FIGLU may be used as a confirmatory test.

Other signs appear at later stages in the succession of steps whereby a biochemical pathway inefficiency progresses to cellular malfunction and cell death and finally to tissue failure. The most susceptible tissue is bone marrow, again owing to the constant high demand for replenishment of mature erythrocytes. Anemia is the end-stage disease of the biochemical lesion resulting from folate deficiency.

A serum folate concentration < 3.0 ng/ml is evidence of deficiency, but false normal values are frequently due to the response of serum levels to transient dietary intake changes that do not correspond to tissue repletion of the nutrient. There is a marked increase in demand for the vitamin during the third trimester of pregnancy. Short-term deficiencies may not produce hematological changes in the mother but are likely to contribute to birth defects.[114] A large epidemiologic study found that in women consuming one or more alcoholic drinks per day, the relative risk of breast cancer was greatly increased for those in the lowest quintile of plasma folate concentration, compared to those in the highest.[115]

Notes:

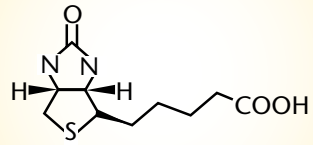
TABLE 2.9 — **FOLIC ACID DEFICIENCY TESTS**

Marker	Deficiency Indication
Homocysteine, urine	> 25 µg/mg creatinine
Homocysteine, plasma	> 15 nmol/mL
Serum folate	< 3 ng/ml
FIGLU	> 50 mg/day following 5–25 gm His
Erythrocyte folate	< 160 ng/ml
Leukocyte folate	< 500 ng/ml

Erythrocyte folate may be used to evaluate deficiency, but the period of deficiency that must pass before abnormal values are observed is longer than that causing elevated homocysteine. Leukocytes contain much higher levels of folate, but the changes parallel those for erythrocytes and, thus, are not valuable early markers of deficiency (Table 2.9). Formiminoglutamic acid (FIGLU) is an intermediate in the degradation of histidine that accumulates in the later stages of folate deficiency, especially when histidine is administered as an oral load (see Chapter 6, "Organic Acids").[116]

REFER TO CASE ILLUSTRATION 2.4

BIOTIN



Active form: Biocytin

Biochemical role: Carboxylation, biotinylation

Example: Pyruvate + CO_2 ➔ Oxaloacetate, Histone ➔ Biotinylated histone

Deficiency tests: β-Hydroxyisovalerate – urine

Adult repletion: 500 to 5,000 µg/d

Physiological Function: Biocytin, released from biotin-containing proteins, is hydrolyzed to biotin and lysine by biotinidase.[7] The active form of biotin, biocytin, is linked to the amino group of enzymes that operate in biochemical pathways of gluconeogenesis, fatty acid synthesis and amino acid catabolism.[117]

Genetic variations in the enzymes involved in the release and recovery of biotin contribute to a functional biotin deficiency state. Other factors operate to modify the flow of biotin from the gut, including bacterial populations that can synthesize the nutrient and the capacity for intestinal absorption. Because of the many biochemical roles of biotin enzymes, the clinical manifestations are highly varied. Early signs of biotin deficiency in biotin depleted rats were abnormalities of hair growth and skin integrity and impaired immune function.[118]

Deficiency: Biotin is the B-complex vitamin most dramatically affected by oral antibiotics. The reason is two-fold. First, most foods are poor sources of biotin, so a low intake is common. Exceptions are biotin-rich foods: egg yolk, kidney, liver and some cheeses. Second, the bacteria of the healthy gut can produce biotin, adding significantly to dietary supply.[119] Thus, antibiotics can disrupt the biotin produced in the gut, but dietary sources are not generally adequate to compensate for this loss.

Assessment of Status: Evidence in support of a functional marker of biotin adequacy has appeared only relatively recently.[120] Urinary elevation of beta-hydroxyisovalerate results from biotin failing to assist in the degradation of a catabolic product of valine. Values of beta-hydroxyisovalerate above 15 µg/mg creatinine in overnight urine indicate potential biotin deficiency. Plasma biotin level is not a sensitive marker of biotin status and has been shown to be resistant even with clinical signs of deficiency.[59, 121-124] Further discussion of the biotin and beta-hydroxyisovalerate relationship may be found in Chapter 6, "Organic Acids."

OF FURTHER INTEREST...

Biotin supplementation, along with chromium, was found to help maintain glycemic control in a double-blind case-study, N = 348. Participants taking 2 mg of biotin and 600 µg chromium picolinate had statistically significant ($P < .05$) reductions of HbA_{1c} and glucose.[253]

It is thought that biotin improves abnormal glucose metabolism by stimulating glucose-induced insulin secretion in pancreatic beta cells, accelerating glycolysis in the liver and pancreas, and enhancing muscle insulin sensitivity.

Biotin is an enzyme cofactor for the following carboxylases:

- Acetyl-CoA (fatty acid synthesis)
- Pyruvate (gluconeogenesis)
- Methylcrotonyl-CoA (leucine catabolism)
- Propionyl-CoA (MMA synthesis)

Notes:

VITAMIN C (ASCORBIC ACID AND DEHYDROASCORBIC ACID)

Active form: Ascorbic acid
Biochemical role: Antioxidant, hydroxylation
Examples: Lysine-Collagen → alpha-Hydroxylysine-Collagen
Deficiency Tests: Vitamin C – serum, leukocyte
Adult Repletion: 1000 to 5000 mg/d

Physiological Function: Ascorbic acid is oxidized to dehydroascorbic acid and ketogulonic acid. The conversion to dehydroascorbic acid is via the intermediate semidehydroascorbate, a free radical with an unpaired electron. The majority of ascorbic acid is converted to dehydroascorbic acid.[50] Dehydroascorbate can be converted back to ascorbic acid, the reaction being glutathione dependent. The ability to reverse back to ascorbic acid from dihydroascorbate gives vitamin C its primary property as a biological reductant. The conversion to ketogulonic acid is irreversible (Figure 2.13).[7] Vitamin C is a powerful water soluble antioxidant for both intra- and extra-cellular reactions and is heavily involved in reactions with reduced iron and copper metallothione enzymes. The synthesis of collagen (proline or lysine hydroxylation), carnitine and neurotransmitters (norepinephrine and 5-hydroxytryptophan) require vitamin C dependent enzymes.[38, 50]

Deficiency: Individual vitamin C requirements vary significantly. In controlled human trials, early signs of scurvy appeared when plasma ascorbic acid levels fell below 0.3 mg/dl, while after 30 days of a diet deficient in vitamin C, skin hemorrhages and emotional changes had begun to occur.[51] Most frequently associated signs of deficiency were petechiae, gum changes, hyperkeratosis and arthralgia.[51, 125]

Assessment of Status: Because of its short physiological half-life and chemical instability, vitamin C has been considered one of the most problematic nutrients to assess in terms of optimal intake for an individual, though previous studies have found good stability of whole blood and serum samples and assessments have been successfully used in large cohort studies.[126] Intake must be relatively constant to maintain tissue levels in a range where short-term effects on amino acid metabolism and long-term effects on collagen synthesis are optimal. There are no functional biochemical markers specific for vitamin C status. Most studies of vitamin C have relied on serum or plasma levels that show a linear relationship with intake over a limited range. Plasma ascorbic acid reaches a maximum concentration at about 1.4 mg/dl. Further intake results in rapid renal clearance. With deprivation of vitamin C, plasma ascorbic acid decreases rapidly and clinical signs of scurvy will appear at continued levels of less than 0.2 mg/dl.[127] Under controlled conditions, measurements of plasma, tissue and urinary vitamin C in healthy patients on various doses of vitamin C reveal bioavailability and tissue

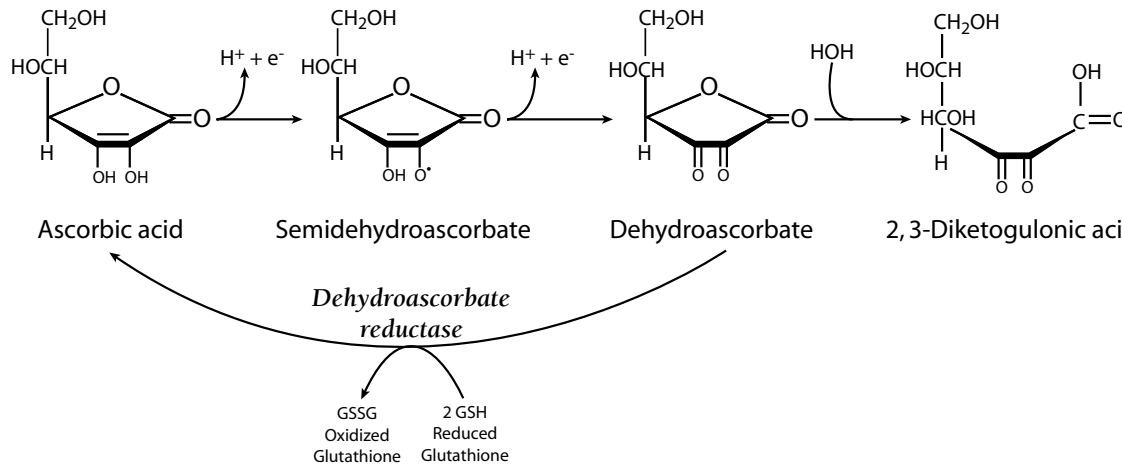


FIGURE 2.13 —Ascorbic Acid Converts to Diketogulonic Acid

Vitamin C refers to both ascorbic acid and dehydroascorbic acid. Ascorbic acid is easily oxidized to dehydroascorbate and reduced back to ascorbic acid with oxidation of glutathione. The conversion to diketogulonic acid is irreversible.

saturation. After keeping subjects on low vitamin C intake for several weeks, such studies have demonstrated complete tissue uptake of doses up to 200 mg with declining bioavailability at single doses above 500 mg.[128] Owing to the high safety and low risk, many studies have shown clinical effects at higher doses. For example, young women who consume two 500 mg capsules of ascorbic acid at breakfast were shown to have more favorable lipoprotein profiles than controls.[129]

Large-scale investigations of mechanisms for cancer prevention by vitamin C depend on reliable interlaboratory ascorbic acid (AA) concentration determinations.[130] The National Institute of Standards and Technology (NIST) has prepared a standard reference material (SRM 970) at two AA concentrations in serum. Level I and Level II of SRM 970 contain 10 and 30 umol/L AA, respectively. When seventeen independent laboratories using a variety of methods analyzed SRM 970 for total AA, the results showed large variability.[131] The availability of SRM 970 should assist improvement of interlaboratory reliability as methods can be more closely investigated for sources of error.

Ascorbic acid disappears from urine early in the process of depletion. Leukocyte levels fall more slowly than plasma ascorbate and drop most dramatically only when signs of scurvy appear. Determination of leukocyte ascorbate has other drawbacks. The assay is technically difficult and requires large volumes of blood. Improper handling of the leukocytes will cause a loss of ascorbate resulting in falsely low values. Also, ascorbic acid is an extremely labile vitamin and extensive efforts must be made to preserve the vitamin, especially in urine where exposure to air increases the rate of oxidation.

Claims of correlation of ascorbic acid tissue saturation and the rate of disappearance of the intradermally injected dye, 2,6-dichloroindophenol, or the disappearance of the dye after topical application to the tongue have been shown to be invalid.[132-135]

Notes:

VITAMIN D (D_3, CHOLECALCIFEROL AND D_2, ERGOCALCIFEROL)

Active forms: 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$);
24,25-dihydroxyvitamin D_3 (24R,25-$(OH)_2D_3$);
1,25-dihydroxyvitamin D_2 (1,25-$(OH_2)D$);
25-hydroxyvitamin D_3 (25-OH-D_3);
25-hydroxyvitamin D_2 (25-OH-D_2),

Biochemical role: Regulation of calcium, immune modulation

Example: Calcium-binding protein (CBP) synthesis

Deficiency tests: 25-hydroxyvitamin D – serum, Bone-specific peptide – urine (indirect)

Adult repletion: 700 - 10,000 IU/d

Physiological Function: There are two primary forms of vitamin D, vitamin D_3 and vitamin D_2. Vitamin D_3, or cholecalciferol, is formed in the skin upon UV exposure. The other, vitamin D_2, or ergocalciferol, comes from foods or supplements.[136] The cholecalciferol form of vitamin D comes from the conversion of 7-dehydroxycholesterol to previtamin D_3 via UV radiation from the sun (Figure 2.14). Previtamin D_3 is then converted to vitamin D_3. Excess vitamin D cannot be achieved by sun exposure because previtamin D_3 and vitamin D_3 become photolyzed to inactive products when levels rise.[137] After binding to carrier proteins (such as vitamin D binding protein or DBP), vitamin D_3 is then transported to the liver where it is hydroxylated to 25-hydroxyvitamin D_3. The kidneys are stimulated by parathyroid hormone (PTH) to produce 1,25-dihydroxyvitamin D_3 from circulating 25-hyroxyvitamin D levels. This conversion can also take place in other cells and tissues (prostate, colon, placenta, bone, macrophages, T-lymphocytes) via cytokine signals.[136] 1,25-dihydroxyvitamin D_3 modulates its effects based on blood calcium levels or physiologic need.[139] 1,25-dihydroxyvitamin D_3 enters the cells and initiates transcription and translation, leading to protein synthesis, which results in effects such as proliferation, differentiation and apoptosis.[138] 1,25-dihydroxyvitamin D_3 leads to the production of calcium binding protein (CBP or osteocalcin) which regulates the active transport of calcium, via vitamin D-dependent absorption.[136] 1,25-dihydroxyvitamin D_3 is responsible for increasing calcium and phosphorus in the blood via intestinal absorption, bone resorption and renal tubular absorption in the kidney. When there is sufficient amounts of 1,25-dihydroxyvitamin D_3, its production will decrease and the production of 24,25-dihydroxyvitamin D_3 will increase.[136] The role of 24,25-dihyroxyvitamin D_3 is not yet fully defined.[140]

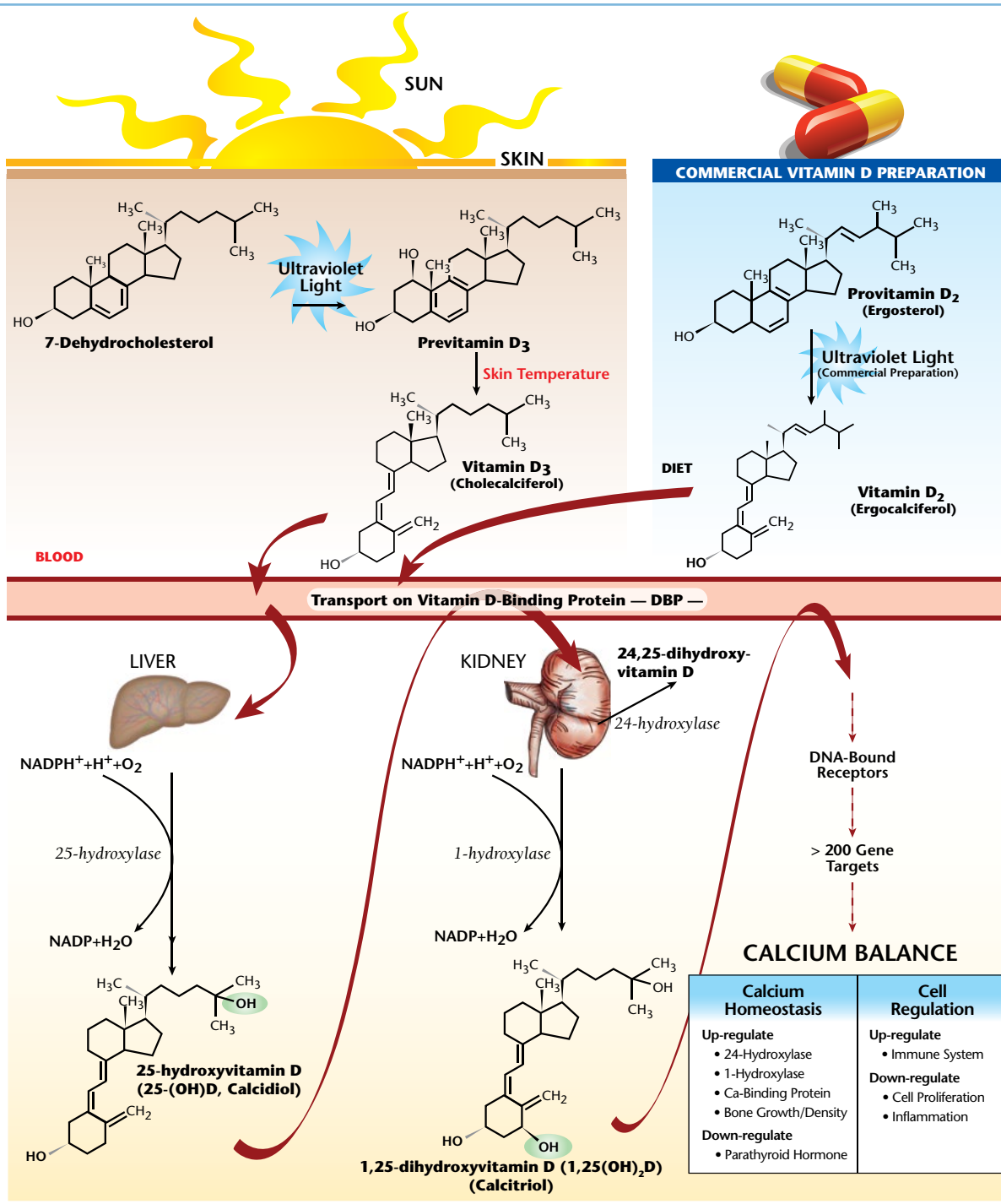


FIGURE 2.14 — Metabolism of Vitamin D

In the skin, ultraviolet rays are required to convert 7-dehydrocholesterol into vitamin D_3 (cholecalciferol). Industrial production starts with ergosterol to produce vitamin D_2 (ergocalciferol) that is added to foods or sold as a nutritional supplement. Both forms are metabolized to their respective 25-hydroxy circulating forms and 1,25-dihydroxy active forms that have similar biological activity. The difference lies in the faster rate of clearance of 25-hydroxyvitamin D_2. Vitamin D_2 is absorbed by the gut and follows a pathway similar to vitamin D_3. Thus, unless otherwise noted 25-hydroxyvitamin D is a combination of both 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2.

Vitamin D_2, or ergocalciferol, undergoes reactions similar to the D_3 pathway. It goes from ergosterol, to ergocalciferol, to 25-hydroxyvitamin D_2 and then to the final active form 1,25-dihydroxyvitamin D_2.

Beyond osteoporosis, negative health effects of vitamin D insufficiency include strong influences on the development of cancer and autoimmune diseases, such as insulin-dependent diabetes and multiple sclerosis.[141] Research has also found a relationship with maternal vitamin D intake and childhood illness. In a study of 1,212 mother-child pairs, children of mothers with higher vitamin D intakes had lower rates of early childhood wheezing illnesses.[142]

Deficiency: There is growing concern over the occurrence of poor vitamin D status.[143-147] Some prominent vitamin D researchers have identified vitamin D deficiencies as a pandemic.[148-152] Vitamin D deficiencies are more common in those with limited sun exposure, a low intake of vitamin D, impaired absorption owing to kidney or digestive diseases, or older adults who have a decreased ability to synthesize this vitamin from the sun.[136, 153] UV exposure is the primary source of vitamin D for most people.[137] Many populations are showing alarming rates of deficiencies.

Research revealed 41% of U.S. outpatients aged 49 to 83 had low circulating 25-hydroxyvitamin D levels. The level is higher in European studies[148 154] In the first year of institutionalization, almost all elderly people are at risk of becoming deficient in vitamin D.[56] A cause of the reemergence of rickets is due to extended breastfeeding without adequate sunlight or vitamin D supplementation.[155] Dietary factors other than vitamin D intake and sun exposure may affect vitamin D status. In a large multi-country study, higher calcium intake was associated with greater fracture rates.[156] Excessive animal protein[157, 158] or calcium intake[159, 160] can cause lower blood levels of 1,25-dihydroxyvitamin D by affecting the rates of its formation and clearance.

Cases requiring massive doses of vitamin D to achieve normal 25-dihydroxyvitamin D levels have been reported.[161] Such cases illustrate the range of individual requirements for vitamin D. Studies giving 10,000 IU/day to adults for up to five months found no toxic effects.[162] A short term whole body sun exposure is reported to provide the equivalent of 10,000 to 20,000 IU/day of vitamin D3.[162, 163] In a review of pharmacological doses ranging from 20,000 IU to 600,000 IU of vitamin D for 3 months to over 20 years, vitamin D intoxication was accompanied with a serum 25-hydroxyvitamin D level > 220 nmol/L.* However, not all the high doses were associated with intoxication and levels of 225 nmol/L have been achieved by sunshine exposure alone.[162] Levels of 135 to 225 nmol/L have been found to be normal in sunny climates.[164] Other research showed no harmful effects at levels of 250 nmol/L.[165] An acute toxic dose in adults is not well established, though the potentially toxic dose is significantly lower for very young children. In a double-blind, placebo controlled study, girls ages 10 to 17 were given 14,000 IU/week (2,000 IU/day) for 1 year without adverse consequences.[166] Toxicity is generally only expected to be a problem in those taking large doses and in those who already have adequate or high levels of 25-hydroxyvitamin D. Serum 25-hydroxyvitamin D of 250 nmol/L has been shown to be a cut off for a toxic concentration.[163, 165, 167]

Supplement doses of vitamin D to achieve recommended 25-hydroxyvitamin D levels vary between individuals. Intakes of 700 to 1,000 IU/day have been shown to achieve serum 25-hydroxyvitamin D level of 90 to 100 nmol/L in 50% of adult populations,[143] while younger adults may need higher intakes. Levels of 4,000 to 10,000 IU of vitamin D were found to be safe in younger adults.[143] In regards to the type of supplement, vitamin D_3 (cholecalciferol) has been found to be more efficacious than vitamin D_2 (ergocalciferol). In a study comparing the effectiveness of the two forms, both were found to produce a similar rise in serum levels.[168] However, vitamin D_3 had a more sustained peak that was still visible on day 14, whereas vitamin D_2 returned to

Notes:

** Conversion factors:*
nmol/L = ng/mL x 2.5
ng/mL = nmol/L x 0.4

baseline by day 14.[168] Other research showed oral vitamin D_3 to be about 1.7 times more efficient than vitamin D_2 for raising serum 25-hydroxyvitamin D.[168,169] There is some reason for concern over excessive intake of vitamin D owing to effects on vascular calcification,[170] especially for individuals who frequently consume milk products.

Although the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in the kidney controls plasma levels of the active hormone, which then control calcium absorption, other tissues carry out the conversion apparently to satisfy their own vitamin D demands. It has been proposed that the level of circulating 25-hydroxyvitamin D that satisfies bone formation may be lower than the concentration needed for adequate supply in extrarenal tissues, such as the prostate, colon and skin. By this rationale, many have proposed that an acceptable serum 25-hydroxyvitamin D should be higher than currently recommended. Estimation of the optimal 25-hydroxy vitamin D level for multiple health outcomes has been estimated to be 90-100 nmol/L.[143] The health benefits associated with vitamin D were noted to begin at approximately 75 nmol/mL and previous research has shown calcium absorption to decrease when 25-hydroxyvitamin levels fall below 80 nmol/mL.[141, 143, 163] Table 2.10 summarizes clinical indications of serum vitamin D levels.

TABLE 2.10 — 25-HYDROXYVITAMIN D RECOMMENDED LEVELS IN SERUM

25-Hydroxyvitamin D		Indication
nmol/L	ng/mL	
< 75	30	Insufficient
75–250	30–100	Sufficient
> 250	100	Toxic

Assessment of Status: The most frequently used assay for vitamin D assessment of status is serum 25-hydroxyvitamin D, a combination of both D_3 (cholecalciferol) and D_2 (ergocalciferol). 25-hydroxyvitamin D is the primary circulating form of vitamin D and the most reliable indicator of its status.[139] It is the most abundant metabolite of the vitamin and is the direct precursor of the active form.

Two common measurements of 25-hydroxyvitamin D are enzyme-linked immunoassay (EIA)[171] or radioimmunoassay (RAI).[172] Improved chromatographic techniques may allow routine analysis of multiple forms of vitamin D in plasma.[173] Researchers have thus identified the need to standardize vitamin D measurements.[174] Radioimmunoassay (RIA) is FDA approved and thus the current recommended testing procedure.[175, 176] Detection of individual 25(OH)D_2 and 25(OH)D_3 can currently only be done using LC/MS/MS. Though not FDA approved, this method is accurate when performed by experienced personnel using a validated procedure.[176]

Many markers may be seen in a vitamin D deficiency state. Severe deficiency of vitamin D indirectly causes increased serum activity of alkaline phosphatase because of the role of this enzyme in bone mineralization. Alkaline phosphatase is also under the influence of a variety of factors, including several disease processes,[3] zinc status,[177] and vitamin C.[178] The calcium binding protein, osteocalcin, is secreted by osteoblasts and its serum concentration is elevated in a number of conditions associated with high bone turnover. Osteocalcin, however, is not as sensitive a marker of bone turnover as serum alkaline phosphatase in Paget's disease.[179] A part of this lack of sensitivity may be the co-dependence on vitamin K status for osteocalcin formation. In elderly females with vitamin D deficiency, secondary hyperparathyroidism is associated with increased serum osteocalcin levels, which indicates increased bone formation. These conditions might contribute to the bone disease of geriatric patients.[180] Other measurements that reflect vitamin D deficiency are low urinary calcium excretion, low serum calcium and phosphorus. Table 2.11 identifies some possible markers that may be modified with a vitamin D deficiency.[153]

The process of bone formation is under complex control by several factors, only one of which is vitamin D. Assays of collagen peptide markers for bone loss[161, 181]

TABLE 2.11 —VITAMIN D DEFICIENCY MARKERS

The Following Markers May Be Seen In Vitamin D Deficiency	
Serum	
25-hydroxyvitamin D	Low
Phosphorus	Low-normal/low
Parathyroid hormone (PTH)	Elevated
Alkaline phosphatase	Elevated
Urinary Markers	
Hydroxyproline, pyridinoline, deoxypyridinoline -N-telopeptide	These are bone collagen by-products that may all be elevated in vitamin D deficiency

are described under the calcium section of Chapter 3, "Minerals." Urinary or plasma proline and hydroxyproline elevations are also found in individuals with increased bone turnover resulting from inadequate calcium supply (see Chapter 4, "Amino Acids"). Iron deficiency can lower 25-dihydroxyvitamin D, possibly by impairing small intestinal absorption and may need to be included in an overall assessment.[182]

Vitamin D levels (along with calcium and PTH activity) impact the rate of absorption of phosphorus from the intestines. The majority of phosphorus is absorbed from the intestines, although the rate depends somewhat on the levels of calcium and vitamin D and the activity of parathyroid hormone (PTH). Calcium tends to follow phosphorus. Thus for every gram of phosphorus ingested in the diet, the body must match that with another gram of calcium. If the calcium is not available in the diet, the body will pull it from the bones. The biggest concern with those eating a chronically low calcium, high-phosphorus standard American diet is a low calcium/phosphorus ratio leading to a consistently high PTH level which can then cause increased bone turnover and decreased bone density.[139]

Refer to Case Illustration 2.5

Notes:

Vitamin E (Tocopherol)

Active forms: RRR alpha-, beta-, gamma- and delta-Tocopherol; alpha-, beta-, gamma- and delta-Tocotrienol

Biochemical role: Antioxidant, especially membrane and plasma lipoprotein protection

Example: Protection of low-density lipoprotein from oxidation

Deficiency tests: Tocopherol, tocopherol/triglyceride – serum,

Adult repletion: 200 to 1,600 IU/d

Physiological Function: Vitamin E can come in the form of tocopherol and tocotrienol. Vitamin E has a phytl side chain and a chroman ring. The position of methyl groups on the chroman ring determines the type of *tocopherol*—alpha, beta, delta or gamma (Figure 2.15). There are some important structural considerations among forms of vitamin E for supplementation. There are three chiral carbons in the tocopherol structure. This means that eight optical isomers are possible. The most abundant naturally occurring stereoisomer of alpha-tocopherol has all three chiral carbons in the R configuration and it is called *RRR*-gamma-tocopherol. The name is frequently shortened to gamma-tocopherol.The totally synthetic vitamin E, obtained without any control of stereochemistry, is a mixture in unspecified proportions of four pairs of enantiomers (i.e. eight diastereoisomers). Such a totally racemic mixture is called all-rac-alpha-tocopherol. Refer to Table 2.15 for relative activities of the principle forms of vitamin E.

Tocopherol acetate has twice the biological activity of all-rac-alpha-tocopherol.[183] Intestinal absorption does not discriminate between the isomeric forms described above. However, hepatic alpha-tocopherol transfer protein (alpha-TTP) selectively transfers alpha-tocopherol to newly secreted VLDL particles.[184] Standard clinical laboratory methods do not discriminate between the various optical isomers. Because of the specificity in the action of alpha TTP, however, plasma concentrations preferentially reflect intake and status of alpha-tocopherol.

Tocotrienols (TCT) are another class of vitamin E analogs. They differ from tocopherols because their phytl side chains contain 3 double bonds (Figure 2.16). Tocotrienols modulate several mechanisms associated with the aging process. This class may suppress reactive oxygen species damage more efficiently than the tocopherols, lowering cholesterol levels, preventing cell adhesion to endothelial cells, suppressing tumor growth and reducing glutamate-induced neurotoxicity.[185] Orally administered

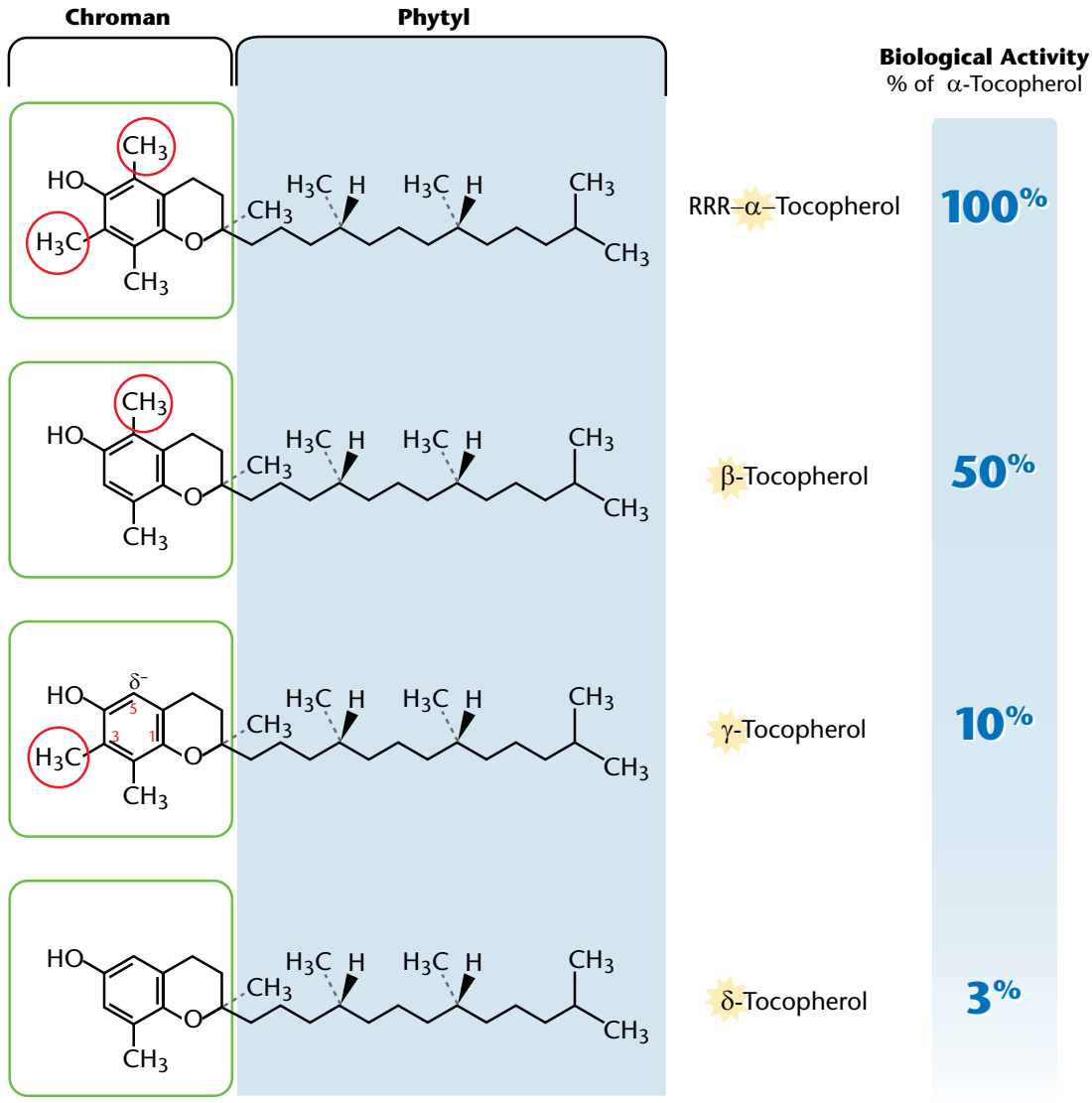


FIGURE 2.15 — Structures and Relative Activities of Tocopherols (TCP)

Tocopherols contain a chroman ring and a phytyl group. The presence of methyl groups at the 1 and 3 positions (circled in red) of the chroman ring characterizes the α, β, γ, and δ isomers. The chroman ring gives vitamin E its antioxidant capabilities via its hydroxyl group, which can donate a hydrogen radical. The phytyl group anchors the vitamin in lipids, such as lipid membranes, lipoprotein and tissue lipids. The biological activity varies depending on the form of tocopherol. Some unique biological properties of γ-tocopherol are related to the negative (nucleophilic) character of the number 5 ring carbon.

tocotrienols appear in plasma at a maximum concentration within about 4 hours.[186]

Deficiency: Vitamin E is transported in plasma in the lipoproteins and it serves as the most important membrane protective antioxidant and free radical scavenger in the body.[187] Although alpha-tocopherol is the major component in foods and human tissues, the beta, delta and gamma isomers are sometimes included in profiles to show detail of tissue composition.

Experimental vitamin E deficiency is difficult to produce in humans because of the intricate system of checks and balances in the antioxidant cascade. Although symptoms of vitamin E deficiency are subtle, many clinical effects are well documented. Cancer,

heart disease, hemolytic anemia, neurological disease (including ataxia) and other neuropathies are some of the human disorders more frequently associated with vitamin E deficiency.[188]

Assessment of Status: Plasma or serum alpha-tocopherol should be above 12 mg/L. Since patients with hypertriglyceridemia have elevated levels of lipoproteins, vitamin E concentrations also tend to rise, leading to overestimation of vitamin E total body status. The ratio of measured tocopherol to triglycerides in plasma or serum may be calculated as a way of correcting for this effect. Vitamin E deficiency is indicated when the ratio of serum tocopherol to total lipids is less than 0.8 mg/g in lipid extracts of serum. Because of the ease of simultaneous analysis, vitamins A, E and beta-carotene and coenzyme Q_{10} are frequently reported as a profile of fat-soluble vitamins. Concentrations of these vitamins in serum are measured by high performance liquid chromatography.[23, 189, 190]

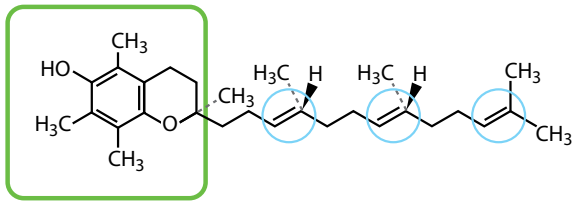


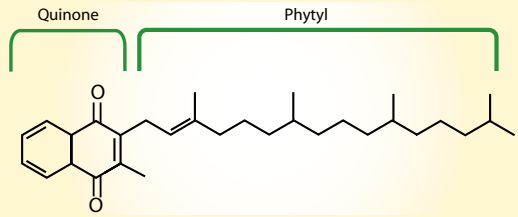
FIGURE 2.16 — **Structure of Tocotrienol (TCT)**

Tocotrienols differ from tocopherols because their phytl side chains contain three double bonds (circled in blue). Isomeric variations similar to tocopherols occur with methyl group removals.

REFER TO CASE ILLUSTRATION 2.1

Notes:

VITAMIN K (PHYLLOQUINONE)



Active form: Protein-bound vitamin K

Biochemical role: Gamma glutamyl carboxylation

Example: Formation of active osteocalcin

Deficiency tests: PIVKA-II – serum, undercarboxylated osteocalcin – serum, Vitamin K – serum, prothrombin – plasma

Adult repletion: 500 to 1,000 µg/d

Physiological Function: Vitamin K is a fat soluble vitamin that primarily acts as a cofactor in blood coagulation and bone matrix proteins. It is made up of a quinone and a phytyl group. The biochemical action dependent on vitamin K involves unique gamma-carboxylation of glutamyl residues of specific proteins. The result of the carboxylation is increased binding of calcium that is known to be required in blood clot formation and bone remodeling.[191] There are two natural forms of vitamin K which differ based on their phytyl group, phylloquinone, synthesized from plants and menaquinone, synthesized from bacteria.

Deficiency: Vitamin K deficiency results in an increase in the time required for blood to clot. The usual clinical manifestation is a tendency to hemorrhage. The measurement of prothrombin time in plasma reveals the vitamin K-dependent activation of prothrombin and other clotting factors. Prothrombin times longer than 12 seconds can indicate vitamin K deficiency (Table 2.12). Vitamin K status is especially important in the elderly because of inadequate dietary intake and absorptive difficulties, frequently complicated by drug therapies. Monitoring vitamin K can help reduce osteoporotic bone fractures by identifying individuals whose bone loss is due to vitamin K deficiency.[192, 193] Calcium loss in such cases can be reduced by up to 50% with vitamin K supplementation.[194]

Assessment of Status: Methods for direct measurement of vitamin K concentration in blood or urine have been reported.[195] Human subjects placed on a low vitamin K diet for several weeks show variable declines in body pools of vitamin K from normal values of ~1.0 µg/kg body weight. During dietary restriction, fecal

excretion of an administered dose of vitamin K falls, but urinary excretion rises, indicating activation of intestinal absorption mechanisms.[196] The more clinically relevant question of body pool size calls for measures of functional status that are becoming available. Radioimmunoassay for undercarboxylated osteocalcin and measurement of γ-carboxyglutamate (Gla) in urine are two ways to see whether the biochemical functions of the vitamin are adequate (Table 2.12). These markers are sensitive indicators of vitamin K status.[197]

TABLE 2.12 — VITAMIN K TESTS

Test	Deficiency Indication
Phylloquinone, plasma	< 1 nmol/L
Gla, urine	< 3 µmol/mmol creatinine
Under-carboxylated osteocalcin, plasma	> 20% Total Osteocalcin
PIVKA-II	< 29 mAU/ml
Prothrombin time	> 12 seconds

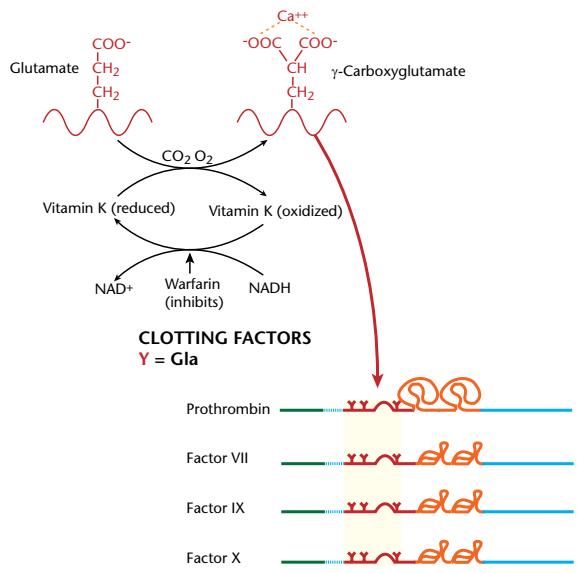


FIGURE 2.17 — The role of Vitamin K in Carboxylation Reactions and Blood Coagulation

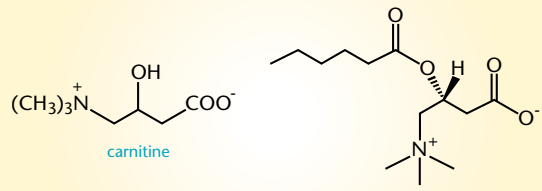
Glutamate residues of proteins may be converted to γ-carboxyglutamate (Gla) by the action of vitamin K bound to carboxylase enzymes. An additional carboxylic acid group is introduced at the gamma carbon position. The Gla residues with dual negative charges have high binding affinity for calcium ions. The process of carboxylation catalysis causes oxidation of the vitamin K that must be cycled via NADH reduction. This is the step that is inhibited by warfarin to prevent blood clotting. The bottom portion shows regions of Gla sequences (red Y-shaped structures) in proteins of the clotting cascade.

Binding of calcium is critical for the function of the sequence of reactions leading to blood clotting and the change from native to calcium-binding clotting factor structures requires vitamin K (Figure 2.17). High affinity calcium binding sites are generated when an additional carboxylic acid group is added to glutamyl residues. Multiple proteins of the blood clotting cascade have sites for the carboxylation reaction shown by the red regions of the protein chains in the bottom of Figure 2.17. Prothrombin is also known as Factor II, thus it is the protein "inactivated" by vitamin K in the "protein induced by vitamin K absence or antagonist-II" (PIVKA-II) assay used to reveal vitamin K status.[198-200] The term PIVKA-II can be confusing since one might say that the carboxylation reaction during the physiological process of blood coagulation is an activation rather than inactivation of prothrombin. A less confusing alternate name, des-gamma-carboxyprothrombin, is sometimes used. The undercarboxylated osteocalcin assay is based on the same concepts as the PIVKA-II assay.

The progression of PIVKA-II results to elevated values with each trimester of pregnancy has led to the suggestion that some women develop a sub-clinical vitamin K deficiency during gestation.[201] Such women will have difficulty maintaining calcium homeostasis while breast feeding. Some tumors produce PIVKA-II, including gastric cancer[202] and hepatocellular carcinoma.[203, 204]

Notes:

CARNITINE



Biochemical role: Fatty acid transport across cell membranes

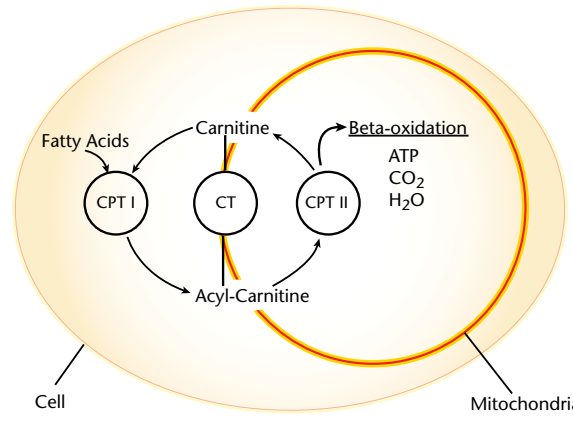
Deficiency tests: Elevated adipate, suberate, ethymalonate

Adult repletion: 250 to 1,000 mg/TID

Physiological Function: The action of carnitine may be described as a key that opens the gate of energy flow in most cells (Figure 2.18). The main function of carnitine is to transport fatty acids across the mitochrondrial membrane for fatty acid oxidation, with the notable exception of brain tissue. The enzymes of this pathway reside inside the mitochondria. Fatty acids, however, must gain entry to the mitochondria by forming fatty acyl-carnitine esters in the mitochondrial trans-membrane space. Limitation of carnitine can slow the process of transfer and therefore the overall energy yielding system. Skeletal and cardiac tissues rely on fatty acid oxidation and therefore have a high concentration of carnitine. The need for and supplementation of carnitine has been studied in many areas, including human metabolism, cardiovascular disease, sports enhancement, peripheral artery disease, diabetes, aging, immunity, HIV, thyroid function, cancer, infertility and others.[205]

Deficiency: Carnitine is a water-soluble compound that is abundant in animal muscle tissue, including red meats (Latin: carne). Carnitine is synthesized from lysine and therefore, it is not strictly classified as an essential nutrient. Evidence of its conditional essentiality comes from the finding of low plasma carnitine in patients on long-term total parenteral nutrition.[205] Skeletal muscle myopathy has been successfully treated with carnitine.[206] Measurement of acyl- and free carnitine is used to detect secondary carnitine deficiencies in certain inborn errors of organic acid metabolism.[207] Many of the toxic effects of chronic alcoholism that result from glutathione depletion are reduced by administration of carnitine.[208] Carnitine has been advocated as a "mitochondrial megavitamin" therapy for adult onset diabetes.[246]

Assessment of Status: Functional carnitine deficiency is revealed by elevated urinary excretion of adipic, suberic and ethylmalonic acids,[247] and is described in more detail in Chapter 6, "Organic Acids." Impairment of fatty acid oxidation is accompanied by high levels of these urinary organic acids, as reported in Reye-like syndrome[248] and other mitochondrial impairment disorders.[249] Mitochondrial fatty acid oxidation disorders have been considered rare in the past, though research is now finding milder forms. Mild forms of short-chain acyl-CoA dehydrogenase deficiencies can be identified via urinary ethylmalonic acid excretion.[209, 210]



CPT I - Carnitine acyl-transferase I
CPT II - Carnitine acyl-transferase II
CT - Carnitine acyl-transferase

FIGURE 2.18 — Carnitine and Beta-oxidation

Fatty acids are converted to fatty acyl-carnitine for transport across the inner membrane of the mitochondria. Carnitine then 'flips' the fatty acid through the membrane to the inside of the mitochondria. Enzymes oxidize fat into ATP, carbon dioxide, and water. Carnitine is recycled back outside the mitochondira. When carnitine is not available fatty acids undergo ω-oxidation, and adipate, suberate, or ethylmalonate will accumulate. See figure 5.5 in Chapter 5, "Fatty Acids," for greater detail.

Notes:

COENZYME Q_{10}

Common name: Coenzyme Q_{10} (CoQ_{10}), ubiquinone

Biochemical role: Oxidative phosphorylation

Deficiency tests: Coenzyme Q_{10} – serum, urinary hydroxymethylglutarate, lactate, succinate, fumarate, malate, pyruvate

Adult repletion: 10 to 300 mg/d

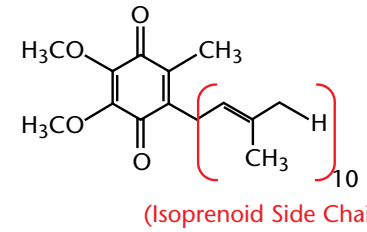


FIGURE 2.19 — **Coenzyme Q_{10}**

The isoprenoid side chain of coenzyme Q (identified in red) determines the type of coenzyme. Thus, the form with 10 isoprenoid groups is called coenzyme Q_{10}. The oxygen-containing quinone ring can accept single electrons becoming a free radical shuttle to the heme centers of cytochromes.

Physiological Function: Coenzyme Q_{10} (CoQ_{10}), also called ubiquinone, is a quinone with an isoprenoid side chain (Figure 2.19). The number of side chains determines the type of coenzyme. The primary function of CoQ_{10} is to shuttle electrons through the electron transport chain (ETC) in the mitochondrial inner membrane. This pathway is also referred to as the oxidative phosphorylation part of the central energy pathway. The electrons are received directly from succinate and choline, or indirectly from several other substrates such as, pyruvate, acyl-CoA and alpha-ketoglutarate. CoQ_{10} moves from one electron carrier complex to the other to ultimately deliver electrons to oxygen, one at a time, in a never-ending cycle of oxidation and reduction (Figure 2.20).

While the electrons are delivered one at a time, they leave in pairs to form ATP and H_20. If CoQ_{10} availability is not adequate, the electrons will not be able to travel in pairs and single electrons will take another, less desirable, pathway that can lead to the generation of superoxide radicals. Optimal functioning of this pathway is critical for the fundamental energy generation that powers all cell functions.

The tissue that has the largest, most critical energy demand —the heart—will first show effects of conditional deficiency of the coenzyme. Preoperative oral CoQ_{10} therapy (300 mg/d) in patients undergoing cardiac surgery increased myocardial and cardiac mitochondrial CoQ_{10} levels, improved mitochondrial efficiency and increased myocardial tolerance to in vitro hypoxia-reoxygenation stress. Average serum CoQ_{10} levels in this experiment for the control and supplemented groups were 0.4 and 1.6 µg/mg protein, respectively.[250] Endurance exercised rats showed increases of CoQ_{10} in heart and skeletal muscle out of proportion with the changes in other electron transport system components, indicating a direct role of the coenzyme in regulation of mitochondrial capacity for forming ATP.[251] There also may be a role of CoQ_{10} as an antioxidant, independent of its function in mitochondrial electron transport.[252]

Deficiency: Research has identified CoQ_{10} as a conditionally essential nutrient. It is found in significant amounts in all nutrient-dense foods. Plants contain a slightly different compound that may serve the electron transport function in animals, though with lower efficiency. Patients with mitochondrial encephalomyopathy owing to electron transport complex I and IV deficiencies show improved mitochondrial function and an increased capacity for fat metabolism when supplemented with 150 mg/d of CoQ_{10}.[211] Lactate and pyruvate elevations are reduced in patients with mitochondrial myopathy when they are given daily oral doses of 120 mg of CoQ_{10}.[212] In a more recent case described as primary deficiency of CoQ_{10}, an 11-year-old male displayed insidious onset of exercise intolerance and

TABLE 2.13 —TESTS FOR CoQ_{10} DEFICIENCY

Test	Abnormality	Indication
CoQ_{10} , serum	Low	Sign of depletion of tissue CoQ_{10}
Hydroxymethylglutarate (HMG), urine	Low High	Metabolic block before HMG Metabolic block after HMG
Lactate, succinate, fumarate, malate, urine	High	Functional insufficiency to meet energy pathway demands

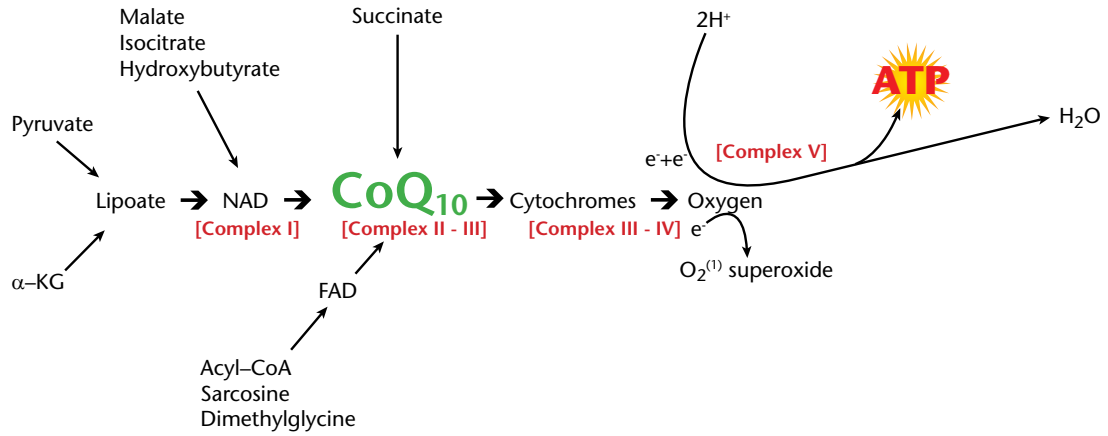


FIGURE 2.20 — Relationship of Coenzyme Q_{10} to Oxidizing Pathways

The metabolic intermediates shown entering the pathway originate from dietary fat, carbohydrate and protein. The electron transfer that terminates energy-yielding pathways must be processed through the CoQ_{10} shuttle. CoQ_{10} moves from one electron carrier complex to the other ultimately delivering electrons to oxygen, one at a time. Even transient, mild insufficiency of CoQ_{10} can impair the massive mitochondrial electron transfer process, allowing single electrons to spill onto other acceptors that can initiate free radical pathologies.

proximal muscle weakness with constitutional fatigue, weight loss and muscle cramps in his lower extremities. Abnormalities in organic acids and respiratory chain enzymes normalized when CoQ_{10} was supplemented at 300 mg/d.[213]

Assessment of Status: The clinical interpretation of abnormal serum CoQ_{10} levels may be enhanced by simultaneous measurement of markers that monitor biosynthetic and functional roles of the cofactor. CoQ_{10} synthesis is dependent on the availability of hydroxymethylglutarate (HMG). Thus, if HMG is low it will slow the rate of CoQ_{10} synthesis. Statin drugs block the conversion of HMG to cholesterol and to CoQ_{10}. HMG accumulates and spills in urine at abnormally high levels that signals reduced CoQ_{10} biosynthesis (Table 2.13). Impairment of the synthetic pathway does not necessarily mean functional impairment. A functional impairment at the level of mitochondrial CoQ_{10} electron transfer will also affect succinate, malate, fumarate and pyruvate, which are the energy pathway intermediates. CoQ_{10} deficiency causes elevation of these intermediates. The direct transfer of electrons from succinate to the FMN reductase enzyme in the electron transport system is slowed when the electron shuttle action of CoQ_{10} is inadequate to meet demands. Chapter 12, "Pattern Analysis." presents further detail about the interpretation of multiple profile data for vitamins and the other nutrient classes covered here.

REFER TO CASE ILLUSTRATION 2.1

Notes:

LIPOIC ACID (THIOCTIC ACID)

Active forms: R-α-lipoic acid; dihydrolipoic acid

Biochemical role: Keto acid dehydrogenase cofactor and glutathione recycling

Example: Pyruvate ➔ Acetyl-CoA + CO_2 GSH ➔ GSSG

Deficiency Tests: Elevation of urinary lactate, pyruvate, branched-chain α-keto acids, α-ketoglutarate

Adult Repletion: 100 to 1,800 mg/d

Physiological Function: Lipoic acid is a disulfide compound that contains a chiral center and thus has two enantiomers, or mirror images. Lipoic acid can also be reduced to dihydrolipoic acid (DHLA) (Figure 2.21). As a primary component of the lipoyl subunits, lipoic acid is required to carry out oxidative decarboxylation reactions catalyzed by several alpha-keto acid enzyme complexes—pyruvate dehydrogenase (PDC), alpha-ketoglutarate dehydrogenase, (KGDC), the branched-chain alpha-keto acid dehydrogenase complex (BCKD)—as well as for the glycine cleavage system (GCS).[214-216] Besides its function in dehydrogenase activities, it is also a powerful antioxidant that has been found to quench reactive oxygen species. It functions as a recycler of endogenous antioxidants, like glutathione and vitamin C.[217, 218] Promising research with supplemental lipoic acid has been conducted on hepatic injury, HIV, glaucoma, cancer, CVD, Alzheimer disease and diabetes complications.[219-225]

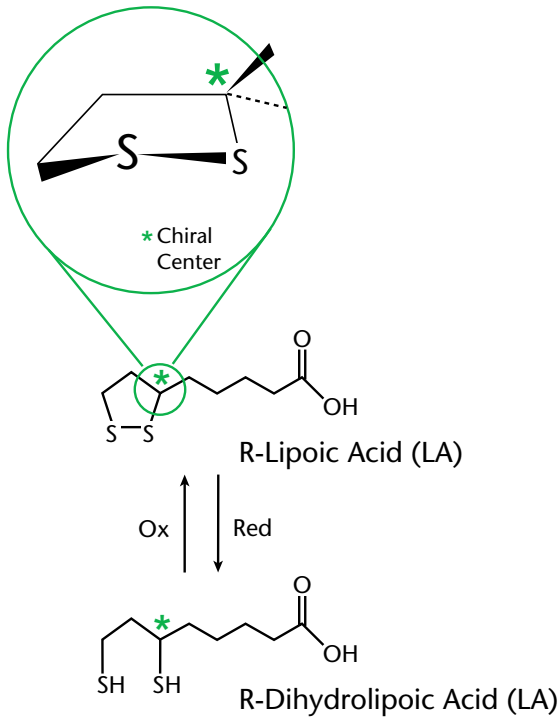


FIGURE 2.21 —Structural Features of Lipoic Acid

The inset shows a close up view of the reactive disulfide group that becomes reduced to two sulfhydryl groups when lipoic acid functions as a coenzyme in dehydrogenation reactions. When the lipoyl group is attached to the five-atom side chain of lysine, the sulfur center extends well away from the polypeptide chain of the apo-enzyme, providing an accessible and movable point for attachment of reactants.

Lipoic acid comes primarily from dietary sources. It is highest in animal tissues, with a smaller percentage found in fruits and vegetables.[226] It can also be synthesized de novo in the mitochondria from the fatty acid octanoic acid (caprylic acid) and cysteine, by way of lipoic acid synthase.[217, 227, 228]

Deficiency: R-alpha-lipoic acid (ALA) is conditionally essential, illustrated by its ability to increase insulin sensitivity in diabetics after short-term (10 d or less) parenteral administration.[229] Oral administration of alpha-lipoic acid has also been shown to improve insulin sensitivity in patients with type-2 diabetes, as well as lowering their serum lactate and pyruvate levels.[230] The mechanism of this effect appears to be direct stimulation of glucose uptake. Both basal and insulin-stimulated glucose transport is stimulated by addition of ALA to cell cultures of adipocytes.[231]

Assessment of Status: The oxidative decarboxylation of pyruvate, alpha-ketoglutarate and branched-chain alpha-keto acids requires lipoic acid as a cofactor.[226] These enzymes are multi-subunit complexes.[232] Genetic differences in the lipoyl-containing subunit can affect the metabolic activity of all three enzymes. An insufficient or deficient amount of lipoic acid, as well as genetic polymorphism in the lipoyl-containing subunit can cause insufficient function of these enzymes, leading to elevated pyruvate, branched-chain alpha-keto acids, alpha-ketoglutarate and lactate.[232, 233] The activity of the pyruvate dehydrogenase complex (PDC) has been shown to be reconstituted by addition of ALA,[234] yet some research has suggested that high doses (600 mg) of lipoic acid can inhibit the PDC and lead to an elevated lactate.[216] In type 2 diabetes patients, elevations of urinary pyruvate and lactate have been normalized by 600 mg of ALA twice daily.[230]

CHOLINE

Biochemical role: Membrane structure, neurotransmitter formation, methyl donor, fatty acid transport

Deficiency tests: Liver phosphocholine; choline – plasma

Adult repletion: 2 g/d, (Best in divided dose)

Physiological Functions: Choline has three methyl groups (Figure 2.22) that are available for transfer to homocysteine or tetrahydrofolate. Choline is essential for cell membrane structure and signaling; as a methyl donor via betaine; as a neurotransmitter (acetylcholine); a precursor to phospholipids (phosphatidylcholine and sphingomyelin); and in the metabolism and transport of cholesterol and lipids.[59, 235] Thus it is not surprising that choline has been studied for the treatment of dementia, heart disease and cancer.[59, 236-238]

Although choline can be synthesized in human tissues by methylation of glycine, there are many conditions that limit the rate of synthesis, making choline a conditionally essential nutrient.[236] Deficiencies of folic acid, vitamin B_{12} and methyl donor compounds increase the potential for choline insufficiency. Lack of availability of the precursor amino acid glycine can limit choline biosynthesis as described in Chapter 4, "Amino Acids."

Deficiency: Reports of requirements for choline come primarily from studies of choline depleted diets, especially those with adequate methionine, B_{12} and folate. Maternal reserves of choline are depleted during pregnancy and lactation.[239] Dietary deficiency of choline reduces hippocampal release of acetylcholine in rats.[240] Such effects may explain the beneficial effects found for CDP-choline (cytidine 5'-diphosphocholine) supplementation in elderly patients with cognitive deficits, inefficient memory and early-stage Alzheimer's disease.[241]

The primary marker of deficient choline is liver damage, identified by elevated alanine aminotransferase (ALT). Choline deficiencies have been found to induce apoptosis in rat cells.[59, 242] Excessive choline supplementation can lead to a fishy body odor, gastrointestinal disturbances, sweating and salivation. Large doses of choline may be contraindicated for those with renal and liver disease, trimethylaminuria, depression and Parkinson disease. Methotrexate has been shown to decrease choline levels and administration of choline has been shown to decrease liver damage caused by the drug.[59, 243]

Assessment of Status: Though choline can be synthesized endogenously, much of the body's demand for it is regulated by the interplay of choline, methionine, folate and B_{12}, thus an assessment of choline status must look at the interrelationship of all of these nutrients.[59] The best marker of dietary choline intake is liver phosphocholine. Plasma choline levels range from 7 to 20 µmol/L, with an average of 10 µmol/L. Plasma levels may not be stable enough to use in assessing choline status because levels increase significantly with dietary intake or supplement use, but rarely fall dramatically, even when fasting.[59, 236, 244, 245]

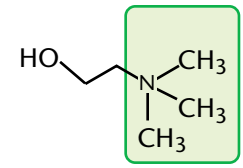


FIGURE 2.22 — **The Methyl Groups of Choline**

The shading indicates the three methyl groups that are available for transfer to homocysteine or tetrahydrofolate, forming methionine or 5,10-methylene-THF, respectively.

Choline supplements come in two forms—choline chloride or choline bitartrate. Lecithin can also be used as a choline supplement.[59] Diets with high lecithin content can double plasma choline concentrations.[245] Food databases are limited in their ability to evaluate choline intake, though 730 to 1,040 mg/day has been estimated to be the average U.S. intake.[59]

Notes:

SUMMARY

Deficiencies or even mild insufficiencies of vitamins can result in a wide range of negative consequences for human health. Even in developed countries, where protein and caloric intake may be adequate, humans can become depleted owing to low dietary intake, poor digestion and absorption of nutrients from an otherwise "healthy" diet, increased cellular demands for a given nutrient, or medications that affect the absorption or catabolism of vitamins. Genetic factors can also be revealed by abnormalities in laboratory tests of metabolic function owing to effects on enzyme activities or regulatory proteins. This chapter has reviewed the biochemistry and physiology, assessment and repletion of a variety of essential and conditionally essential vitamins.

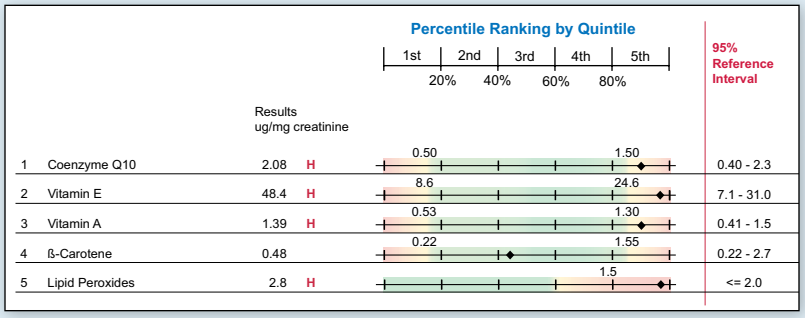
Assessment tools include direct measurement in blood, challenge or loading tests, ratios, related hormones, cellular assays, enzyme assays and measurements of amino acid precursors in urine or blood. Direct measurement of vitamins in specimens such as serum reveals circulating concentrations. Functional markers can further inform the practitioner whether circulating levels of a given vitamin are adequate to sustain an individual's metabolic demand for the vitamin.

Notes:

Case Illustrations

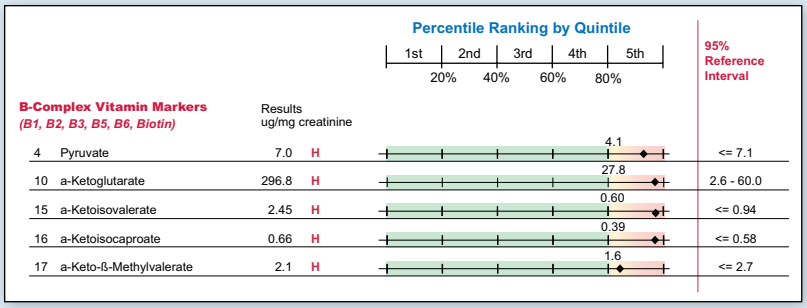
Case Illustration 2.1 — Fat-Soluble Vitamin Supplementation Pattern

This pattern was found in a 52-year-old man, suffering from cardiovascular disease who had been taking daily oral supplements of vitamins A, E and CoQ_{10} for several months. Note that, in spite of high antioxidant vitamin levels in serum, he still has elevated lipid peroxides indicating risk of further degeneration owing to oxidative challenge. ❖



Case Illustration 2.2 — Functional Markers of B-complex Deficiency

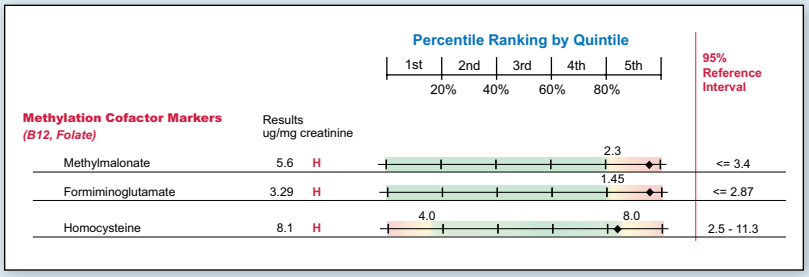
A 62-year-old female with chronic fatigue and depression is found to have significant elevations of all five of the keto acids reported on her profile of organic acids in urine. Aggressive thiamin alone may resolve the biochemical lesion and produce favorable clinical resolution of the fatigue and depression. Since the keto acid dehydrogenase enzyme system also requires vitamins B_2, B_3, pantothenic acid and lipoic acid, however, the usual treatment is a high potency B-complex preparation. Retesting may be done as soon as 90 days for adjustment of dosages required to stabilize the keto acids in their normal ranges. ❖



Notes:

Case Illustration 2.3 — Multiple Methylation Cofactor Deficiency

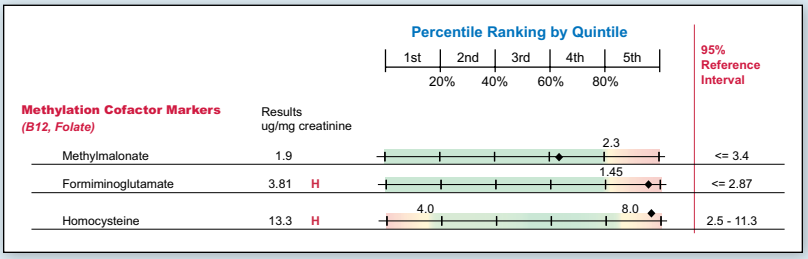
These results were found for the same 52-year-old male with a cardiovascular disease shown in Case Illustration 2.1.



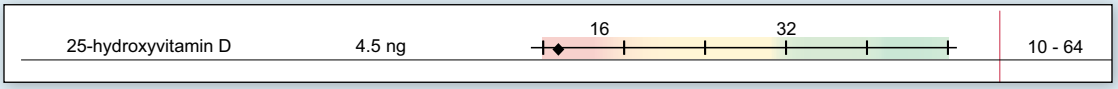
Here his plasma homocysteine is seen to be slightly elevated. The concurrent urinary organic acid results showing elevated markers for insufficiency of vitamin B_{12} (Methylmalonate) and folate (Formiminoglutamate) predict progressive worsening of the laboratory homocysteine measurements and of his cardiovascular disease, if not corrected. ❖

Case Illustration 2.4 — High Homocysteine Associated with Folate but Not Vitamin B_{12} Deficiency

This 72-year-old male came for a wellness check-up. His workup included a plasma homocysteine and a urinary organic acid profile. In his case the nutritional therapy should focus on a course of aggressive folate, but it does not need to include high doses of vitamin B_{12} according to the normal value found for methylmalonate. Comparison of this pattern with that shown in Case Illustration 2.3 provides insight regarding the range of individual variation revealed by metabolic assessments. ❖



Case Illustration 2.5 — Vitamin D Measurement to Avoid Insidious Bone Loss



A 32-year-old female scheduled a routine office visit with no current health problems. Her serum vitamin D level was found to be very low. Left without the simple and inexpensive supplementation with vitamin D, this pattern foretells chronic bone loss that is generally insidious for decades before incidents of fracture or diagnostic bone scan techniques reveal bone loss that is difficult to reverse, not to mention increased risks of depression and immune dysregulation. Thus, this type of patient presents opportunity for the clinically optimal intervention that can result in total avoidance of major degenerative diseases. The test result provides evidence on which to base aggressive vitamin D supplementation for several months with follow up testing to demonstrate normalization of serum 25-hydroxyvitamin D concentration. ❖

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Chapter 3

Nutrient and Toxic Elements

Kara N. Fitzgerald, Cass Nelson-Dooley and Richard S. Lord

CONTENTS

Notes:

"Laboratory testing can reveal element deficiencies and toxicities by direct measurement of element concentrations in body fluids or tissues, or by measuring biochemical markers that give evidence of the element's metabolic activity, be it toxic or essential."

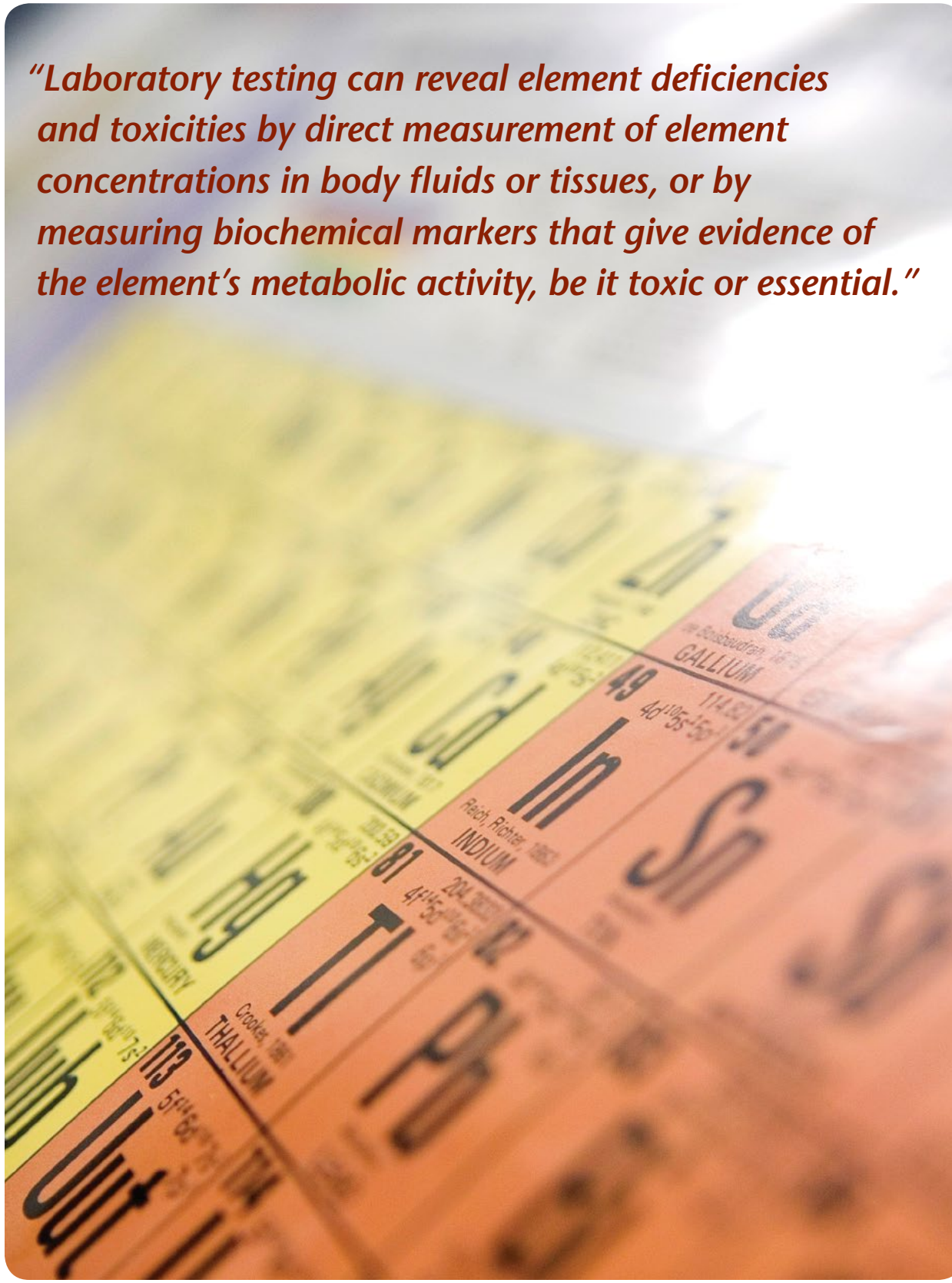


Table 3.1 — Summary of Essential Element Assessment and Treatment

	Element	Primary function	Laboratory Assessments		Repletion Dosage*		
			Direct Markers	Functional Biomarkers	Children 2–12 yrs	Adult Male	Adult Female
Nutritionally Essential Elements	Calcium (Ca)	Bone and tooth formation, blood clotting, nerve and muscle contraction	Hair, urine	Bone resorption markers, serum 25-hydroxyvitamin D, PTH	500–1200 mg	800–1300 mg	800–1300 mg**
	Magnesium (Mg)	Muscle, nerve action, coenzyme for carbohydrate and protein metabolism	RBC, whole blood, plasma, serum, urine	Refractory hypokalemia	200–600 mg	350–750 mg	300–700 mg**
	Potassium (K)	Intracellular fluid control, nerve & muscle contraction, energy metabolism	RBC, serum, hair	NA	50–150 mg	150–300 mg	150–300 mg
	Iron (Fe)	Heme function, cofactor for enzymes, cytochromes, RBC production,	Ferritin	Total iron binding capacity, transferrin saturation, hemoglobin, hematocrit	10–20 mg IDA*: 5 mg/kg	10–30 mg IDA*: 90–200 mg	18–50 mg** IDA*: 90–200 mg
	Zinc (Zn)	Cofactor for many enzymes; structural role in proteins including DNA	RBC, hair, plasma	Delta 6 desaturase activity (LA:GLA) Zn -metallothionein	8–30 mg	15–65 mg	15–65 mg**
	Copper (Cu)	Enzyme cofactor (erythrocyte superoxide dismutase)	RBC, serum ceruloplasmin	Elevated urinary HVA/VMA ratio; bone resorption markers	1.0–5 mg	2.0–10 mg	2.0–10 mg
	Manganese (Mn)	Cofactor for many enzymes—able to replace Zn in SOD and Mg in pyruvate carboxylase	RBC	Abnormal urinary ammonia markers, elevated arginine: ornithine ratio	1–10 mg	5–13 mg	5–13 mg
	Selenium (Se)	Cofactor for glutathione peroxidase, thyroxin oxidase	Serum, whole blood, hair	Selenoprotein P, urinary selenosugars	50–150 µg	50–400 µg	50–400 µg
	Molybdenum (Mo)	Sulfur metabolism, purine degradation	Hair	Elevated xanthine: uric acid ratio	20–200 µg	50–400 µg	50–400 µg
	Chromium (Cr)	Insulin receptor binding factor	RBC, urine, hair	Insulin, blood glucose	50–500 µg	200–1000 µg	200–1000 µg
	Iodine (I)	Thyroid hormone	Urine	Serum T3, T4, TSH, blood spot thyroglobulin	90–600 µg	150 µg–50 mg*	150 µg–50 mg*
Elements of Uncertain Human Requirements***	Boron (B)	Steroid metabolism, osteoporosis	Serum, urine, hair	Bone resorption markers	NA	1–12 mg	
	Nickel (Ni)	Growth, reproduction (animal studies only)	Urine, plasma, hair	None	NA	< 5 µg	
	Lithium (Li)	Psychosis, depression	Serum, plasma, urine, hair	None	NA	400–1000 µg	
	Vanadium (V)	Glucose transport and metabolism	Whole blood, serum, urine	Blood glucose, blood lipids	NA	9–250 µg	
	Strontium (Sr)	Osteoporosis	Serum, urine, hair	Bone resorption markers	NA	125–680 mg	

* *Repletion dosages are commonly used amounts to assist the normalization of an individual with demonstrated nutrient insufficiency. Amounts shown are daily oral doses of bioavailable element salts commonly used in research and clinical settings. The percent of element present in a given element chelate may vary depending on form. Check with product manufacturer. See individual element sections for further discussion. Iron deficiency anemia (IDA).*

** *In pregnant or lactating women: Calcium up to 2000 mg, magnesium 600 mg, zinc 50 mg. Daily iron requirements during pregnancy may be 30 to 60 mg but revert to non-pregnant amounts 2 to 3 months after delivery, even if lactating.*

*** *Refer to individual sections for further discussion of reasons for supplementation.*

General Concepts

Across all demographic strata in industrialized nations, element deficiencies are recognized as being involved in the pathogenesis of many health conditions, including heart disease, hypertension[3] and cancer.[4-12] Certain populations demonstrate a relatively high incidence of elemental deficiencies, including pregnant women,[13-15] children and adolescents,[16,17-19] the elderly,[20] and those who are immunocompromised.[21-23] However, in the United States the recommended dietary allowance (RDA) (currently designated DRI*) is not met by diet alone in most population groups for magnesium, calcium and potassium.[24, 25] Element deficiencies during the perinatal period can contribute to behavioral, immunological and biochemical abnormalities that can last in adulthood.[26] Essential element deficiencies during embryonic development can cause mental retardation[27] and may contribute to the severity of genetic abnormalities.[26] The causes of such widespread element deficiencies vary with each element, but may be categorized as those which decrease supply and those that increase demand. Figure 3.1 illustrates how the overall contributions of various factors combine to dictate the progression toward iron deficiency or repletion. Supply is lowered by depleted soil,[28] toxic element interference,[29] poor food choices[25] and compromised digestion, including iatrogenic-induced gastric acid reduction.[30, 31] Most of these factors affecting demand also generally impact other essential elements.

The term "mineral" that has been used in many discussions of the nutritionally essential elements actually refers to natural compounds formed through geological processes. Since the nutritionally essential chemical elements covered in this chapter occur in the human body in forms quite distinct from those found in the crust of the earth, the more general term "element" will be used. The same may be said of the toxic elements. Thus, the terminology "nutrient and toxic elements" in clinical assessments of blood, urine, hair

** While the term dietary reference intake (DRI) has been adopted for discussions of intake levels, the older term, recommended dietary allowance (RDA), will be used instead because of its continued widespread use by clinicians and laypeople. The term tolerable upper limit (UL) is used when discussing current recommended upper intake levels.*

Table 3-2 — Summary of Toxic Metal Assessments and Protective Measures

Metal	Toxicity Symptoms	Body Burden Assessment	Biochemical Marker	Protective Measures[1, 2]	Mechanism
Aluminum	Abnormal speech, myoclonic jerks, osteomalacia, progressive encephalopathy, Alzheimer's disease, Parkinson's disease	Whole blood, serum, hair, urine	Total urinary porphyrins	Phosphorous Calcium	Lowers intestinal absorption
Arsenic	Peripheral arteriosclerosis ("blackfoot disease"), "rice-water" stools, proteinuria, hyperkeratosis, "milk and roses" hyperpigmentation, garlic breath odor, stomatitis	Whole blood, urine, hair, nails	Urinary uroporphyrin, coproporphyrin I/III, hexacarboxyporphyrin	*Emblica officinalis*, selenium, NAC, glutathione	Competes for binding sites
Cadmium	Femoral pain, lumbago, osteopenia, renal dysfunction, hypertension, vascular disease	Whole blood	Coproporphyrin I	Zinc, antioxidants	Competes for binding sites
Lead	Microcytic hypochromic anemia, renal dysfunction, hypertension, anorexia, muscle discomfort, constipation, metallic taste, low IQ (children)	Whole blood, urine, hair	Urinary coproporphyrins, (sometimes I), zinc protoporphyrin, ALA	Calcium, ascorbate, alpha lipoic acid, iron adequacy	Lowers intestinal absorption
Mercury	Mental symptoms (erethism, insomnia, fatigue, poor short-term memory), tremor, stomatitis, gingivitis, GI and renal disturbances, decreased immunity	Whole blood, urine, hair	Urinary pentacarboxy-porphyrin, coproporphyrin III, precoproporphyrin	Selenium, NAC	Protects against cellular toxic effects

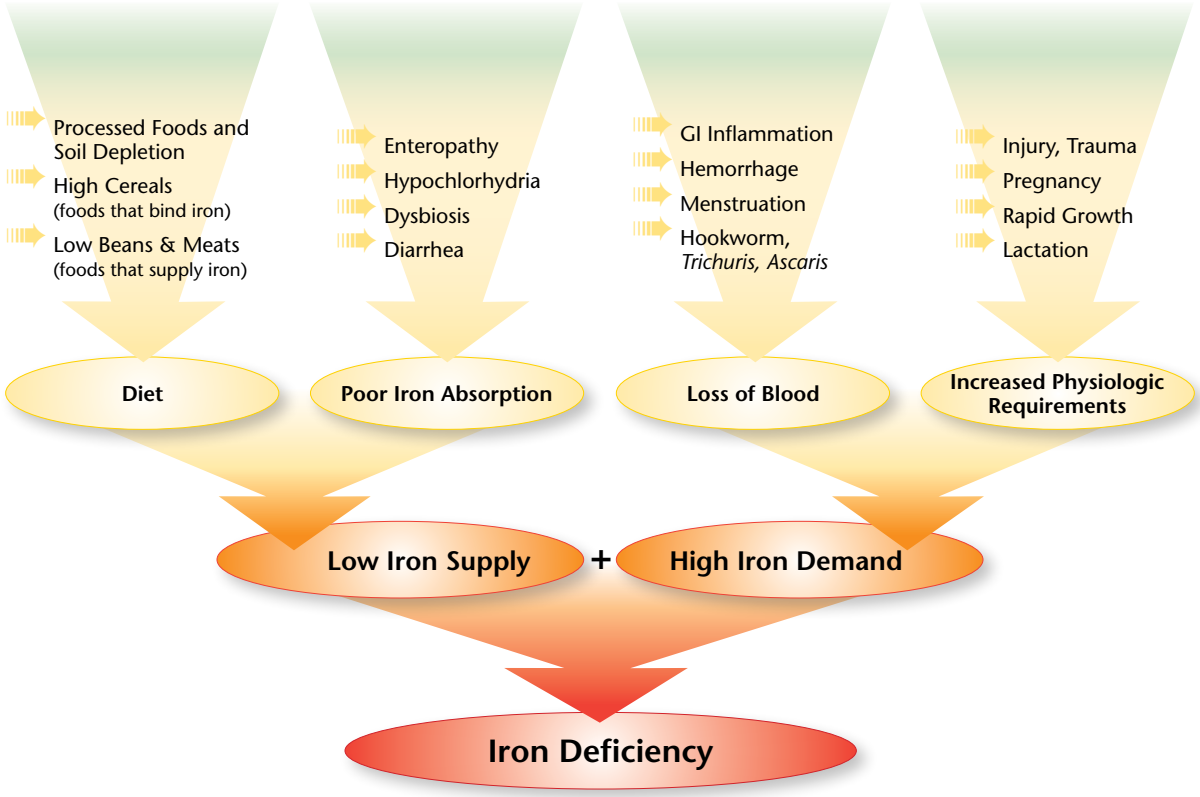


Figure 3.1 — Factors Contributing to Iron Deficiency

Various combinations of factors can add up to elemental deficiency, as illustrated here for iron. To move a patient from states of deficiency to repletion, multiple actions may be required, the most common of which is oral supplementation of absorbable forms of iron or other elements as indicated by laboratory evaluations. The ultimate goal is to guide the patient to improved habits of diet and lifestyle that improve supply while managing factors that increase demand.

or other body fluids or tissues corresponds to most clinical laboratory reporting conventions.

As for all of the discussions of nutrient use in patient treatment in this book, the object is to present practicing clinicians with practical information for assisting the nutrient-deficient patient's return to optimal function. This objective is to be distinguished from those of government and scientific panels that may attempt to derive safe and effective levels of intake to prevent frank deficiency or possible toxicity. Disagreement exists among experts worldwide as to daily element requirements versus tolerable upper limits for the general population.[32] For instance, the current RDA for total magnesium intake in males is 420 mg/d. This level is higher than the published tolerable upper limit of 350 mg/d of supplemental magnesium.[33] Accurate laboratory testing to assess body status pre- and post-supplementation can ensure repletion by demonstration of normal test results, along with clinical observations to monitor patient improvement.

Clinical laboratory testing likewise clarifies questions of excessive use of nutrient elements and significant exposure for toxic elements, whereas functional markers can demonstrate metabolic poisoning. In routine clinical practice it is generally agreed that testing should identify chronic low level effects as well as diagnose the frankly toxic individual. Various government authorities have set levels that are agreed to raise alarm about imminent severe toxic effects of organ failure or life-threatening irreversible damage. However, chronic exposure to low levels of toxic elements can lead to disparate clinical disorders with manifestations vastly different from the symptoms of acute toxicity.[34] Thus, the clinical laboratory may establish certain toxic element

limits for potential concern plus higher limits for alarm.

Laboratory testing can reveal element deficiencies and toxicities by direct measurement of element concentrations in body fluids or tissues, or by measuring biochemical markers that give evidence of the element's metabolic activity, be it toxic or essential. For example, urinary iodine can provide evidence of intake, whereas serum free T3, free T4 and thyroid-stimulating hormone are iodine functional biomarkers. The various options for essential element assessment and dosages for repletion are summarized in Table 3.1 and are further elaborated under each element section below. Likewise,

TABLE 3.3 — SUMMARY OF ESSENTIAL ELEMENT DEFICIENCIES AND TOXICITIES*

Element	Deficiency		Toxicity
	Causes	Signs and Symptoms	Signs and Symptoms
Calcium (Ca)	Decreased intake, malabsorption, renal failure, thyroid disorders, parathyroidectomy, hypovitaminosis D, medications (e.g., heparin, glucagon)	Tetany, osteomalacia, arrhythmia, dry skin, brittle nails, mood changes (depression or irritability)	Anorexia, constipation, nausea and vomiting, confusion, depression, fatigue, polyuria, arrhythmia, dehydration
Magnesium (Mg)	Decreased intake, malabsorption, alcoholism, renal tubular leak, aldosteronism, hyperparathyroidism, medications (e.g., diuretics)	Muscular twitching and weakness, arrhythmias, irritability, convulsions, poor growth, insomnia, depression, hypertension, cardiovascular disease	Nausea, vomiting, hypotension, weakness, hyporeflexia, confusion, lethargy, decreases heart and respiration rates
Potassium (K)	Decreased intake, losses from vomiting, diarrhea, renal losses, diuretics, aldosteronism, Cushing's syndrome, congestive heart failure	Muscle weakness, arrhythmia, tetany, hypertension	Paresthesia, confusion, cardiac depression, weakness, nausea, abdominal pain, diarrhea
Iron (Fe)	Decreased intake, blood loss, increased requirement due to pregnancy	Anemia, fatigue, depression, palpitations, tachycardia	Cirrhosis, heart failure, skin pigmentation, diabetes, arthropathy, tarry stools, nausea and vomiting, lethargy, bradycardia, hypotension, dyspnea, coma
Zinc (Zn)	Decreased intake, malabsorption, acrodermatitis enteropathica, diarrhea, sickle cell disease, pregnancy	Rashes, anorexia, lethargy, growth retardation, alopecia, impaired immunity, slow wound healing, altered taste, night blindness, functional hypothryroidism	Cu deficiency, impaired immunity
Copper (Cu)	Decreased intake, malabsorption, prematurity, malnutrition	Anemia, neutropenia	Nausea/vomiting, hepatic necrosis, abdominal pain, splenomegaly, jaundice, weakness, tremors of arms or hands, slow movement, speech impairment, Kayser-Fliescher rings
Manganese (Mn)	Decreased intake, malabsorption	Hypercholesterolemia, weight loss	Hallucinations, neural damage
Selenium (Se)	Decreased intake, malabsorption	Cardiomyopathy, osteopathy, decreased cell-mediated immunity, functional hypothyroidism	Brittle hair and nails, hair loss, fatigue, peripheral neuropathy, rashes, halitosis similar to garlic odor, irritability
Molybdenum (Mo)	Decreased intake, malabsorption	Sulfur metabolism irregularities, mental disturbance, coma	Copper deficiency, altered nucleotide metabolism, gout, oxidative stress
Chromium (Cr)	Decreased intake, malabsorption	Glucose intolerance, sugar cravings	None
Iodine (I)	Decreased intake, impaired conversion	Neurologic, developmental, reproductive	Monitor thyroid hormone levels when supplementing with iodine. See Iodine section.

* *Deficiency and toxicity symptoms listed in this table are primarily severe manifestations, infrequently seen in developed nations. Presentations associated with "sub-clinical" element abnormalities are discussed in the following sections.*

Table 3.4 — Reports Showing Associations of Essential Element Insufficiency with the Top Causes of Death in the United States (2005*)

Cause of Death*	Year of Publication: Number of Subjects						
	Calcium	Magnesium	Zinc	Selenium	Potassium	Chromium	Copper
Heart disease	2006: 110,792[36]	2006: 4,035[37] 2003: 7,172[38] 1999: 2,316[39, 40] 1992: 930[41]	2006: 4,035[37] 2005: 70[42]	2005: 70[42] 1996: 3[43]	1979: 21[44]	1992: 76[45] 1991: 63[46]	2001: 80[47]
Malignant neoplasms	2007: 2,110[48] 2006: 45,306[49]	2005: 61,433[50]	2006: 857[51]	2006: 218[52, 53] 1998: 974[54]			2006: 3,352[55]
Cerebrovascular diseases	2006: 110,792[36] 1999: 85,764[56]	1998: 13,922[57] 1998: 43,738[58]		2004: 1103[59]	2002: 5,600[60] 2001: 9,805[61]		1995: 149[62]
Chronic respiratory diseases		1997: 20[63]		1995: 79[64] 1983: 81[65]			
Diabetes mellitus	2006: 83,779[66]	2004: 39,345[67] 1994: 20[68] 1992: 37[69]	2004: 39,345[67] 1994: 20[68]	2005: 1,247[70] 2005: 92[71] 2005: 194[72]		2006: 36[73] 1997: 180[74]	
Alzheimer's disease		2005: 6[75]		1986: 25[76]			2005: 32[77]
Nephritis, nephritic syndrome			2005: 72[72]	1985: 78[78]			
Liver disease			1995: 253[79]	1992: 188[80]			
Essential hypertension	1991: 1,928[81] 1985: 80[82] 1982: 90[83]	2005: 12,344[12] 1999: 7,731[84] 1998: 60[85] 1992: 30,681[86]	1995: 62[87]	1998: 57[88] 1992: 3,016[89]	2001: 17,030[90]	1991: 63[46]	1995: 62[87]

discussions of assessment methods and protective measures for each toxic element are summarized in Table 3.2.

Before entering into discussions of individual elements, tables of information are presented to convey the spectrum of elemental deficiency clinical effects and relationships to mortality. Table 3.3 summarizes signs and symptoms of deficiency and toxicity for the nutritionally essential elements. Small tables containing parts of this information are placed at the beginning of each element discussion. Table 3.4 lists specific studies that have shown relationships of elemental deficiencies to top causes of death in the United States.

Practitioners frequently raise questions regarding the best specimen or functional biomarker for screening versus confirmatory evidence of deficiency or toxicity and the clinical significance of abnormalities detected. The first section of this chapter focuses on the general concepts of element metabolism. Next, general considerations of specimen and test choice are discussed, followed by detailed discussions of each nutrient and toxic element, with emphasis on effective approaches to assessment of individual patient status.

The elements are grouped by their designations as essential, uncertain, toxic or potentially toxic and, within those categories, by their relative concentrations in the human body (macroelement or trace element). An introduction to the toxic element section includes a discussion on mechanisms of toxicity. For most elements, the discussion is divided into sections on biochemical and nutritional background, clinical indications, and the consequences of deficiency or toxicity. For some elements, the routes of absorption and excretion and homeostatic mechanisms regulating total-body status and transport are discussed. Assessment of element status

Notes:

is covered, with attention to functional biomarkers and various specimen types. Element sections are concluded with discussions of interventions by dietary modification, the use of nutritional supplements, or toxic element removal strategies.

BIOCHEMISTRY AND TOXICOLOGY OF ELEMENTS

Whole-body content of the major, or macro, elements such as calcium and magnesium are in gram amounts, whereas trace elements such as copper and manganese are in milligram amounts. To the analytical chemist, however, the term "trace element" indicates that, because the element cannot be measured with high accuracy, it must be designated as "less than detection limit" in the specimen submitted for analysis. As instrumental sensitivities improve, trace element may then imply one that was previously undetectable. Those that are difficult to detect with more sensitive methods may be called "ultratrace elements." The elements that fall into such analytical categories vary from one tissue to the next, but the terminology is useful for general discrimination of relative amounts.

Thirteen trace elements are currently known to be nutritionally essential for human health. Usually present at concentrations of micrograms per gram of tissue or less (< 1 ppm), these elements are iron, copper, zinc, iodine, selenium, boron, cobalt, chromium, molybdenum, manganese, vanadium, silicon and nickel.[5] Macrominerals phosphorus, magnesium and calcium are present in total-body content of tens or hundreds of grams. The macrominerals sodium, potassium and chloride are classified as electrolytes because of their roles in maintaining ionic equlibria in physiological systems.

Plants and animals have evolved highly efficient mechanisms for extracting nutritionally important elements that are often present in soil and food in minute amounts. A defining characteristic of a trace element is that a very small amount is necessary for proper function of the whole organism. Only a few milligrams of an element such as zinc in a large organ such as liver or muscle can make the difference between normal and abnormal function. When a zinc ion is not available to function as an enzyme cofactor, or a toxic element such as cadmium displaces the available zinc, the result is amplified because of the interruption in flow of metabolic products. When those products are cell regulators that modulate yet other systems, the end effect can be a tissue-wide slowdown generated by loss of only a tiny amount of active zinc.

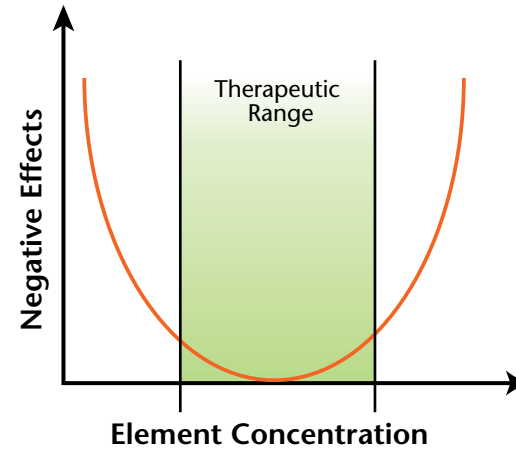


FIGURE 3.2 — Effects of Elemental Deficiency or Excess

Negative consequences usually are associated with deficiency or excess of any element, as illustrated by the U-shape of the effects curve. For example, copper deficiency may be associated with increased oxidative stress due to low activity of the cuproenzyme superoxide dismutase. On the other hand, excess free copper ions produce oxidative stress via the Fenton reaction, which generates hydroxyl radicals. Thus, elements like copper have a fairly narrow concentration range for optimum function. Laboratory evaluations may be needed to demonstrate that neither extreme is a threat. For further discussion of dose response effects, refer to the Chapter 8 section, "Dose-Response Curve and the Hormesis Model."

The major roles of essential elements in biological systems are (1) electron acceptors in oxidative/reductive homeodynamics; (2) enzyme cofactors; (3) crystalline structures, especially in bone; and (4) ionic migrations necessary for nerve signal transmission or cell regulatory responses. Trace elements such as iron, copper or molybdenum serve biological oxidation-reduction functions due to their ability to easily accept and release electrons (i.e., change valence states). In oxidation steps, electrons are lost, and in reduction steps, electrons are gained. Several trace elements, including zinc and selenium, serve to stabilize enzyme structures or position substrates in enzyme-active sites. Non-metal elements (selenium, iodine) and metalloids (silicon, boron) may form covalent bonds with carbon and oxygen, and they may be found as part of a structural protein or enzyme

(i.e., selenium as selenocysteine in enzymes, and silicon in connective tissue). Magnesium and calcium are found in three states: bound to proteins, bound in the crystalline matrix of bone, and as free ions in all body fluids. In their free ionic forms, calcium and magnesium are regulators or modifiers of enzyme activity.

Essential elements tend to display bell-shaped dose-response curves. The curve represents overall functional response to varying intakes of a given element. Low intake results in a deficiency state, whereas high intake results in toxicity symptoms. In the case of copper, both deficiency and toxicity can cause increased oxidative stress via different mechanisms (Figure 3.2). The optimal amount of trace element intake is in the middle of the curve, where function is maximized and toxicity is minimized. Any element can become harmful if intake exceeds the body's ability to utilize, store or eliminate the element. Nutrient elements are no exception to this rule. For some elements, the difference between deficiency or toxicity spans a 100-fold range of consumption.[95] For others, the range is only 10-fold. The essential element selenium can produce signs of toxicity from daily intakes of only 5 mg. This exceeds the US RDA by only a factor of 25.[96] Of course, some elements are characteristically toxic and cause harm even in very small exposures. Lead, cadmium and mercury rank highest on this list. Modern life in an industrialized society has resulted in increased exposures due to the enormous amounts of toxic metals released into the environment.

Daily fluctuations of essential element intake have small impacts on blood serum concentrations that become trends over several days. Trends in serum and transport or storage protein element content become larger tissue-level trends over longer time intervals. Human elemental status is complicated by complex homeostatic mechanisms (see the section "Iron" below) that sequester elements in specific tissues or change their binding to transport proteins with transient factors such as inflammation. Feedback effects that alter rates of intestinal absorption serve as key homeostatic mechanisms. For example, copper absorption can vary from 12 to 70% of a standard ingested dose.[97] Toxic element assessment, likewise, presents challenges. Because toxic elements are bound to tissue proteins, their levels in body fluids may not reflect total-body burden. Thus, multiple tests, using different specimen types, are recommended for the most accurate and reliable represention of a person's elemental body status.

Nutrient and Toxicant Interactions

Not only are element deficiencies frequently encountered, they also tend to occur in multiples (see Case Illustration 3.1). Multiple element deficiencies may occur when the diet is poor or digestion and absorption are impaired. In an element-deficient state, there may be an up-regulation of transport proteins in the gastrointestinal tract, which may allow for greater absorption of toxic elements.[98] Thus, essential element deficiency can increase vulnerability to toxic element exposure.

The deficiency or excess of one element can affect the utilization of other elements. Multiple elements may interact with a single type of transport protein, although one may bind more strongly than another. For instance, the excessive intake of zinc antagonizes intestinal copper absorption to the point that a copper deficiency can result despite adequate copper intake.[99] Copper deficiency exacerbates iron deficiency anemia. Iron deficiency, in turn, exacerbates the toxic effects of lead and cadmium and increases absorption of manganese.[98, 100] Beneficial interactions also exist. For example, selenium mitigates the toxic effects of mercury[29] and arsenic,[101] whereas zinc minimizes the effects of cadmium toxicity (Table 3.2).[102] Vitamin status also affects elemental status as in the promotion of intestinal absorption of iron by vitamin C and the stimulation of intestinal calcium transport by vitamin D.

Clinical symptomatology arising from chronic low-grade essential element deficiency and toxic element burden can be difficult to identify, as numerous organ systems can be involved. Genetic predisposition, nutritional insufficiencies and exposures to toxicants of other types are factors that can also impact element imbalances and contribute to clinical symptomatology.

Notes:

An assessment of total toxicant stress on an individual's metabolism is sometimes referred to as the "toxic load." The toxic load takes into consideration an individual's genetic makeup, including polymorphisms that may compromise detoxification capacity, nutrient availability and exogenous toxin exposure, including metals, chemicals and other xenobiotics.

Intervention Options

When elements contained in food pass through the normal digestive process, they undergo several changes in chemical bonding as various metal complexes are formed. The low pH of normal postprandial stomach fluid serves to weaken the native protein-bound elements in food and cause increased free ionic concentrations with chloride counter ions in the gastric fluid. Thus enzyme-bound manganese in whole grains tends to become soluble manganese chloride during gastric digestion. The pH of chyme leaving the stomach is raised by the action of bicarbonate-rich pancreatic fluid (in response to secretin hormone action). The chloride salts can then form new complexes with negatively charged amino acids (aspartic and glutamic acids) and organic acids. Thus, the mixture that passes down the small intestine contains a variety of mineral salts such as calcium aspartate, magnesium glycinate, vanadium citrate, and myriad other complexed forms, depending on the characteristics of each element. Before they are transported across the intestinal mucosa, most elements undergo at least an additional transition in complexed form. The calcium in milk is thought to undergo multiple transitions, one of the most available forms for assimilation being calcium orotate. Abundant orotate in cow's milk contributes to the high availability of milk calcium. Digestive factors that may dramatically decrease element absorption include inadequate (or diluted) stomach acid, low alkaline pancreatic output, or low pancreatic digestive enzymes.[30, 103, 104]

Notes:

Refer to Case Illustration 3.1

Dietary supplements of elements (as elemental salts) are available in many forms. Because simple forms such as magnesium oxide or magnesium sulfate (Epsom salt) are very poorly absorbed, complexed or chelated forms are usually recommended.[105] The most common of these are the lactate, gluconate, citrate, picolinate or amino acid (especially aspartate) salts, sometimes called chelates. Chelated element production methods can be important considerations when choosing vendors of dietary supplements. Simply mixing an elemental oxide with an amino acid does little good to promote absorption of the element. The formation of organic ligands with metal ions requires careful adjustment of pH with introduction of the new complexing agent at the proper time, similar to that described above for the normal digestive process. Oral supplementation ranges for properly complexed forms of elements are shown in Table 3.1.

Element Status Assessment

Choosing the Best Specimen for Element Testing

There is no single best specimen for simultaneous, optimal status assessment of essential elements and toxic heavy metals. For each element, there are merits and limitations of the specimens commonly tested, such as serum or plasma, urine, hair and whole blood or its cellular constituents. Sections devoted to each element describe their unique characteristics and the evidence for clinically useful testing. According to the weight of published evidence, serum is the specimen most frequently used, although it is used primarily to determine excessively high levels. The value of urine for questions of essential element adequacy is very limited. The best specimen for detecting essential element deficiency depends on the element. Comparing results from multiple types of specimens can provide a more complete picture of elemental status.[106] Enhanced sensitivity may be

Of Further Interest...

Treatment for low element status mainly consists of aggressive dosing of easily assimilated forms of the deficient elements, as well as encouraging greater intake of food sources and correction of digestion or absorbtion problems. Elements chelated to Krebs cycle intermediates (e.g., magnesium citrate) may be the best absorbed forms. Signs of inadequate stomach acid production include rapid satiation or complaints of gas and bloating that prevent further eating. Reflux may also be a sign of inadequate stomach acid production. The pH capsule test is sometimes used to assess gastric acid secretion. Acid-generating agents include betaine hydrochloride and bitter herbs.

Since low stomach acid is frequently associated with poor pancreaticobiliary output, pancreatic enzymes that assist protein digestion may also be needed. Bile replacement or dietary oils that stimulate bile flow can further assist with protein digestion by breaking down dietary fat that adheres to food particles. Supportive interventions such as essential elements, amino acids, fiber and antioxidants are discussed below.

A lifestyle change can be of significant benefit in cases of chronic elemental malabsorption. The simple practice of thoughtfully chewing food and pausing for several minutes in a relaxed state of mind after eating major meals can improve stomach retention through stimulation of parasympathetic nervous system activity. Further, these actions increase needed acidification of chyme to release bound elements and start the protein digestion process that produces the digestive chelates needed for presentation to intestinal mucosal cells. Convincing patients who are habituated to the busy Western lifestyle to take such pauses of time may be challenging.

obtained by measuring 24 hour excretion following oral or IV challenge with nutrient elements or with chelating agents that mobilize toxic elements.

Blood

When selecting a single multi-element profile for nutrient status testing, whole blood or red blood cell (RBC) specimens are valuable. Whole blood is commonly used for baseline, non-challenged toxic element assessment. A flowchart based on initial decisions regarding the need for toxic metal assessment is presented in Figure 3.3. Since each essential element functions synergistically with other elements and nutrients, multi-element profiles coupled with functional biomarkers can provide the best insight into abnormalities in mobilization, utilization and excretion.

It may be difficult to demonstrate chronic exposure to toxic metals in samples of hair, blood and urine despite clinically significant body burdens because they tend to accumulate in specific tissues (especially bone, liver and kidneys). If the main routes of elimination are compromised, testing of body fluids may show falsely normal values. In these situations, testing that shows a metabolic impact of the toxic element is helpful. See Chapter 8, *Toxicants and Detoxification*, for discussion of how urinary porphyrin profiling may be used for this purpose.

Hair

Hair specimens can be useful in routine screening for toxic metal exposure. A specimen composed of 1.5 inches of hair closest to the scalp gives information about the past 3 months of exposure. Keratin, which is rich in sulfur-containing cysteine residues, is the major component of hair. In the active phase of hair growth, the follicular cells have a high rate of metabolic activity. When elements circulating in blood reach the hair follicle, they bind with high affinity to keratin, becoming trapped in the extruded shaft of hair. Due to its high sulfhydryl group content and, consequently, its great affinity for multivalent metals, hair concentrates toxic metals at least 10-fold above concentrations found in blood.[107] For assessing a history of toxic metal exposure, hair serves the purpose of being a chronological "recorder" that has been used in forensic studies to show the time of exposure. For these reasons, when exogenous metal contamination is excluded, hair is described in toxicology textbooks as a very useful specimen for toxic metal testing.[108] With attention to exogenous contamination, the analysis of hair for status assessment of some nutrient elements can also be useful.

Because of the ease of sampling and the apparent rich information content in a profile of 25 or more elements, hair element testing has become a favorite tool among some practitioners. Unfortunately, some

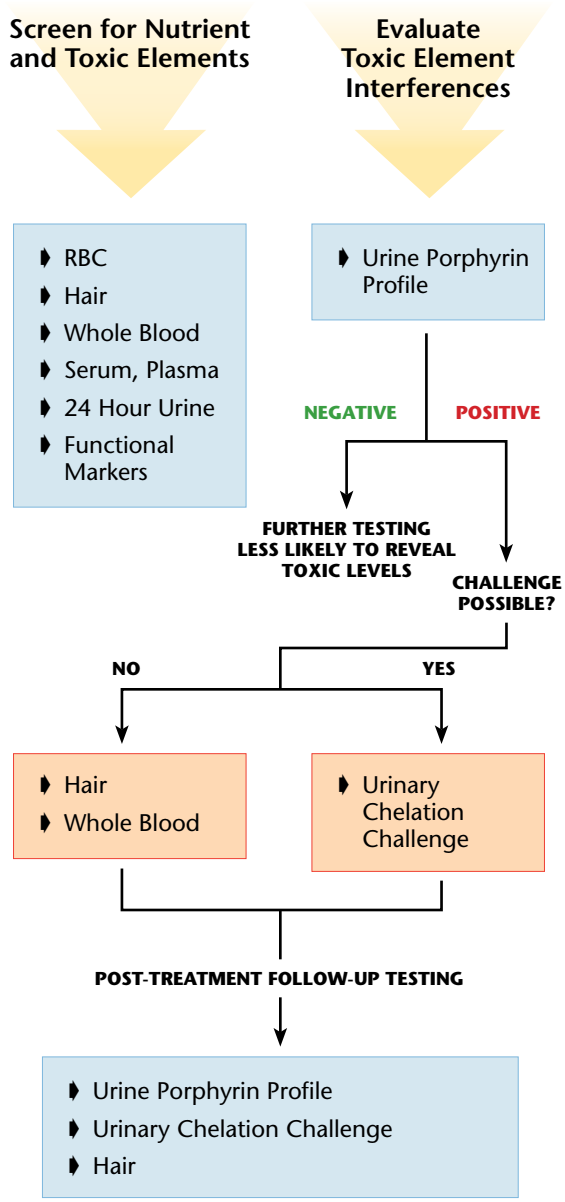


FIGURE 3.3 — **Specimens for Element Status Testing**

Choosing specimens for evaluation of elemental status depends on the clinical focus of concern. If no significant toxic element exposure is suspected, then one or more of the specimens for essential elements may be used. Element-specific focus can alter which of these specimens is best and functional markers may be employed. Using porphyrin profiling to initiate investigation of toxic elements provides evidence of significant toxic effects. Chelation challenge urinary excretion testing or toxic element panels on hair or whole blood may be ordered to further assess toxic element exposures.

laboratories have greatly overstated the diagnostic potential of hair element analysis. The calculation of long lists of meaningless ratios and the inclusion of detailed, misleading metabolic interpretations have led to investigations that paint a poor picture of the testing. In 2001, an analysis of a split hair sample sent to six commercial laboratories was reported.[109] Some of the results showed poor correlation between laboratories, and the interpretive comments varied widely, leading the authors to recommend private restraint and regulatory restrictions on all laboratories offering elemental testing of hair. Although some restrictions may become necessary to avoid misguided interventions, the conclusions of such reports should be evaluated in context of the approximately 3,000 reported studies of hair elemental analysis that have demonstrated its strong potential as a useful clinical tool. Many of these reports have been the subject of independent reviews.[110-113]

Urine

Urine presents a different aspect of element balance. Urine can vary with recent dietary intake. Thus, for individuals whose day-to-day dietary intake is relatively constant, normal levels of urinary zinc and chromium can reflect nutritional adequacy for these elements, since urine is the main route of excretion for many elements when intake is plentiful. On the other hand, for individuals with large daily variations in diet, urinary levels are more difficult to interpret. For example, consumption of oysters or shellfish might show high urinary measurement of zinc, but may not reflect a patient's overall low status of zinc. Twenty-four-hour urine specimens are commonly used in research, although accurate results rely on the patient's ability to reliably collect their urine for 24 hours and measure the volume accurately. Over 90% of most chelating agents are cleared by the kidneys in a few hours. Further urine collection can be counterproductive due to dilution of the toxic element concentrations passed in the first few hours. Thus, a six- to eight-hour urine specimen collection is not only more

Notes:

convenient, but also those specimens may be superior for assessment of toxic element excretion following a chelation challenge when results are expressed as micrograms per milligram of creatinine.

Chelation Challenge ("Provocation") Tests

Chelation challenge tests offer a more sensitive way to assess total-body burden of toxic metals or for assessing a patient whose toxic metal exposure occurred at an unknown point in time. Oral or intravenous (IV) administration of a chemical that has strong affinity for toxic elements causes a small quantity to redistribute into the blood as a stable complex and be eliminated in the urine, where it can be measured. Low-molecular-weight chelating agents specifically bind and mobilize certain toxic metals from tissues into circulation. Typically, a challenge consisting of an oral or IV dose of the chelating agent is administered. Urine is collected for the next 6 to 8 or 24 hours and analyzed for toxic metals. The more convenient 6- to 8-hour collection is effective because a large percentage of the chelating agents are cleared into the urine in the first few hours after administration. Since essential minerals are lost from the body during the therapy, it is desirable to determine the extent of losses of both toxic and essential elements by performing analysis at baseline (pre-challenge) and with chelation (post-challenge).

An elevation in one or more toxic metals in the post-challenge urine, when compared with baseline, may confirm the diagnosis of elevated toxic metal body burden with potential toxic consequences. The baseline may be the laboratory reference limit for unchallenged urine or the patients own unchallenged element concentration. Rules such as a four-fold increase over unchallenged levels have been proposed for judging clinically significant effects from a single oral chelator challenge.[114] Due to the ubiquitous presence of toxic metals in the environment, chelation is expected to yield toxic metal levels over ranges set on non-chelated individuals.[115] If clinical symptomatology is not compelling, further testing for functional evidence of a toxic burden, such as a urinary porphyrin profile, may be considered prior to initiating aggressive treatment. Combination of chelating agents such as DMSA and EDTA treatments may effectively reduce toxic metal body burdens. For example, treatment with EDTA followed by a dose of DMSA may increase lead excretion.[116] Even as clinicians chelate toxic elements, rigorous testing further confirms the difficulty in predicting the behavior of metals in the body (see Case Illustration 3.2). The field of chelation and metal detoxification has been covered in reviews,[2] and further information can be found at the American Board of Clinical Metal Toxicology and the American College for Advancement in Medicine.

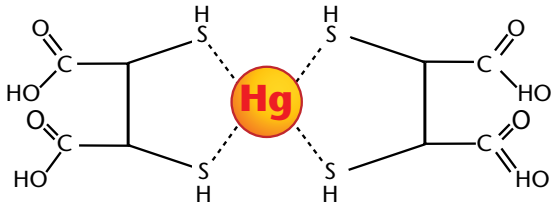


Figure 3.4 — **The DMSA-Mercury Complex**

Each DMSA molecule contains two electron-rich sulfur atoms. In the form shown, the sulfur atoms of the two DMSA molecules form four ligands with the central divalent mercury atom. The combination of its chelating ability and high renal clearance make DMSA an appropriate treatment for removal of a number of toxic metals.

DMSA: Meso-2,3-dimercaptosuccinic acid (DMSA) is an FDA-approved chelating agent that mobilizes heavy metals, such as lead and mercury, from human tissues.[117-122] DMSA transiently diffuses into and effectively competes with the tissue-binding sites to release toxic metals that are normally sequestered in bone, nerve, liver, etc. Effectiveness in mobilization of cadmium, lead

Table 3.5 — Commonly Used Chelating Agents[2]

Name	Abbreviation	Administration	Metals Bound
2,3-Dimercapto-1-propane sulfonic acid	DMPS	IV, Oral	Arsenic, lead, mercury
Meso-2,3-dimercaptosuccinic acid or succimer	DMSA	IV, Oral	Arsenic, copper, lead, mercury
Dimercaprol (British anti-Lewisite)	BAL	IV	Arsenic, copper, lead, mercury
D-Penicillamine (Cuprimine, Depen)	DPA	IV	Arsenic, copper, lead, mercury
Desferoxamine	DFO	IV	Aluminum, iron
Calcium disodium ethylenediaminetetraacetic acid	$CaNa_2$-EDTA	IV, rectal suppositories	Cadmium, lead, manganese

and mercury has been demonstrated, and the safety of DMSA use in children with elevated blood lead has been validated.[123] In addition to its heavy metal-binding capacity, DMSA has also been found helpful in reducing toxicity of organic compounds in rats,[124] and in restoring immune function in lead intoxication. DMSA

OF FURTHER INTEREST...

There are many chelation challenge procedures employed by clinicians to assess toxic metal body burden. Since chelation agents will bind and remove essential elements in addition to the toxic elements, some clinicians discontinue essential element intake prior to urinary collection, although if the individual is deficient in elements, discontinuation may be contraindicated. However, if chelation therapy is initiated beyond the challenge test, regular supplementation and laboratory assessment of essential elements is important. In addition to essential element supplementation, other supportive nutrients such as antioxidants, vitamins and fiber are commonly used. Chelation therapy training is available through a variety of organizations, including the American Academy of Environmental Medicine and the American College for Advancement of Medicine. Pediatric chelation protocols are available through physician-directed organizations such as Defeat Autism Now.

DMSA Challenge Procedure:

- Empty bladder, discard urine.
- Take 1 500 mg DMSA capsule with a glass of water between meals (at least 1 hour before or after eating).
- Take a second 500 mg capsule 4 hours after the first.
- Collect total urine for 24 hours after taking the first capsule. Empty the bladder for the last time at the same time of day the first DMSA capsule was taken on the previous day.
- Mix and transfer a portion of urine to a specimen tube for analysis.

Variations on DMSA protocol:[128, 129]

1. Take 500 mg DMSA twice daily for 1–2 days prior to collection day, and then follow the above protocol.
2. Base DMSA dosing on body weight, using 10–30 mg per kg of body weight per day as a single dose. Collection of urine should commence after DMSA dose is taken, and continue for the next 24 hours.

Example for 110 lb. pt.: weight in kg (lb./2.2) x 10 mg/kg = 500mg DMSA

EDTA Challenge Procedure:[114, 130]

- Evaluate renal capacity by creatinine clearance.
- Prepare IV solution:
  - 500 cc bag of sterile water
  - $CaNa_2EDTA$, 1–3 grams, according to renal capacity
  - Multi-trace element solution containing zinc, copper, chromium, cobalt, manganese, selenium, molybdenum may be added to the last 1/3 of the infusion
  - Magnesium chloride solution 2 grams
  - Ascorbic acid solution, 6–7 grams
  - B-vitamin solutions, including 1,000 µg B12, 100 mg pyridoxine, 250 mg dexpanthenol
- Administer at 166 cc/hour.
- Variable components include heparin, lidocaine and calcium gluconate.

is routinely administered either orally or IV. When completing a challenge protocol, urine is collected after an approved time period, depending on DMSA delivery. Although DMSA is cleared by the kidneys in about 4 hours, some clinicians favor a 24-hour over a 6- to 8-hour urine collection. DMSA and EDTA chelation challenge protocols are described in the "Of Further Interest…" sidebar.

EDTA: Intravenous ethylenediamine tetraacetic acid (EDTA) has been used as a therapy for atherosclerosis and other age-associated diseases.[125] The substance is used in an IV drip solution in the form of the disodium magnesium or disodium calcium salt, which bind with variable avidity to most divalent metal ions (See Figure 3.5). Clinical efficacy has been demonstrated for EDTA chelating therapy in a case of occupational parkinsonism due to manganese exposure.[126] EDTA is a common food additive.

Some concern has arisen concerning the potential for single-dose EDTA causing redistribution of endogenous toxic metals to tissues such as brain. However, one study designed to demonstrate such a phenomenon in rats failed to find any evidence for transient increase in brain lead with EDTA treatment.[127] DMSA and EDTA chelation challenge protocols are described in the "Of Further Interest …" sidebar.

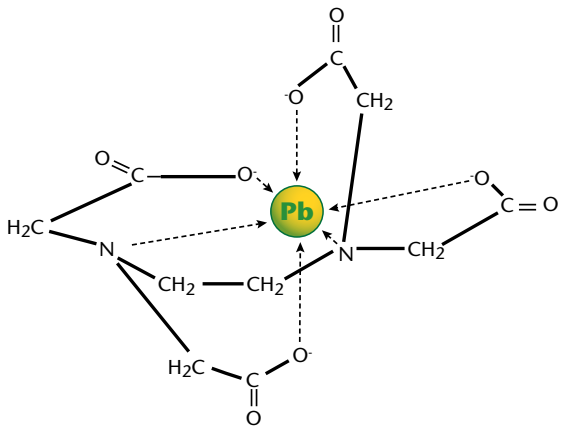


Figure 3.5 — The EDTA-Lead Chelate

EDTA allows hexadentate ligand structures, meaning there are six atoms simultaneously coordinated with the central metal ion. The EDTA molecule is called a chelate from the word "chelos," or claw, because it is able to exert a claw-like grip on the central metal atom. The high affinity for many divalent metal ions means that they will readily transfer from most biological binding sites and remain bound to EDTA until excreted in urine. The much weaker calcium complex used in IV administration allows displacement of calcium by the more tightly bound heavy metals.

Refer to Case Illustration 3.2

Urinary Porphyrin Profiling

Hemoglobin is an iron protoporphyrin embedded in protein chains (globulins).[131] The high rate of activity for the porphyrin biosynthetic pathway leading to heme makes it an excellent candidate for functional testing of impediments to the enzymes that constitute the pathway. Approximately 85% of heme biosynthesis occurs in erythroid cells, with hepatic cells accounting for the majority of the remainder. The cytochrome P450 systems in the liver require heme. Small activities of the pathway are found in most tissues to supply heme for the electron transport system. Even slight slowing of the rate for any one of the porphyrin pathway enzymes can cause abnormal concentrations of its substrate to be detectable in urine. Since specific toxins have been found to cause inhibitions of specific porphyrin pathway enzymes, profiles of the various intermediates can reveal whether an individual has had exposure to lead,[132] mercury[133] or various organic toxins.[134] Porphyrin elevations constitute a functional impairment of a critical system that can have clinical effects such as anemia. Direct measurements of organic or metal toxins, on the other hand, may reveal exposure that has no clinical consequence. Various clinical effects of toxic metals may be traced to their impairments in this pathway. A detailed description of porphyrin testing is presented in Chapter 8, "Toxicants and Detoxification."

Notes:

The Major Elements

Calcium, magnesium, potassium, sodium and phosphorus are present in the human body in hundred-gram quantities. Since many hundreds of grams of calcium, magnesium and phosphorus are present in the bones of an adult, assessment of an individual's status is a matter of determining whether they are in time-averaged positive balance so that net loss from bone is avoided. For sodium and potassium, in contrast, there is no comparable large, dynamic pool, so short term low intake would produce large deficits of these elements except for elaborate mechanisms (mainly renal) for regulating their retention. In addition, we find many interactions between specific elements and other nutrients and toxicants. Thus, several levels of complexity complicate clinical judgements about designing special support with major elements to maintain optimal wellness.

Calcium (Ca)

Adequacy assessment: Hair or urine calcium, bone resorption markers, serum 25-hydroxyvitamin D, PTH

Optimal forms: Gluconate or citrate salts

Clinical indications of deficiency: Osteoporosis, hypertension

Food sources: Dark green vegetables, whole grains, nuts, (milk)

Total-body calcium (Ca) is about 980 grams, which is greater than any other element. The vast majority of calcium resides in bone.[32, 135] Calcium is essential for bones and teeth, heart, nerves, muscles and blood clotting.[136, 137] Excitable and non-excitable cells require calcium (via calcium-activated potassium channels) to transmit impulses along nerves as well as participate in smooth, cardiac and skeletal muscle cell contraction or relaxation. Calcium's actions are as wide ranging as neuronal excitation, neurotransmitter release, innate immunity, hormonal secretion, and tone of smooth muscle cells in the vasculature, airways, uterus, gastrointestinal (GI) tract, and urinary bladder (Figure 3.6).[138, 139]

Notes:

Calcium signaling is vital for hearing, given its role in tuning cochlear hair cells.[138] Calcium plays a major role as a cofactor in the coagulation cascade.[140] In addition to its use as an adjunct treatment of osteoporosis,[140] calcium supplementation has been used to modulate the consequences of colorectal cancer,[141, 142] kidney stones[143] and hypertension[144] (including preeclampsia[145]).

Clinical Associations and Assessment of Calcium Status

Calcium circulating in blood is composed of approximately equal amounts of free calcium (ionized) and albumin-bound calcium. Since low albumin levels frequently, but not always, correspond to low calcium levels, albumin testing should always be included with serum calcium measurements. When blood calcium drops, parathyroid hormone (PTH) is secreted to initiate osteoclastic activity. PTH also stimulates activation of vitamin D to calcitriol, increasing intestinal calcium absorption to assist PTH.[146] Working in tandem, PTH and calcitriol also decrease urinary excretion of calcium.[147] Calcium-lactate-gluconate and calcium carbonate supplementation (500 mg/d) of a calcium-deficient human population resulted in a significant rise in mean serum calcium and fall in serum alkaline phosphatase compared with controls.[148] Calcium and phosphorus levels in serum are decreased in patients with liver cirrhosis without correlation with 25-hydroxyvitamin D levels.[149] The involvement of calcitriol cannot be ruled out in the cirrhotic patients, however, since metabolic acidosis, which may accompany cirrhosis, was later shown to decrease renal formation of calcitriol.[150]

Refer to Case Illustration 3.3

Serum calcium is generally measured as part of a comprehensive metabolic panel, although it is not a sensitive test for detecting early-stage calcium deficiency. Serum calcium may be unaffected by physiological shifts that affect calcium balance. For example, eclampsia is associated with lowering of urinary calcium excretion, whereas serum calcium is unaffected.[151] Hypercalcemia can signal hyperparathyroidism because PTH increases calcium levels. Malignancies may also increase serum calcium by either metastasis to bone (resulting in bone resorption) or by ectopic production of PTH-like peptide by tumors.[152] Symptoms of high calcium have been

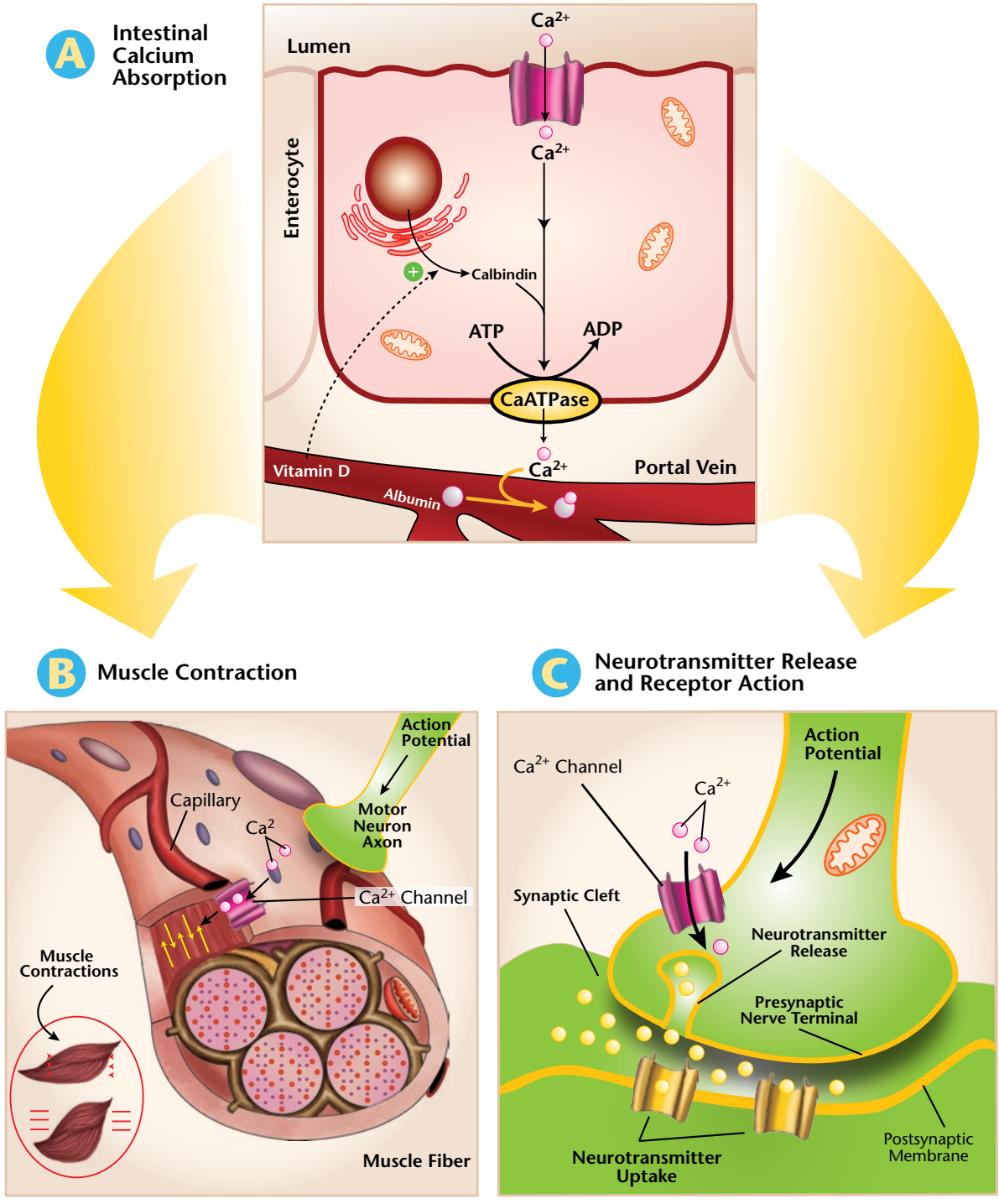


Figure 3.6 — Cellular Actions of Ionic Calcium

Calcium (as Ca^{2+}) is absorbed in the intestine and transported by calbindin to the enterocyte basolateral membrane where it is pumped into portal blood by a calcium ATPase transporter (**A**). Vitamin D increases calcium absorption by inducing calbindin synthesis. After absorption, calcium serves many functions in the body, including muscle cell contraction (**B**) and neurotransmitter release (**C**). Ionic calcium also acts as a 2nd and 3rd messenger in intracellular signaling (not shown).

described as weakness, lack of energy, poor appetite, nausea and vomiting, constipation, frequent urination, or abdominal or bone pain.[137]

Low serum calcium levels have been seen in cases of hypoparathyroidism, intestinal malabsorption, rhabdomyolysis, alkalosis and acute pancreatitis.[147] Further causes are low intake of calcium, vitamin D, or magnesium, or high intake of phosphorus.[136]

Calcium is primarily an extracellular element because cells carefully regulate calcium's entry into the cell for specific impulse transmission. It has been speculated that high levels of RBC calcium in hemodialysis patients may be due to decreased Ca-ATPase activity or toxic agents affecting cell membrane permeability.[153] In a study of hypertensives and diabetics, RBC potassium, calcium, and magnesium were closely linked during the fasting state.[7] High levels of RBC calcium were seen in 18 mild hypertensives compared with 14 healthy controls.[154] Significantly lower levels of red blood cell calcium were found in 46 women with premenstrual syndrome versus 50 controls.[155] Since no consistent clinical association has been established for low RBC calcium, low limits may not be assigned by the clinical laboratory. Because of potential membrane fluidity contributions to perturbed calcium transport, patients with high RBC calcium may need investigation with RBC fatty acid profiling (see Chapter 5, "Fatty Acids").

Urine calcium indicates the amount of calcium eliminated via the kidneys. Measurements of urinary calcium are useful for monitoring therapy with patients known to be in negative calcium balance,[156] or to measure excretion in dosing studies.[157] The importance of associated ions for calcium absorption is indicated by the greater urinary calcium excretion associated with calcium sulfate-rich mineral water compared with milk intake.[158]

In hypercalcemia, excess calcium is eliminated primarily through the urine.[137] High rates of active vitamin D formation may induce hypercalciuria.[159, 160] Kidney stones may be composed of calcium oxalate or calcium phosphate, and high excretion of calcium appears to increase the risk of developing kidney stones.[140] Urinary levels of calcium oxalate and calcium-creatinine ratios were found to be significantly higher in stone-forming children compared with normal children and may be very useful in monitoring patients with urolithiasis.[161] High urinary calcium resulting from low magnesium intake is associated with stone formation, and supplementation with magnesium and potassium citrate decreased calcium stone reformation by 90%.[162, 163] The complexity of factors regulating calcium excretion complicate interpretation of urinary calcium. For example, in a cohort of 68 women with a history of calcium-based renal stones, 24-hour urine calcium levels were not found to accurately reflect dietary calcium intake.[164]

Unlike the narrow range for serum calcium, hair calcium can vary from levels below 100 ppm to elevations as high as 5,000 ppm. Because of the unique physiological controls maintaining circulating calcium, levels in hair do not change in direct relation to intake, so low levels do not generally correlate with calcium deficiency. High hair calcium may be a marker for low calcium intake and negative calcium balance. Parathyroid hormone strongly regulates the hair growth cycle, providing a mechanism for regulation of calcium incorporation into growing hair.[179] Hair calcium levels rise during adolescence, and, prior to age 60, adult women have significantly higher mean levels of calcium in hair than men of similar age.[165-167] The greater calcium mobilization due to estrogen fluxuations in females is associated with 1.5 times higher calcium and magnesium in hair than males.[165] Calcium levels were found to be greater in females than males and tended to increase with age in a Pakistani study of triplicate hair samples from 58 male and 30 female Pakistanis from 3 to 100 years old.[180] Patients with rickets and osteomalacia have elevated hair Ca, whereas those with hypoparathyroidism have significantly decreased Ca concentrations in hair.[168] Low calcium intake and the associated elevation of PTH may induce high levels of calcium in hair. High dietary phosphorus and low calcium intake (a pattern known to induce negative calcium balance) resulted in as much as three times more calcium in hair compared with controls.[169] Both healthy females and Parkinson's disease females had 1.5 times higher calcium and magnesium in hair than males.[165]

Studies have shown low hair calcium is related to increased incidence of coronary artery disease.[170, 171] In one study of 4,393 males in the UK, high water hardness as well as higher hair calcium concentration and higher sunshine hours were significantly associated with lower coronary heart disease mortality. Arterial calcification increases the likelihood of a coronary event.[172,173]

Bone loss may not always be accompanied by high hair calcium. Because of the complexity of cellular responses that can lead to bone loss, there may be

mechanisms unrelated to PTH (and hair calcium changes). For example, the urinary N-telopeptide type I collagen marker rose by 319% along with a 9.3% decrease of bone density 9 months after gastric bypass surgery for morbid obesity. These changes were not associated with increased serum immunoreactive PTH or lowered serum 25-hydroxyvitamin D.[174] Instead, changes in circulating TNF-α or adiponectin are proposed to explain the bone loss under these special conditions.[175] Such mechanisms may leave hair calcium unchanged in spite of high rates of bone loss. Also, factors other than PTH changes may result in the decline of hair calcium and magnesium with increasing age, as found in patients with Parkinson's disease.[165]

One confounding factor for interpreting measurements of hair calcium is that high mineral content in water (hard water) is known to be taken up by hair and can vary widely across geographic location.[176] Exogenous deposition of metals such as calcium, lead, molybdenum and strontium in hair have been shown to correlate with levels in home water supplies.[177]

Indirect Tests of Calcium Status

Perhaps more than most elements, calcium balance evaluation requires multiple markers, both functional and direct. Biochemical tests of calcium tell only circulating levels of calcium and not the status of calcium in the body's principal reservoir, the skeleton. Since calcium makes up a large percentage of bone, direct measurement of bone mineral density (BMD) provides a measure of calcium status, althouth they are too insensitive to detect short-term changes (several weeks). Evaluating BMD in cases such as osteoporosis requires the use of tests such as a dual-energy x-ray absorptiometry (DEXA) or bone density scan.[137] Biochemical testing with markers such as alkaline phosphatase, hydroxyproline and hydroxylysine (see Chapter 4, "Amino Acids"), N-telopeptide, deoxypyridinoline, and, if indicated, PTH provide more tools for detecting short-term effects and for monitoring treatment of osteoporotic patients. Other tests recommended in the evaluation of osteoporosis are 25-hydroxyvitamin D, phosphate, creatinine, liver function tests, complete blood count, estrogen, testosterone (in men), and thyroid-stimulating hormone.[178]

Calcium Repletion

Optimal forms of calcium include gluconate and citrate salts. Calcium repletion was achieved in a group of 53 women using milk, calcium carbonate or calcium carbonate with 1,200 IU of cholecalciferol when compared with controls.[157] A note of caution is warranted regarding the recommendation of milk consumption for achieving optimal calcium status.[181-183] Although milk has appreciable calcium content, the net effect of milk product intake on bone health is quite negative in large-population comparison epidemiological studies.[184] This subject is highly controversial, with ongoing strong advocacy for positive calcium effects from regular milk intake by children[185] and adults.[186] In addition to the complexity of food intake and disease effects on calcium regulation, economical and political factors may need to be considered before reaching conclusions about milk intake.[184]

Notes:

MAGNESIUM (MG)

Adequacy assessment: RBC, whole blood, plasma, serum, urine

Optimal forms: Lactate or aspartate salts

Clinical indications of deficiency: Muscle cramping, twitching, depression, hypertension, cardiovascular disease, and diabetes

Food sources: Whole grains, nuts, legumes, molasses, brewer's yeast

One of the most abundant elements on earth, magnesium (Mg) is the fourth most plentiful cation in mammals, and it is the dominant intracellular cation. Magnesium plays a key role in more than 350 enzymes, primarily as Mg-ATP complex in energy-dependent activities (see Figure 3.7).[187] According to the Third National Health and Nutrition Examination Survey, the mean magnesium intake for males and females is 323 and 228 mg/d, respectively, far less than the dietary reference intakes of 420 and 320 mg/d, respectively.[188] Daily intakes of magnesium range between 84 and 598 mg.[189] Total-body magnesium is about 25 grams, of which more than 50% resides in bone, 35% in skeletal muscle and 1 to 2% in blood. 20% of blood magnesium is protein bound. The bone pool is not completely bioavailable when general depletion occurs.[190] Similar to calcium, bone magnesium decreases with age. Soft tissue magnesium concentration varies between tissue types, and cellular transport is regulated by numerous factors, including insulin, growth factors and catecholamines.[191, 192] Sex hormones have also been demonstrated to impact total serum and ionized magnesium in women, depending on the time in menstrual cycle.[193, 194] The physiologic mechanism behind hormonal regulation is not currently understood, although it may be associated with effects on magnesium-sensitive enzymes, as in vitro studies have demonstrated significant tissue magnesium variation in the presence of sex hormones.[195]

Magnesium is primarily absorbed in the jejunum and ileum, likely via active transport, although the exact mechanism is unknown.[187, 196] Dietary magnesium absorption is usually in the range of 35 to 40%. Parathyroid hormone, calcitonin and antidiuretic hormone (ADH) increase magnesium absorption. Passive gastrointestinal paracellular absorption also occurs with high intraluminal magnesium concentrations, allowing for greater systemic uptake, which is why there is some concern regarding toxicity when using high-dose magnesium for constipation.

The kidney is the principle organ involved in magnesium homeostasis. Drugs such as loop diuretics (thiazide) that manipulate electrolyte resorption also provoke renal magnesium loss.[197, 198] Alkalosis increases magnesium resorption, whereas acidosis increases excretion.[199] Changes in dietary intake elicit altered renal activity, increasing or decreasing resorption as needed.

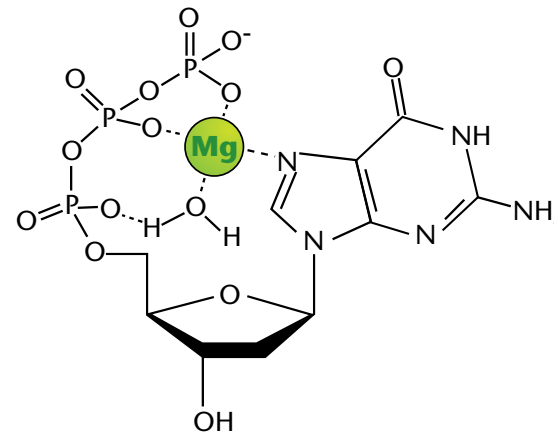


FIGURE 3.7 — **The Magnesium-ATP Complex**

The Mg-ATP complex is a tetradentate ligand, where four of the six coordination positions of magnesium are bound to three ATP oxygen atoms and one ring nitrogen atom. The binding to the first phosphate group is mediated through a water molecule interposed between it and the Mg^{2+} ion.

Magnesium is involved (as Mg-ATP) in virtually every metabolic process occurring in the body, including active transport such as the sodium-potassium ATPase pump, and cell signaling, including cAMP in protein phosphorylation. Magnesium is involved in multiple steps of RNA and DNA synthesis. It also plays structural roles in DNA, cell membranes and chromosomes. Magnesium plays numerous key roles in enzymes involved in protein, carbohydrate and fatty acid metabolism. Examples include the amino acid transaminases, pyruvate dehydrogenase, and delta-6 desaturase enzymes. Magnesium is a key cofactor in both methylation and sulfur amino acid metabolism, and is thus involved in the production of glutathione and S-adenosylmethionine. Magnesium is also required for formation of active cofactors from vitamins B_1, B_2, B_3, B_6 and pantothenic acid.[200]

Clinical Associations of Magnesium

Magnesium is often cited as the element most deficient in modern diets.[201] Estimates indicate that 40% of the population in the United States have magnesium status low enough to increase risk of degenerative diseases, including osteoporosis.[202] Magnesium deficiency is implicated in the pathogenesis of the leading causes of death in the United States (see Table 3.4). Magnesium intake was shown to be directly related to improved insulin sensitivity in children[203] and inversely related to incidence of developing type 2 diabetes mellitus in the 1,223 men and 1,485 women of the Framingham offspring cohort.[204]

Extracellular magnesium, comprising about 1 to 2% of total-body magnesium, is very tightly regulated. However, subtle shifts in blood magnesium or a reduced calcium-magnesium ratio are found in diabetes and other blood sugar dysregulatory conditions such as polycystic ovarian syndrome.[205, 206] The National Health and Nutrition Examination Survey, in which more than 9,000 Americans participated, demonstrated increased incidence of diabetes for individuals with serum magnesium below 0.88 mmol/L.[205, 207]*

Since magnesium is involved in the active transport of other ions, electrolyte imbalances are often associated with magnesium deficiency. Hypomagnesemia can masquerade as refractory hypokalemia. In such cases, active transport is compromised due to insufficient magnesium, leading to reduction of intracellular potassium levels and renal potassium resorption.[208] In these instances, magnesium insufficiency frequently presents as cardiovascular and neuromuscular symptomatology, including arrhythmia, hypertension and tetany.[209] The antihypertensive effects of magnesium may also be related to its involvement in moving calcium out of the cell, allowing smooth muscle dilation. Further, magnesium is able to bind calmodulin and modulate calcium signal transduction. Because of multiple tissue effects, early signs of magnesium depletion are vague and often missed, as they may be confused with other disorders. In one study, 47% of 1,033 patients submitted for serum electrolyte analyses were found to be hypomagnesemic, using a cutoff of 0.74 mmol/L.[210]*

Frank deficiency may be caused by diarrhea, loop diuretics and alcoholism. Therapeutic drugs (diuretics, chemotherapeutics, immunosuppressive agents, antibiotics) can cause hypomagnesemia due to increased urinary loss.[211] In addition to the well-known relationship of magnesium with calcium, magnesium has also been demonstrated to increase iron[212] and manganese[213] gastrointestinal absorption and balance in magnesium-deficient rats. Magnesium toxicity is rare, and usually of iatrogenic origin.[214]

Assessment of Magnesium Status

Assessing magnesium status is challenging. Serum magnesium is the most commonly used specimen for magnesium measurement.[190] However, low serum magnesium is not a sensitive test for magnesium deficiency for a number of reasons: Only 1% of total-body magnesium is extracellular, and it is tightly regulated by many factors, including parathyroid hormone, which normalizes serum magnesium levels during low intake. Whereas a low serum magnesium level is suggestive of a deficiency, higher levels of serum magnesium correlate poorly with total-body magnesium stores.

A better index of whole-body magnesium status is the concentration of magnesium inside cells. Magnesium stores in muscle account for approximately 27% of whole-body magnesium, representing a significant pool of biologically active magnesium.[215] There are a number of reports associating magnesium levels in mononuclear white blood cells (MBCs) with magnesium status.[216-219] A significant correlation exists between the magnesium concentration in MBCs and muscle. Like MBCs, red blood cells contain appreciable amounts of magnesium. The cellular composition of blood is 99% RBCs and less than 1% MBCs. Thus the magnesium pool in RBCs is significantly greater than that in MBCs. Although they may not accurately reflect changes in total body status, RBC specimens are frequently used to assess magnesium. Changes in RBC magnesium have been linked to hypertension, premenstrual syndrome and chronic fatigue syndrome.[196,220] RBC magnesium has been demonstrated to be a more sensitive indicator of deficiency

Notes:

** For Mg, 1 mmol/L = 24 µg/g = 24 ppm*

than serum in insulin-dependent diabetic children.[221] It has been shown that RBC magnesium correlates better with bone magnesium, the major storage site of body magnesium, than muscle magnesium.[222]

REFER TO CASE ILLUSTRATION 3.4

With attention to caveats regarding the interpretation of results, hair can be a useful specimen for magnesium evaluation. Hair magnesium is representative of long-term dietary habits, but the measured levels may be inversely correlated with intake because they respond to parathyroid hormone that is pulling magnesium (and calcium) from bone to make up for insufficient dietary intake. The above paradoxical presentation was demonstrated in an animal study and one human study.[169, 223] Hair magnesium and calcium in female Parkinson's patients and controls was also shown to be 1.5 times higher than their male counterparts, despite the fact that these two elements have been found to be deficient in Parkinson's patients using other specimens.[165, 224, 225] Furthermore, deficiency of magnesium is generally associated with calcium deficiency because they commonly are present together in foods. This leads to the clinical association of osteoporosis with insufficiency of both elements.[202, 226] Thus, elevated hair magnesium and calcium may be indicative of mobilization of the elements from bone to replenish depleted stores in metabolically active tissues. Conversely, low hair magnesium may not be indicative of magnesium deficiency.

An important mechanism for magnesium conservation is kidney tubular reabsorption.[227] During periods of magnesium depletion, kidney magnesium excretion is markedly reduced. Therefore, in the presence of normal renal function, reduced urine magnesium is indicative of low magnesium intake. It should be kept in mind, however, that this test can reflect recent decrease in dietary magnesium despite normal cellular levels. This limitation can be overcome by conducting a magnesium load-retention test. As originally described,[8] this functional test consists of infusing 2.4 mg Mg/kg lean body weight and collecting pre- and post-injection 24-hour urines that are assayed for magnesium and creatinine. Retention of magnesium greater than 25% of the load indicates magnesium deficiency. The increase of post-challenge over pre-challenge urine magnesium should be greater than 180 mg/d. However, due to time requirements and specimen collection challenges, this test is impractical for most clinics. A modification of this test consists of an equivalent oral dose of magnesium in place of the IV route. Although adjusting for variations in gastrointestinal absorption of magnesium between patients does present a challenge, this form of the test is considerably easier to conduct and deserves further study.

A number of functional magnesium biomarkers have been described for assessing magnesium status, including C-reactive protein (CRP), thromboxane B2, endothelin-1 and Na/K ATPase. Although all of these are impacted by magnesium status, other factors can also impact levels, such as CRP elevation in inflammation.[228] There remains a need for a sensitive and specific functional biomarker of magnesium status.

Magnesium Repletion Dosing

Repletion dosing for oral magnesium is up to 750 mg per day for adults; higher dosing may lead to diarrhea. The chloride, lactate, aspartate, glycinate and gluconate chelates of magnesium have high bioavailability, whereas the oxide, carbonate and hydroxide forms are poorly absorbed, and therefore may be used as osmotic laxatives.[196, 199, 229] IV magnesium is commonly given in much higher doses, up to 10 grams or more over 24 hours in hypomagnesemia with intact renal function, closely monitoring serum levels.[230]

POTASSIUM (K)

Adequacy assessment: RBC potassium, serum, hair

Optimal forms: Citrate or chloride salts

Clinical indications of deficiency: Hypertension, stroke, kidney stones, osteoporosis

Food sources: Avocado, banana, mango, corn on the cob, dried beans, potatoes, tomatoes

Potassium (K) is the most abundant intracellular cation in eukaryotic cells. Only 2% of total-body potassium is extracellular.[231] The average human body potassium concentration of 800 mmol/L (or 140 grams) accounts for the recommended dietary intake of nearly 5,000 mg/d. Dietary deficiency of potassium frequently goes undiagnosed because the element is ubiquitous in fruits and vegetables, and symptoms are generally vague and non-specific. Consuming a diet high in processed foods and in sodium, can easily result in a mild, chronic hypokalemia.[231]

Potassium is vital for triggering the action potential for muscle and nerve cell activity. Potassium channels include voltage-gated, calcium-activated, and ligand-gated/G-protein coupled potassium channels. Potassium channels are membrane proteins important in neurotransmitter release, cell excitability, hormone secretion, heart rate, and tone of vasculature and smooth muscle.[138] Potassium channel distribution in neuronal dendrites and how these channels dictate multiple factors in neuronal activity have been reviewed.[232] Alterations of potassium channels are associated with cardiac failure,[233] cerebral ischemia[234] and nerve injury.[235]

Clinical Associations of Potassium

Potassium insufficiency has been implicated in stroke,[58, 59, 236] cardiac dysfunction, hypertension,[237, 238] renal stone formation,[143, 239] hypercalciuria,[240] and postmenopausal osteoporosis.[231, 241-243] Potassium is important in maintaining acid-base equilibrium and in insulin sensitivity,[231, 244-246] and it has been suggested to play a role in neurological complaints (including schizophrenia and brain injury)[247] and gastroesophogeal reflux disorder (GERD).[248-250] Increased potassium intake reduces incidence of stroke and decreases hypertension,[58, 59, 61, 236, 251] possibly by a potassium-induced decrease in platelet aggregation.[237] In 59 volunteers, supplementation of potassium chloride, at 24 mmol/d for 6 weeks, lowered mean arterial pressure by 7.01 mm Hg, systolic blood pressure by 7.60 mm Hg, and diastolic blood pressure by 6.46 mm Hg.[238] It has been argued that magnesium and potassium therapy should be "first-line therapy" in drug-induced arrhythmias or digitalis overdose as well as in treatment of congestive heart failure.[252]

Hypokalemia (serum potassium less than 3.5 mEq/L) is the most severe consequence of potassium depletion, resulting in neuromuscular, gastrointestinal and cardiac conduction dysfunction. It can be caused by diarrhea, vomiting, low intake, increased renal excretion or even hypomagnesemia, due to magnesium's involvement in potassium pump activity. Drugs can induce hypokalemia, especially diuretics,[253] and can increase the risk of cardiovascular events such as stroke and myocardial infarction.[254]

Hyperkalemia (serum potassium of more than 5.5 mEq/L) is potentially life threatening and is caused by decreased potassium excretion or increased extracellular potassium due to inappropriate IV therapy, medications or potassium-sparing diuretic use. The morbidity and mortality caused by hyperkalemia is due to the consequences on cardiac function. High extracellular potassium is rectified with calcium chloride, alkalizing agents or diuretics.[255] There is no evidence that hyperkalemia can arise from intake of potassium-rich foods.

Assessment of Potassium Status

Red blood cells may be the best specimen for assessing intracellular potassium status.[256] In men, a significant inverse relationship was found between RBC potassium and hypertension and blood pressure, whereas there was a positive association between RBC sodium and hypertension in women. However, these associations were more profound in older versus younger participants (n = 1,805).[257] A study of 100 adolescents showed low RBC potassium to be associated with hypertensive parents, and therefore a possible sign of future hypertension development.[258] The potassium-dependent cardiac repolarization phase measured by electrocardiogram was found to correlate with RBC potassium levels in an elderly population. On the other hand, the correlation with serum potassium levels was poor.[256, 259] Whole-blood potassium can be useful for whole-body potassium assessment since it includes RBC potassium; however, care must be taken to assure that conditions inducing serum potassium fluctuations are considered.

In mild to moderate chronic renal failure, serum potassium will elevate.[260] Because potassium is primarily cytosolic, dietary effects on serum potassium are slight at best.[237] Nor is serum potassium a good marker for assessing hypertensive risk associated with potassium status.[261]

Notes:

If renal failure is not present, urinary potassium excretion accurately reflects potassium intake on the day the specimen was collected.[262] Urinary potassium reflects potassium intake and fruit and vegetable intake.[263] Some epidemiologic studies have shown that urinary potassium is significantly less in African-Americans, whether on random or controlled diets.[237, 264-270] Urinary potassium correlates with bone density at femoral, neck and lumbar spine as well as total-body bone density, independent of lean body mass in children.[263] Urinary potassium was found to be low in patients with untreated interstitial cystitis, presumably due to epithelial leakage of potassium, whereas heparin treatment resulted in increased urinary potassium.[271] Kidney stone formers are characterized by high urinary calcium, and potassium appears to decrease stone formation by decreasing urinary calcium levels. Conversely, low potassium intake results in higher calcium urinary levels.[143, 239]

Hair potassium levels decrease after prolonged alcoholism, apparently showing chronic deprivation of hair follicles during their active growth phase.[272] Studies with cystic fibrosis indicate that when there is normal potassium retention by the soft tissues, hair potassium remains normal.[273]

Potassium Repletion Dosing

The minimum potassium requirement is 1,800 to 2,000 mg/d,[33] whereas the adequate intake dose is 4,700 mg/d.[274] Food sources supplying these levels are well tolerated, but treatment by oral supplementation is limited by gastrointestinal tolerance. In severe deficiency, IV potassium administration allows more rapid repletion, but careful monitoring of serum levels is needed during administration because sudden elevation of serum potassium outside the usual narrow physiological range can lead to severe cardiac distress. Citrate, chloride and bicarbonate salts of potassium have been used for therapy and for investigations of potassium effects. Oral doses of 96 mmol (7,200 mg/d) for potassium chloride or potassium citrate were found to decrease blood pressure in hypertensives.[275]

SODIUM (NA)

Sodium (Na) along with chloride comprise the major electrolytes of the body's extracellular fluid (ECF). Sodium deficiency is rarely considered outside of unusual circumstances of losses due to vomiting and diarrhea or sweating. In such cases, the imbalance in ECF and intracellular fluid (ICF) allow water to pass into the cells in excess, leading to symptoms of water toxicity, including apathy, muscle twitching and loss of appetite. When both sodium and water are lost, total blood volume decreases, causing hypotension, tachycardia and other heart disturbances.[135] Prolonged imbalances in ECF and ICF can become serious emergencies. Excessive sodium intake is widely considered to be a risk factor in certain cases of hypertension, and frequent monitoring with 24-hour urinary sodium measurements is recommended to help educate patients who need to lower sodium intake.[276] Magnesium deficiency has been demonstrated to impact electrolytes, including sodium, potassium and calcium.[208]

PHOSPHOROUS (P)

Phosphorous (P) ranks second in abundance in human tissue after calcium, with total-body stores of 700 grams. Eighty-five percent of phosphorous resides in bone as hydroxyapatite, a combination of phosphate and calcium ions, whereas the remaining 15% exists in every cell in the body. Phosphorus as inorganic phosphate is essential for DNA, RNA and ATP formation. Phosphate is also a key constituent in cell membranes as phospholipid and is involved in most second messenger signaling pathways, including cAMP. Serum phosphorus is regulated by PTH, but not as closely as calcium. Phosphorus is highly bioavailable with 60 to 70% being absorbed in the GI tract. The element (as phosphate) is plentiful in most foods as it is similarly utilized for nuclear material and second messenger activity in both plants and animals. Phosphorus is also present in processed foods, particularly soft drinks.[33]

An inverse relationship exists between calcium and phosphorus. A low serum calcium-phosphorus (Ca/P) ratio, brought on by diet, is implicated in bone loss and has been referred to as "nutritional secondary hyperparathyroidism," where high-normal blood PTH levels are associated with increased bone resorption and decreased serum calcitriol. This condition is a concern for adolescent girls, as the combination of poor calcium intake and excess soft drink consumption can compromise peak bone mass accumulation. Examples of high phosphorus foods include macaroni and cheese (322 mg per cup), pizza (216 mg per slice) and cola (46 mg per 12 ounces). The two latter foods also contain high calcium, whereas the soda does not. Elderly patients with a low serum Ca/P ratio are at increased risk for osteoporosis[33, 277]

Phosphate deficiency may occur in the elderly, or those taking phosphate-binding antacids. Given the ubiquitous role of phosphate, symptoms are disparate, resulting primarily from decreased ATP production and involve neuromuscular, skeletal and hematological systems.

Blood and urine specimens are used to evaluate phosphorus status as part of profiles to diagonse parathyroid, bone and calcium disorders, vitamin D imbalances, and kidney abnormalities.[147]

Trace Elements

Iron (Fe)

Adequacy assessment: Ferritin, hemoglobin, hematocrit, total iron binding capacity, transferrin saturation

Iron excess: Transferrin saturation

Optimal forms: Ferrous gluconate, fumarate, and citrate salts; combine with ascorbate

Clinical indications of deficiency: Fatigue, delay in growth or cognitive development, weakness, arthralgias, organ damage

Food sources: Organ meats, brewer's yeast, wheat germ, egg yolk, oyster, dried beans, and some fruits

It has been estimated that 6 of 100 Americans are in negative iron balance, whereas 1 of 100 have iron (Fe) overload.[278] Iron overload can be caused by a common genetic disorder in the United States. There are only about 2.5 to 4 grams of iron in the healthy human body, yet this element has critical functions, and the human body has an intricate system of maintaining homeostasis. Human understanding of iron and anemia has a long history, and therefore a wealth of information is available on its absorption, transport, storage and biochemical roles, as well as appropriate laboratory evaluation.

Hemoglobin contains 70% of total-body iron. Another 3.9% is found in myoglobin and in mitochondrial proteins involved in energy metabolism and respiration such as cytochromes, catalase, peroxidase and metalloflavoprotein enzymes.[279, 280] Plasma iron is largely bound to transport proteins (mainly ferritin, transferrin and albumin), leaving only 0.1% of total-body iron as free iron in plasma.[281]

Dietary sources of iron include heme iron (meat) or non-heme iron (iron-rich plants), which is less bioavailable. Homeostasis of iron is carried out by up- or down-regulation of transferrin and ferritin receptors on cell surfaces to balance absorption, storage, circulation and excretion of iron. Absorption of non-heme iron is mediated by the divalent metal transporter 1 (DMT1) (Figure 3.8), among others. This transporter is up-regulated in iron deficiency. Toxic elements such as cadmium and lead share the same transporter, and it may be the reason that iron deficiency predisposes humans to cadmium and lead toxicity. By the same token, an iron-replete diet may protect from other element toxicities.[98, 282, 283] There is no mechanism to excrete excess iron by the body, though small amounts of iron are lost through urine, bile and sloughing of intestinal mucosal cells in the feces. This loss amounts to less than 1 mg/d,[281, 284] so the daily need of iron is about 1 to 1.5 mg for healthy adults. The RDA is much higher, reflecting low GI absorption of iron in healthy individuals. Premenopausal women are subject to a much greater loss of iron during menstruation.

Toxic elements can "piggy back" on the homeostatic mechanisms for iron regulation and can pose a second adverse consequence for the patient with either extremely high iron stores or for the patient with iron deficiency. DMT1 mediates absorption of iron, manganese, cadmium, and lead,[98] and some toxic elements use transferrin as their carrier protein (e.g., aluminum).[295]

Iron Deficiency Anemia

Iron deficiency anemia (IDA) has effects on tissue and cardiac health, physiological growth, productivity, maternal and fetal mortality, cognitive development, and attention span.[281, 284] Although hemoglobin (Hb) is routinely measured to monitor the critical stages of anemia, Hb is not the most sensitive marker of iron deficiency which advances in stages, starting with decreased iron stores (ferritin) and ultimately ending in effects on erythrocytes. Figure 3.9 shows the stages of iron deficiency,

Notes:

including depletion of iron stores, iron-deficient erythropoeises and, finally, IDA. Severe anemia is associated with hemoglobin levels less than 8 g/dL. At this stage metabolic acidosis occurs because of failure for compensation of decreased oxygen-carrying capacity.[285]

Iron deficiency is a common nutrient deficiency in the United States and worldwide, affecting mainly older infants, young children and women of childbearing age. Causes of iron deficiency can be grouped into four categories: (1) low dietary intake of iron, (2) gastrointestinal blood loss, (3) poor absorption of iron and (4) increased iron requirements (see Figure 3.1). Inadequate dietary levels of vitamin B_{12}, folic acid, vitamins A, C, or E, or trace elements contribute to development of IDA. Malaria and hemoglobinopathies also decrease iron levels.[286] Testing iron status is most important in those at high risk of developing iron deficiency anemia: pregnant women, infants, adult females and adolescent females, in that order.[280]

Chronic Iron Excess

In addition to clinical concern over iron deficiency, the question of excessive iron has received attention because of the realization that free iron greatly increases non-specific oxidation reactions that are involved in free radical degenerative effects.[287] The Fenton reaction is

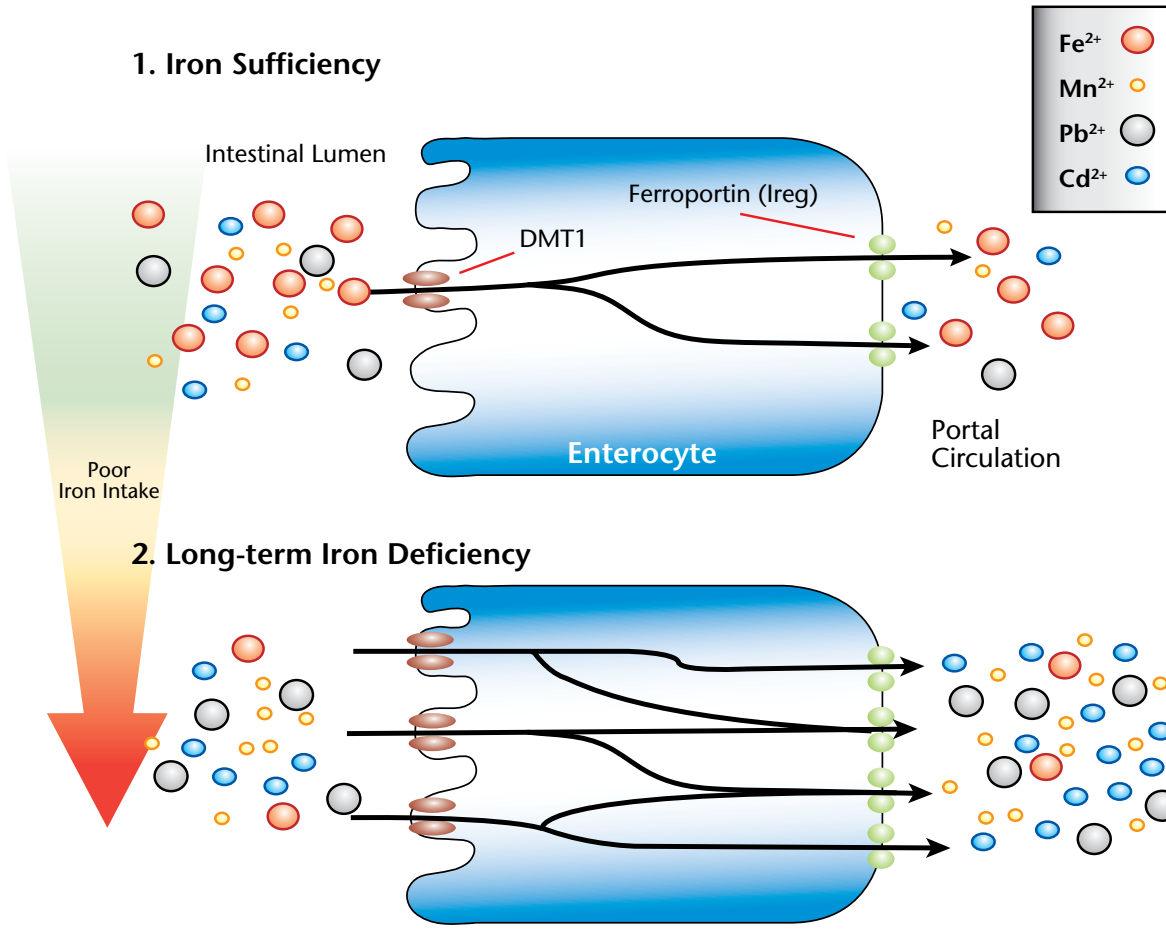
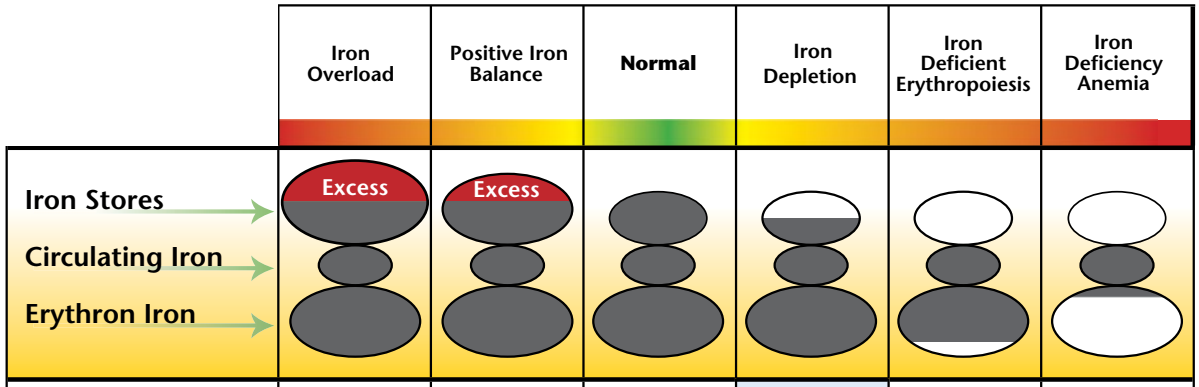


FIGURE 3.8 — **Effects of Transport Protein Changes in Iron Deficiency**

In the apical surface of enterocytes, DMT1 is the primary transporter for non-heme iron and it also can transport manganese, cadmium and lead. The figure illustrates the difference between the iron-sufficient state (1) and iron deficiency (2). Iron-deficiency stimulates expression of DMT1 resulting in greater rates of absorption of iron. The concurrent increased absorption of lead and cadmium can produce higher risk of toxic tissue exposure. At the basolateral surface, other transport proteins, especially ferroportin mediate movement of iron, manganese and toxic elements into portal circulation.

responsible for the production of a hydroxyl group free radical from the highly reactive ferric (3+) iron. Through this mechanism, high levels of iron are believed to cause oxidative damage to the endothelium, resulting in premature cell aging.[288, 289]

A specific protein-bound iron accrual occurs in hereditary hemochromatosis that has been found to occur at a frequency of 0.44% in non-Hispanic whites in North America, but at lesser frequencies in other groups.[290] Iron overload disorders have been covered in



	Iron Overload	Positive Iron Balance	Normal	Iron Depletion	Iron Deficient Erythropoiesis	Iron Deficiency Anemia
RE Marrow Fe	4 +	3 +	2–3 +	0–1 +	0	0
Transferrin IBC µg/100 mL (TIBC)	< 300	< 300	330 ± 30	360	390	410
Serum Ferritin µg/L	> 300	> 150	100 ± 60	20	10	< 10
Iron Absorption %	> 15	10–15	5–10	10–15	10–20	10–20
Plasma Iron µg/100 ml	> 175	> 150	115 ± 50	115	< 60	< 40
Transferrin Saturation %	> 60	> 45	35 ± 15	30	< 15	< 10
RBC Protoporphyrin µg/100 ml RBC (ZnPP)	30	30	30	30	100	200
Sideroblasts	40–60	40–60	40–60	40–60	< 10	< 10
Erythrocytes (Hgb)	Normal	Normal	Normal	Normal	Normal	Microcytic Hypochromic

Figure 3.9 — Iron Biomarkers in Iron Deficiency and Iron Overload

Moving from left to right, the table presents the progression of iron status from toxic overload to iron deficiency anemia. The three elliptical regions represent stored, circulating (free) and erythron (erythrocytes plus erythropoietic tissue) iron pools. Circulating and erythron iron are the last to be depleted. The data in the table are laboratory cutoffs and shaded regions show the most sensitive markers for iron excess (transferrin saturation %) and the stages of iron depletion. Ferritin is elevated in iron overload, but it also rises due to inflammation. In the "Normal" column, iron status is replete but not excessive, and all laboratory values are within normal limits. Iron depletion, iron deficient erythropoiesis, and iron deficiency anemia are reflected by sequential changes in biomarkers as deficiency worsens. Erythrocytes are the last to show evidence of iron deficiency. Thus ferritin is a better indicator of iron deficiency than hemoglobin.[278,280]

various review articles.[291, 292] Symptoms include extreme fatigue, arthralgias, and loss of libido.[293] Because consequences of iron overload include hepatic fibrosis/cirrhosis and diabetes, neonatal screening for hemochromatosis has been recommended. Life expectancy is normal if hemochromatosis is detected early. Typically, serum transferrin and iron saturation is used to detect early iron excess. If abnormal, these tests are followed by, or used in conjunction with, serum ferritin to detect hemochromatosis. Serum ferritin over 300 ng/mL for men and over 150 ng/mL for women is abnormal.[294,292] If repeat tests (while fasting) are abnormal, genotype testing can be used as a confirmatory test of hemochromatosis.[292]

REFER TO CASE ILLUSTRATION 3.5

Acute Iron Toxicity

Acute iron toxicity in the adult is possible but requires enormous quantities of iron. The lethal dose of elemental iron is 200 to 250 mg/kg. The tolerable upper limit (UL) is 45 mg/d of iron, based on the occurrence of gastrointestinal side effects.[296] The main victims of iron toxicity are children who overdose on 10 or more iron tablets. The symptoms of overdose manifest largely in the gut as necrotizing gastroenteritis, vomiting, abdominal pain, bloody diarrhea, shock, lethargy and dyspnea. The remedy for iron toxicity is bowel irrigation, administration of desferrioxamine (DFO), and phlebotomy.[284] A more recent iron chelator class is available, one of which is called deferasirox, and may become the main treatment modality for those with hemochromatosis. Dietary treatment for iron excess includes removing iron supplementation, vitamin C, and alcohol while reducing intake of organ meats and red meats. Increased phytate consumption such as legumes, whole grains, brown rice and wheat bran may also be used to lower non-heme iron absorption.

Notes:

TABLE 3.6 — NORMAL LEVELS FOR VARIOUS IRON MARKERS[147]

Test	Reference Limits	
	Non-SI Units	SI Units
Serum Total Iron and Transport Capacity		
Male	80–180 µg/dl	14–32 µmol/L
Female	60–160 µg/dl	11–29 µmol/L
Total iron-binding capacity (TIBC)	250–460 µg/dl	45–82 µmol/L
Transferrin		
Male	215–365 mg/dl	2.15–3.65 g/L
Female	250–380 mg/dl	2.50–3.80 g/L
Transferrin Saturation (TSat)		
Male	20%–50%	
Female	15%–50%	
Ferritin		
Male	12–300 ng/mL	12–300 µg/L
Female	10–150 ng/mL	10–150 µg/L

Assessing Iron Status

Due to the body's complex homeostatic controls on elemental concentrations in body tissues and fluids, testing of multiple specimens may be needed to accurately assess status. Iron provides a good example, as summarized in Table 3.6, showing markers for detecting ID, IDA, iron excess and hemochromatosis.[147, 278, 297, 298]

Routine hematological markers of iron status include Hb, hematocrit, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and erythrocyte count.[299] The biochemical tests are generally more accurate, though more expensive and difficult.[297] They include serum iron, serum transferrin, transferrin saturation, serum ferritin,[300] total iron-binding capacity (TIBC), transferrin receptor,[301] erythrocyte protoporphyrin (EP) and the zinc protoporphyrin heme ratio test.[302] Bone marrow aspiration or measuring hemoglobin response to iron administration are definitive diagnostic markers of iron deficiency.[303] However, bone marrow aspiration may be excessively intrusive, and the oral iron challenge test increases free radical oxidative damage to lipoproteins and cell membranes.[304]

Hemoglobin is a measure of iron incorporated into the red blood cells. This test is low cost, easy, and fast and appears to be better than hematocrit.[297] For IDA, hemoglobin must be measured before and several weeks

after iron supplementation. In one study, premenopausal women were depleted in iron and then fed iron-supplemented diets. The relative sensitivities for detecting depletion of iron were, in decreasing order, as follows: ferritin, percent transferrin saturation (TSat), plasma iron, hemoglobin, hematocrit, and erythrocyte zinc protoporphyrin.[305] Reticulocyte hemoglobin may produce less variability than serum ferritin and TSat.[306,307]

Some limitations of hemoglobin are that it detects only late-stage iron deficiency and has been reported to detect only 25 to 37% of iron-deficient patients.[131,297] Schneider and colleagues showed that in a sample of 425 toddlers from low-income families in California, hemoglobin had a low sensitivity for predicting iron deficiency (ID) of 23 to 40%, meaning that about 60 to 77% would not have been identified based on hemoglobin testing alone.[298] Warning against the use of hemoglobin in screening for ID is significant, especially because ID, not only IDA, can cause developmental delays[308] and attention deficit hyperactivity disorder (ADHD) symptoms.[309]

Decreased serum ferritin serves as an early sign of declining stores of body iron. Serum ferritin reflects iron stores because it is the major iron storage protein, and it appears to be the most sensitive test to determine iron-deficiency anemia[147] (Figure 3.9). Exceptions to this rule are cases of cancer, infection, inflammation and liver disease,[310, 311] where serum ferritin can appear normal, but iron administration may reduce mortality. Also, because serum ferritin increases with the severity of protein-energy malnutrition,[312] it is risky to rely on serum ferritin levels and/or transferrin saturation in patients with inflammation (such as hemodialysis patients), who may have falsely elevated iron status, but actually need iron administration.[306] Further, serum ferritin can be normal in patients with cardiac iron overload leading to cardiomyopathies.[313] Such responses highlight the challenge for determining the true iron sufficiency state.[306]

Serum ferritin increases with inflammatory markers such as C-reactive protein and IL-10, presumably mediated by IL-1 and TNF-α.[306] In 58,000 hemodialysis patients, serum ferritin of 200 to 1,200 ng/mL and transferrin saturation levels of 30 to 50% had no association with cardiovascular or all-cause mortality when adjusted for markers of inflammation and malnutrition. These patients had improved survival with IV iron ($\leq$ 400 mg/month) when compared with the control group.[306, 312] Other conditions reported to affect biochemical indicators of iron deficiency are infection, malignancy, malnutrition, alcoholism or liver disease.[297]

Transferrin saturation (TSat) and serum ferritin have been called the cornerstones of iron status assessment. Transferrin is the transport protein of iron. TSat is the percentage of transferrin bound to iron and is a good indicator of iron availability to the bone marrow. Fasting TSat is the earliest indicator of hemochromatosis. High serum ferritin is less sensitive, but should be considered if inflammation can be ruled out as the cause of elevated serum ferritin. DNA testing alone can miss 20 to 40% of whites and most blacks with hemochromatosis, so TSat is preferred.[314] Another common measurement of iron status is the total iron-binding capacity (TIBC). TIBC is the maximum amount of iron that serum proteins will bind.

Serum iron concentration is decreased in many, but not all, patients with IDA, chronic inflammatory disorders and myocardial infarction. Elevated serum iron occurs in hemochromatosis, acute hepatitis, blood transfusions such as in sickle cell anemia, and excessive supplemental iron ingestion. Measurements of serum iron, TIBC and TSat are most useful for screening iron overload disorders, rather than for the diagnosis of depleted total-body iron pools.[315]

Erythrocyte protoporphyrin (EP) and serum zinc protoporphyrin (ZnPP or ZP) are metabolic markers of the effect of iron deficiency on the pathway for heme biosynthesis. The insertion of ferric iron, a final event in heme formation, is under the control of ferrochelatase. In the absence of iron, this enzyme inserts zinc instead of iron, producing ZP. Thus, an abnormal ZP or ZP-heme ratio is a sensitive, functional marker capable of detecting preanemic iron deficiency.[316] The tight linkage of changes of ZP with ferritin shows that an early effect of iron deficiency is iron-deficient erythropoiesis.[317] In a study of 2,613 children and 5,175 women, EP was found to be a superior screening tool for iron deficiency in children aged 1 to 5 years, whereas Hb and EP were equally specific and sensitive for screening non-pregnant women.[297] Other studies have demonstrated that ZP and EP are more sensitive than Hb for detecting iron deficiency, especially at early stages.[318] In one study, EP was more sensitive but less specific than Hb for detecting IDA.[318, 319] The sensitivity of ZP and hematocrit was 81% and 16%, respectively, for identifying young children responsive to iron therapy.[320] For patients already depleted in iron, the earliest sign of repletion was heme synthesis via protoporphyrin levels.[305] For more

detailed information about the porphyrin pathway, see Chapter 8, "Toxicants and Detoxification."

Abnormal serum ferritin and ZnPP identified hemodialysis patients who would show an increased hematocrit when intravenous iron dextran was administered.[321] Ferritin reflects the iron stores, whereas ZnPP indicates whether the ID in a given patient is clinically relevant or not. The measured ZnPP, hemoglobin concentration and red cell indices can reveal the extent of a clinically relevant ID.[322]

Iron Repletion Dosages

Depending on the stage of ID, 1 to 5 mg of dietary iron intake may be actually absorbed, the mean for men being somewhat less than for women (1.0 vs. 1.4 mg/d).[280] Men absorb less iron per day because their iron stores are greater than those of women. An anemic person, with low iron stores and low hemoglobin, can absorb 50% more non-heme iron than a person with adequate iron stores.[296] Administration of vitamin C improves iron bioavailability and has been shown to significantly improve markers of iron status.[305,323] Prophylactic vitamin C supplementation alone may prevent ID.[324]

Other commonly used forms of iron supplementation are ferrous fumarate and ferrous gluconate. Treatment regimens may use elemental iron as high as 200 mg per day for adults and 100 mg per day for children. However, in extreme cases of IDA, up to 480 mg per day has been used to achieve rapid results.[280] Frequent monitoring of iron status should be done if this treatment is continued for several months.[284] Response to iron supplementation should be almost immediately evident in the hemoglobin levels (Figure 3.9) and the patient will feel an increase in energy, but iron stores will not be replete for months. Intramuscular iron injections have been shown to be more effective in improving ferritin levels than oral supplementation over 1 month of treatment, but proper protocol must be followed to minimize pain and avoid tattooing.[325]

Notes:

ZINC (ZN)

Adequacy assessment: Metallothionein, plasma, hair, or RBC Zn, delta 6 desaturase activity

Optimal forms: Gluconate, amino acid chelates

Clinical indications of deficiency: Depressed growth, poor immune function, alopecia, eye and skin lesions, diarrhea

Food sources: Red meat, oysters, whole grains

Zinc is necessary for growth and development of all living organisms due to its role in numerous catalytic and regulatory enzymes and in protein folding and receptor binding. It is a cofactor for more than 300 known enzymes. Of trace elements, zinc is second only to iron in body content at 2 to 3 g, found largely in the intracellular compartment of tissues such as the liver, pancreas, kidney, bone and muscles, with smaller concentrations in the eye, prostate, fingernails, hair and skin.[330]

Zinc fingers that regulate gene expression represent a key structural role of zinc in proteins. They contain zinc atoms usually bound to cysteine or histidine residues of the protein (see Figure 3.10). It appears that approximately 1% of the human genome codes for zinc fingers[326] that have important roles in the functions of DNA, RNA, and transcription of receptor factors for retinoic acid and 1,25-dihydroxycholecalciferol. They are also involved in regulation of receptor binding, cell cycling and cell-cell signaling.[327]

Clinical Associations of Zinc

Symptoms of mild and severe zinc deficiency include depressed growth, teratogenesis, poor carbohydrate metabolism, altered cognition, poor immune function, alopecia, impotence, eye and skin lesions, and diarrhea.[328-330] Low dietary zinc has been associated with hypogonadism,[33] low testosterone,[331] low free T4[332] and low IGF-1,[333] revealing the importance of zinc in steroid-hormone gene transcription.[330] Acrodermatitis enteropathica is a genetic disease that results in zinc malabsorption. It is characterized by eczematoid skin lesions, alopecia, diarrhea, bacterial and yeast infections, and can be fatal,[33] but is reversible with zinc supplementation.[327]

Although the immune dysfunction seen in zinc deficiency is not well understood, animal studies show that zinc deficiency results in thymic atrophy and lowered T-helper cell function.[334] In children, zinc supplementation improves T-lymphocyte responsiveness.[335] Interleukins 1 and 6 are mediators in zinc metabolism, initiating zinc uptake into liver, bone marrow and thymus, possibly as

a host defense mechanism.[327, 336] However, zinc supplementation to increase immune function has conflicting evidence; in HIV patients, low serum zinc is associated with increased mortality,[337, 338] and zinc supplementation (45 mg/d) decreased the number of opportunistic infections.[339]

Although research supports a toxic dose of zinc of 100 to 300 mg/d and an upper limit of 40 mg/d, 100 mg/d is commonly used for short-term repletion. The main toxic effect is that zinc interferes with copper absorption. Zinc supplementation of 2 g/d causes GI symptoms and vomiting. Toxicity is rare, primarily seen in hemodialysis patients where the dialysis fluids were contaminated with zinc. Symptoms include anemia, fever and central nervous system effects. Zinc exposure from welding has been documented.[33]

Homeostatic Mechanisms

Zinc is believed to enter enterocytes by active transport when body status is low and by the paracellular (passive) route in the zinc-replete state. Thus, as with other elements, zinc deficiency is offset by increasing absorption, whereas zinc excess causes increased excretion. The metal-binding protein, metallothionein (MT) binds zinc in the enterocyte and transports it to the basolateral border, where it is actively exported from the cell and

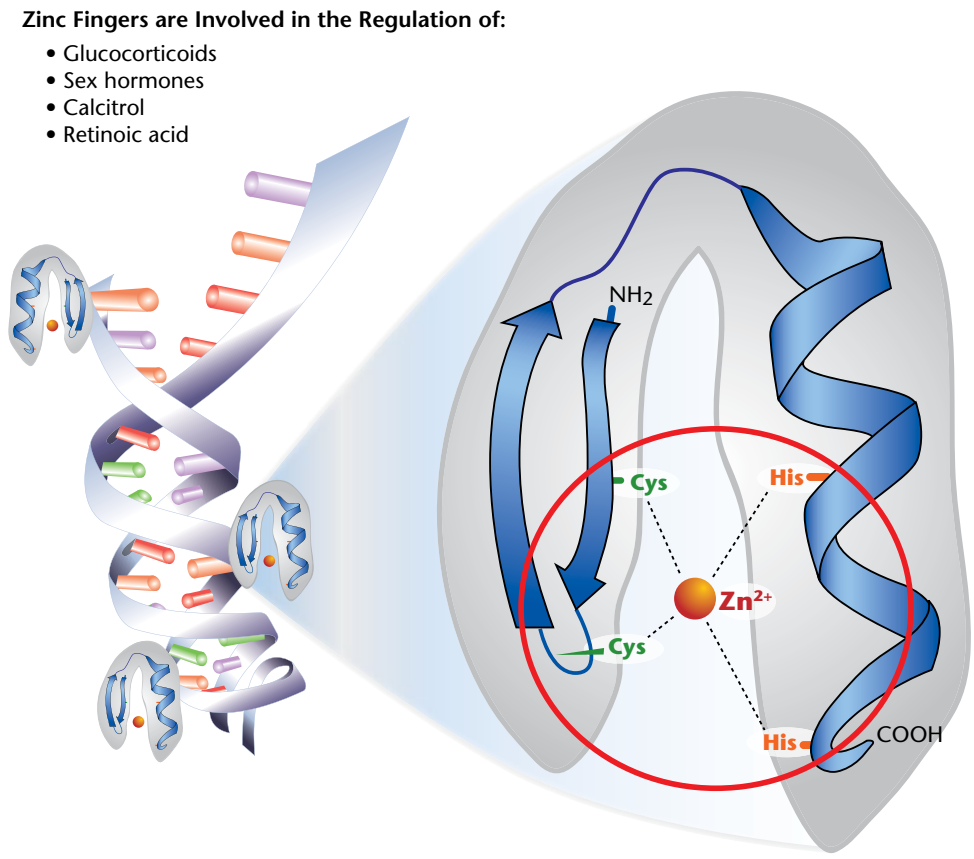


Figure 3.10 — Zinc Fingers

A double strand of DNA with multiple zinc fingers (Znfn) bound to the DNA surface is shown. The Znfn close-up shows the finger-like loops of amino acids stabilized by a single zinc atom. The zinc binds to cysteine and histidine side chains. Zinc fingers are epigenetic transcription and post-transcription factors involved in regulating gene expression. In other cellular locations, zinc fingers are involved in cell regulation and signal transduction. They are among the most abundant proteins coded for in the eukaryotic genome, and mutations of zinc fingers are associated with numerous conditions, including schizophrenia, myeloid leukemia, Wilms' tumor and other cancers.

bound to albumin for delivery to tissues (Figure 3.11). Albumin is the principal carrier of zinc, although some zinc may be carried by transferrin and prealbumin. Diseases of malabsorption such as Crohn's disease impair zinc status.[33] Zinc fecal excretion ranges from 1 mg/d in deficient individuals to 5 mg/d in zinc-replete individuals.[329] Fecal zinc losses occur in the form of pancreatic secretions and intestinal cell turnover.[340] Urinary zinc comprises less than 10% of fecal zinc excretion.[341]

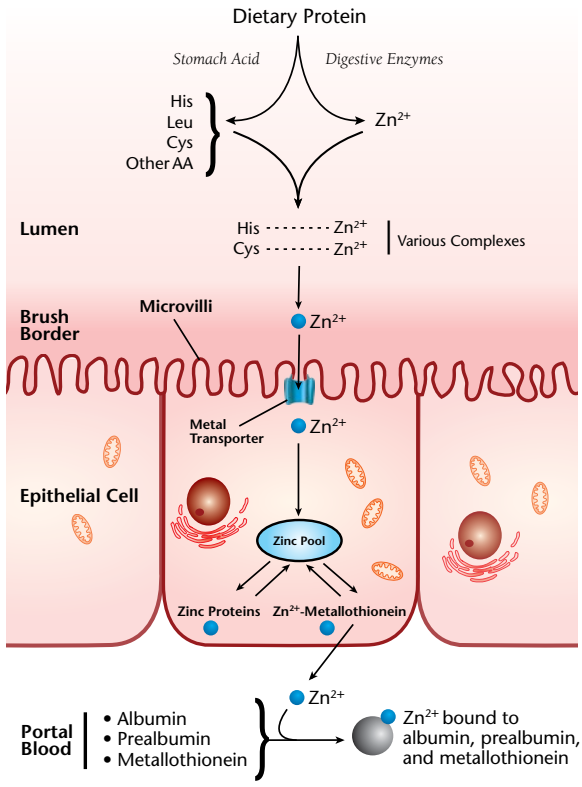


FIGURE 3.11 — Zinc Absorption and Distribution

Dietary zinc arrives at the absorptive region of the small intestine complexed with various counter-ions, especially the amino acids released from digestion of dietary proteins (see Chapter 7, "GI Function"). Divalent metal transporters in the enterocyte brush border release zinc ions into the cytosolic zinc pool where they equilibrate with zinc transiently bound to metallothionein or incorporated into zinc metalloproteins. Metallothionein transports intracellular zinc from the lumen to the basolateral border for exit. In the blood, zinc is bound to albumin, its principal protein carrier. The rates and directions of transfer respond to changes in dietary zinc supply and blood concentrations of zinc.

Assessing Zinc Status

To date, there is no generally accepted standard index for zinc status, although levels of zinc in whole blood, plasma, blood cells and urine tend to fall in severe zinc depletion. Accurate assessment of zinc status is difficult and studies are often contradictory. MT appears to be a valuable marker of zinc status.[327, 330, 342] Plasma and serum are thought to be insensitive indicators of zinc status.[327, 330, 342] However, some studies have shown plasma to respond to both depletion and repletion of zinc,[343, 344] and for this reason, some experts recommend using both plasma zinc and erythrocyte MT as an indicator of zinc status.[106, 327] Erythrocyte zinc has been shown to be both responsive[342] and non-responsive[343] to zinc depletion.[327, 330,345] Erythrocyte zinc may be useful primarily due to high concentration of zinc enzymes such as carbonic anhydrase. Other enzymes such as erythrocyte membrane alkaline phosphatase can serve as supportive measures when assessing zinc status.[344] Urinary zinc has also been shown to be a useful biomarker,[330, 344] and even to correlate with plasma zinc levels,[343] yet it has also been shown to be a poor indicator for early stages of zinc depletion.[342] Hair zinc correlates with zinc supplementation-induced growth,[330] yet others have shown no relationship between hair zinc and zinc status.[343] Ultimately, gathering multiple measurements of zinc status is ideal in an effort to put together the puzzle of a patient's total-body zinc status.

Blood accounts for a mere 0.5% of the total-body content of zinc. Blood zinc is partitioned between plasma (12–22%), leukocytes and platelets (3%) and erythrocytes (75–88%). Plasma zinc may fluctuate with dietary intake or inflammation and can be decreased by 50% after an injury.[33] Many zinc nutrition studies have relied on measurements of plasma zinc levels.[112] Plasma zinc represents the dynamic balance of systems regulating absorption of dietary zinc, tissue uptake and efflux.[342, 346] Normal fasting plasma zinc concentration is 650 to 980 µg/L or 0.65 to 0.98 ppm.[106, 330] Zinc levels are lowered after meals.[347] Although plasma zinc concentration is clinically useful, the results can be complicated by conditions of acute stress and illness, such as following myocardial infarction, where zinc can rise because of increased binding protein levels.[348]

Furthermore, since plasma zinc is largely bound to albumin, any treatment that alters albumin levels will alter plasma zinc concentration. For example, corticosteroids, oral contraceptives and pregnancy lower plasma

zinc.[349] Serum levels rise then decrease after meals.[33] Compared with controls, serum zinc was elevated in patients with atherosclerosis obliterans.[350] In 15 young males, plasma zinc decreased significantly on 3.2 mg zinc/d for 42 days. However, a decrease wasn't seen in the groups fed 7.2 or 15.2 mg zinc/d.[342]

Erythrocyte metallothionein (MT), termed a zinc trafficking molecule, has been used to distinguish between mild deficiency and positive balance of zinc.[327] MT is important for regulation of zinc and copper metabolism (Figure 3.11) and may protect against toxic metal effects by binding them until they can be excreted. MT contains multiple metal-binding sites, so that 1 mole of MT binds 7 moles of zinc.[342] In rat studies, dietary zinc determines MT concentration by regulating MT mRNA.[342, 351, 352] Monocyte metallothionein mRNA also responds to zinc supplementation.[353] MT also can carry cadmium, mercury and other toxic elements. In humans, zinc depletion has been shown to decrease MT by 46%,[342] or 68% after only 7 days of a zinc-deficient diet.[354] In young males given varying doses of zinc, erythrocyte MT decreased significantly with the dose of only 3.2 mg zinc/d for 42 days, whereas no decrease was seen in groups given 7.2 or 15.2 mg zinc/d. During zinc depletion, a diet supplying only 0.55 mg zinc/d for 12 days, decreased erythrocyte MT.[342] This demonstrates that only when dietary zinc is very low does MT decrease. See the section "Copper" for further discussion of MT.

Zinc status may be more accurately revealed by RBC zinc rather than plasma zinc measurement.[355, 356] Mononuclear blood cells (MBCs) and erythrocytes containing zinc dependent proteins make an alternative for determining zinc status. For instance, RBCs contain about 10 times more zinc than plasma due to their content of carbonic anhydrase and other zinc-containing enzymes. Additionally, since they remain in the circulation for 110 to 120 days,[357] RBC zinc levels reflect long-term zinc stores.[344] Low zinc in red blood cells was seen in 1 of 3 cystic fibrosis patients (n = 51).[355] High erythrocyte zinc has been found in cases of cancer metastasis,[358] hypertension[359] and renal failure.[356] High erythrocyte zinc has been associated with increased hypothyroidism in hospitalized patients, and hyperthyroid patients have been found to have lower erythrocyte zinc.[360, 361] Like RBCs, MBCs have zinc levels that can indicate zinc status.[362] MBCs have considerably faster turn over rates (~10 days), and therefore exhibit intermediate sensitivity to changes in zinc nutriture compared with erythrocytes.

Eighty five percent of erythrocyte zinc is bound to carbonic anhydrase (CA) and 5% to superoxide dismutase (ZnSOD), where zinc plays a structural role. CA is involved in acid-base balancing, and ZnSOD catalyzes the dismutation of super oxides to hydrogen peroxide and water.[327] In a study of non-dialyzed chronic kidney disease patients (n = 38), high levels of carbonic anydrase correlated with zinc levels in erythrocytes.[356] Other zinc-containing enzymes in blood such as alkaline phosphatase, nucleoside phosphorylase and ribonuclease are responsive to changes in zinc status.[363] Zinc-deficient patients who received zinc supplementation showed increases in serum alkaline phosphatase activity that paralleled the degree of zinc repletion.[364] Similar studies that measured alkaline phosphatase activity in erythrocytes have shown this membrane enzyme to be responsive to zinc depletion, even when other markers, including plasma, erythrocyte and platelet zinc concentrations failed to change in early zinc deficiency.[344, 365] Another zinc-dependent marker with promise as a sensitive indicator of mild zinc deficiency is the plasma enzyme, 5′-nucleotidase.[366]

When low erythrocyte or plasma zinc is found, testing the patient's plasma amino acids may be indicated. Zinc may be absorbed as a peptide, bound to amino acids such as cysteine and methionine.[367] Therefore, normal levels of methionine, taurine, cysteine and histidine may support zinc absorption (Figure 3.11).[33, 367, 368] On the other hand, excessive levels of histidine intake can deplete zinc.[369]

Notes:

Measurement of 24-hour urine zinc can be clinically useful, provided that chronic renal disease, liver cirrhosis, sickle cell anemia and other conditions known to cause urinary hyperexcretion of zinc are ruled out.[370] After an oral zinc load, psoriatic patients showed lower plasma zinc levels at 2 and 4 hours than healthy controls, indicating that the condition produced tissue depletion.[371]

Urinary excretion of zinc decreases as a result of zinc deficiency.[372] Zinc urinary levels are high in muscle protein catabolism brought about by starvation or trauma.[330] Some studies show that in zinc depletion, urinary zinc decreases, and in zinc excess, urinary zinc is high,[342] indicating a renal mechanism in regulating total-body zinc. Urinary zinc, but not copper, was found significantly higher in stone formers (n = 30) than in controls (n = 27).[373]

Hair zinc has been reported by a number of studies to reflect the dietary zinc intake in both animals and humans. Low levels of zinc in the hair of children were shown to correlate with poor growth in the Near East,[374] and to correlate with poor growth, anorexia and hypogeusia (decreased taste) in the United States.[375] When low hair zinc status was used as an impetus to supplement children with zinc, the children underwent increased growth velocity after zinc supplementation. Therefore, growth in children upon zinc supplementation serves as a functional marker of zinc. Low zinc in hair has also been shown in individuals with a high dietary phytate-zinc molar ratio, a factor known to decrease zinc absorption.[330] Such findings have been corroborated in zinc-deficient children with Crohn's disease.[376] Acute zinc deficiency, however, can arrest hair growth, causing levels to remain in the normal range. Thus, hair zinc may not be a reliable indicator of zinc status in patients with periodically deficient zinc intake.[374]

Because zinc is involved in the delta-6 desaturation of fatty acids, the ratio of a precursor essential fatty acid (linoleic acid) divided by its delta-6 desaturase product (i.e., gamma linolenic acid) offers a functional assessment of zinc adequacy. A high fatty acid LA/GLA ratio indicates functional need of zinc. Inhibition of delta-6 desaturase can occur not only from zinc deficiency, but also from excess saturated, monoenoic or *trans*-fatty acids. Other cofactors required in desaturation are magnesium, niacin, pyroxidine and vitamin C. For further discussion of this metabolic marker see Chapter 5, "Fatty Acids."

Zinc Repletion Dosing

The acetate, gluconate, picolinate and sulfate forms of zinc are acceptable dietary supplement compounds. Zinc gluconate has been used at zinc equivalent doses of 5 mg /d,[344] 10 mg/d,[343] or 50 mg/d[353,354] before the first meal of the day to replete patients while supplying a diet of zinc-containing foods. Repletion has also been achieved using elemental zinc (50 mg/d) and self-elected diet.[342] Zinc picolinate is not any better absorbed than other forms.[367] Absorption of zinc can be maximized by reducing dietary phytate. Dietary modification to increase intake of zinc-rich foods is, of course, a worthy effort to improve a patient's zinc status.

Excess iron or calcium intake can decrease zinc absorption. Cadmium and copper also compete for the same carrier protein and can decrease zinc absorption. Conversely, excessive zinc intake can reduce copper absorption, leading to copper deficiency.[33] The RDA ratio for zinc:copper is about 9:1 for adult men and women. Zinc in human milk is more bioavailable than in cow's milk, presumably due to the greater ease of protein digestion. Long-term alcoholism can result in decreased zinc absorption and increased zinc excretion.[377]

Urinary concentrations of amino acids[33] important for zinc absorption, such as cysteine and histidine, may be valuable adjunct data when zinc is low or high. Intake of dietary proteins that yield cysteine and methionine, such as beans and peas or eggs, may improve zinc absorption.[367]

COPPER (CU)

Adequacy assessment: *Copper deficiency:* RBC Cu, Serum ceruloplasmin, elevated urinary HVA:VMA ratio, bone resorption markers *Copper excess:* urine

Optimal forms: Aspartate or sulfate salts

Clinical indications of deficiency: Refractory anemia depigmentation, impaired glucose tolerance, cardiac-related problems, elevated cholesterol

Food sources: Liver, cashews, black-eyed peas, sunflower seeds

Toxicity sources: copper piping, copper cooking utensils

Protective measures: NAC, antioxidants, tetrathiomolybdate, zinc

The average adult has about 100 mg of total-body copper (Cu). Copper is required for over 30 metalloproteins involved in oxidation-reduction reactions; neurotransmitter, energy, myelin and bone or connective tissue production; immune function; and hematopoeisis.[32, 378] Cuproenzymes are primarily involved in one-electron exchanges, frequently utilizing oxygen and ascorbic

acid. As such, cuproenzymes have key roles in antioxidant defense systems, including superoxide dismutase (SOD), which reduces the free radical superoxide to hydrogen peroxide and water.[379] Similar to iron, copper's ability to transfer electrons makes it critical in metabolic pathways, yet also contributes to its potential toxicity. Toxicity is kept in check by homeostatic mechanisms, which include transport, chaperone and storage proteins such as cuprometallothionein.[380]

Homeostatic Mechanisms

The metal-binding protein metallothionein that was discussed in the section "Zinc" above is key player in copper homeostasis, as it binds and stores intracellular copper, minimizing the presence of free copper ions. Metallothionein is a relatively small protein with multiple binding sites for elements with different valences and ligand field geometries. The synthesis of MT is up-regulated in states of excess to sequester cellular Cu^{2+} (thereby decreasing copper) and in deficiency to increase absorption.

Metallothionein is capable of binding multiple elements, including toxic metals.[385] It has very high affinity for cadmium and mercury. Therefore, in copper- or zinc-deficiency states, an increase in metallothionine may result in an increased uptake of toxic metals such as cadmium. Cystine residues in MT must be reduced by selenoproteins so that MT can bind metals. Thus, in a selenium-deficient, oxidative environment, elements are released from metallothionein, which also contributes to metal toxicity.[380-384]

Excess copper intake or impairment in homeostatic mechanisms can lead to the presence of increased free copper ions causing pro-oxidative events such as the Fenton reaction.[386] Copper deficiency on the other hand also leads to oxidative damage due to insufficient cuproenzyme activity. Thus, copper levels must be maintained in quite a narrow range for optimal function.[387] Refractory anemia is the most common copper deficiency presentation, whereas subclinical deficiency states may be more difficult to diagnose. Copper excess and deficiency can present with similar symptoms, since both states contribute to oxidative stress.[388] Laboratory evaluation of copper is essential for proper assessment of copper status, preferably employing both functional markers and direct measures.

Ceruloplasmin, also referred to as ferroxidase, is a multicopper oxidase essential for normal iron homeostasis and copper transport.[389] The metabolic fates of copper and iron are intimately related, and deficiency of copper or ceruloplasmin results in cellular iron deficiency and, ultimately, anemia.[379] This mechanism explains how elevated zinc, which decreases copper absorption, can lead to iron-deficiency anemia. The RDA recommends a zinc to copper ratio of about 9:1.

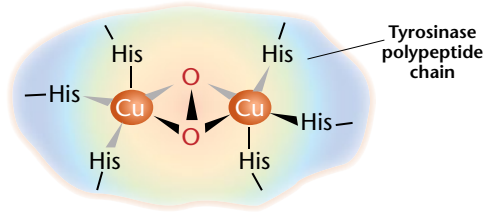


Figure 3.12 — The Copper-Histidine Tyrosinase Active Site

Tyrosinase is an oxidase enzyme containing six histidine residues that coordinate two copper ions to generate the oxygen-binding site. In humans, tyrosinase oxidizes tyrosine enroute to the formation of the pigment melanin. Reduced enzyme activity is associated with hypopigmentation of skin and hair. Genetic mutations of this enzyme are associated with albinism. Tyrosinase is widespread in plants and animals, and is responsible for the browning (oxidation) of certain fruits, such as apples, and potatoes when exposed to oxygen.

Ceruloplasmin contains six atoms of copper, three of which provide active binding sights for electron transfer. It is a powerful antioxidant, scavenging hydrogen peroxide and other reactive oxygen species while inhibiting lipid peroxide and DNA damage wrought by free copper and iron ions. Ceruloplasmin also oxidizes certain compounds, such as in the conversion of cysteine to the disulfide cystine.[390] Additionally, ceruloplasmin is an acute-phase reactant protein, and may elevate in inflammatory conditions.[391, 392]

At least four atoms of copper are required to complete the structure of cytochrome-*c* oxidase, the final and rate-limiting electron transport protein in mitochondrial ATP production.[393] This cuproprotein might be considered the most important enzyme in mammals, since it sits at the final gateway for energy substrate flow, and its action governs the life cycle of cells via mitochondrial signaling of apoptosis.[394]

Copper also acts as a modulator of neuronal transmission in central nervous system (CNS) neurons, being involved in N-methyl-D-aspartic acid (NMDA) receptor modulation[395] and catecholamine biosynthesis.[396, 397]

As a final note on copper physiology, the toxic effects of adriamycin on cell growth are due to the formation of an adriamycin-copper complex that inactivates mitochondrial cytochrome-*c* oxidase.[398] The effect is greatly reduced in vitro by adding glutathione, which effectively competes for copper.[399]

Clinical Associations of Copper

While frank copper deficiency is considered to be rare, its most frequently seen in children, patients on total parenteral nutrition (TPN) and those with malabsorptive conditions or who are under aggressive, long-term zinc therapy. Menkes disease is a copper deficiency condition caused by an X-linked recessive genetic defect in the MNK protein (a P-type ATPase), which causes copper to be exported across the blood-brain barrier, gastrointestinal tract and placenta. Presentation is multisystemic, with alterations to hair and skin and involving CNS, musculoskeletal, cardiovascular, hematological and immunological derangements. Symptoms appear in the first few months of life, and death may follow in early childhood.[32,400,401]

An extensive body of evidence exists for copper deficiency associated with cardiovascular disease: aortic fissures and rupture, arterial foam cells and smooth muscle migration, cardiac enlargement and rupture, coronary artery thrombosis, and myocardial infarction.[36, 402-405] Mild copper deficiency can also contribute to elevated cholesterol, impaired glucose tolerance,[406] and erythropoietin and iron resistant normocytic anemias, as well as neutropenia, thrombocytopenia, peripheral neuropathy, defective elastin and bone demineralization.[99] A type of myeloneuropathy resembling that seen in vitamin B_{12} deficiency, but responding to copper supplementation, has also been reported.[407]

Chronic copper exposure via copper piping or copper cooking utensils can lead to poisoning. Symptoms include nausea, vomiting and diarrhea. Long-term exposure to elevated copper can result in hepatic cirrhosis.[32, 388] Wilson's disease, a condition of copper excess, is an autosomal recessive disorder of copper metabolism resulting from insufficient copper excretion via hepatocytes into bile due to a defect in the same copper-transport enzyme as in menkes, P-type ATPase. This pathway is the sole mechanism of copper excretion. When it fails, toxic levels of copper accumulate and produce neurodegenerative conditions and cirrhosis. Excessive accumulation of copper in the hepatocyte cytoplasm causes cellular necrosis and leakage of copper into the plasma. Copper is also deposited in extrahepatic tissues, including the cornea (Kayser- Fleischer rings) and the brain. Patients develop retinal degeneration and CNS extrapyramidal and cerebellar degeneration with cognitive dysfunction.[400, 401] The excess free copper ions can behave as free radicals, resulting in cancer and heart disease. Both zinc and molybdenum (as tetrathiomolybdate) are used to decrease elevated copper levels.[32, 378, 408] The competition of zinc and molybdenum with copper for binding sites on proteins is used to treat copper toxicity conditions such as Wilson's syndrome, cancer and other inflammatory conditions.[408]

Children with autism, a condition associated with increased oxidative stress, frequently have an elevated serum copper-zinc ratio[409] coupled with reduced ceruloplasmin, pointing to a derangement in copper homeostasis.[410] Elderly patients are more likely to have inefficient copper homeostatic mechanisms, producing elevated serum free copper,[408] which can lead to increased oxidative stress. Copper excess or deficiency due to homeostatic derangements have been implicated in the pathogenesis of Alzheimer's disease.[345, 411,412] The pathophysiology of neurodegenerative conditions has been associated with copper dysregulation, including amyotrophic lateral sclerosis (ALS), Parkinson's disease and Down's syndrome, and idiopathic seizure disorder.[412,413] Copper-binding drugs such as tetrathiomolybdate, penicillamine, and trientine have been developed to treat the toxicity of Wilson's disease, but they tend to bind or displace the non-essential toxic elements as well. Copper-binding drugs inhibit angiogenesis, fibrosis and inflammation and are used to treat conditions such as rheumatoid arthritis, diabetic neuropathy and heart disease indicating toxic effects of excess copper and other toxic metals.[414, 415] Since copper is excreted via bile, compromised gall bladder activity contributes to toxicity. Bile duct ligation in rats leads to higher total-body copper.[378, 408]

Notes:

REFER TO CASE ILLUSTRATION 3.6

Assessing Copper Status

The most frequently used biomarkers of copper status are plasma or serum copper and serum ceruloplasmin levels. Levels of these analytes plateau at adequacy, so toxic concentrations are difficult to establish. A high serum copper concentration with elevated copper to ceruloplasmin ratio is indicative of copper excess.[388] Without a measure of ceruloplasmin, serum copper may change independent of dietary intake. Since ceruloplasmin is an acute-phase reactant protein, higher levels are found during periods of inflammation. Ceruloplasmin synthesis is suppressed with protein inadequacy or corticosteroid excess. Estrogen, however, tends to elevate ceruloplasmin,[345] explaining why females tend to have higher serum copper levels. In Wilson's disease, the P-type ATPase defect causes ceruloplasmin to be lowered, whereas free copper is elevated.[389]

RBC copper may be a useful as a marker for copper deficiency because of the tight relationship with copper-superoxide dismutase (SOD). Most of the copper in erythrocytes is associated with this enzyme. In most routine clinical situations, RBC copper appears to be closely associated with liver copper.[416] Direct measurement of SOD in erythrocytes provides meaningful insight into copper status.[388,417]

Several studies have reported hair copper as a measure of copper status.[418-423] However, two situations can occur that will produce misleading results: (1) copper loading diseases, especially Wilson's disease, will produce results not reflective of total-body copper stores, since copper is largely sequestered in the liver,[424] and (2) exogenous contamination such as that found in some hair treatments causes false elevations. Although urinary copper is occasionally used to assess body status,[423] it is primarily useful for diagnosis of copper toxicity, such as in Wilson's disease, where excretion is significantly elevated.[425]

The cuproenzyme dopamine beta-hydroxylase converts dopamine to norepinephrine. Homovanillic acid (HVA) is the main urinary dopamine catabolite, and vanillmandelic acid (VMA) is a norepinephrine and epinephrine catabolite that can be measured in urine. An elevated HVA/VMA ratio (> 4) is an early and sensitive marker for the copper deficiency as seen in Menkes disease.[426] An elevated HVA/VMA ratio has also been observed in copper deficiency in animal studies.[427] This ratio may prove to be a highly valued indicator of copper status, though the ratio may be affected by other factors.

Healthy adults in good copper status showed no change in bone resorption markers when they were supplemented with additional copper.[431] Copper is involved in bone metabolism via lysyl oxidase, an enzyme that forms the stabilizing cross-links between collagen strands. Markers of bone loss, including urinary deoxypyridinoline, pyridinoline, hydroxylysine and hydroxyproline have been shown to be abnormal in urine and serum in copper-deficient humans and animals.[428-430] Copper deficiency tends to produce lower levels of the collagen cross-link markers because the cross-links are formed by copper-dependent enzymes.

Copper Repletion Dosing

Commonly used bioavailable forms of copper include aspartate or sulfate salts. Dosing range is 2 to 10 mg/d for adults. Excessive zinc intake with concurrent low copper intake can induce copper deficiency. The RDA recommendations for zinc and copper are in a ratio of 9:1.

Manganese (Mn)

Adequacy assessment: RBC Mn; BUN, urinary ammonia markers, arginine/ornithine ratio

Optimal forms: Sulfate, lactate, succinate, gluconate and citrate salts

Clinical indications: *Deficiency:* increased oxidative activity, *Toxicity:* neurotoxicity, including parkinsonism

Food sources: Tea, whole grains, legumes, nuts, green vegetables

The average adult contains 10 to 12 mg total-body manganese (Mn),[32] primarily concentrated in tissues requiring high energy, including brain, and also found in liver, pancreas and kidney.[432] Manganese is a group VII transition metal, existing in a number of different oxidation states, but in biological systems, the most prevalent are +2 and +3. Chemically, manganese is similar to iron, so an imbalance in one may induce imbalance in the other. For example, iron deficiency may increase manganese transport, both in the GI and CNS, creating the potential for a toxic manganese burden.

Manganese is a cofactor for enzymes involved in metabolism of amino acids, lipids and carbohydrates. Manganese-dependent enzyme families include oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Examples of manganese-containing enzymes are arginase, glutamine synthase and mitochondrial superoxide dismutase (referred to as SOD2 or MnSOD). Physiological activities include immune function, regulation of blood sugar and cellular energy, reproduction,

digestion, bone growth, and protection from oxidative challenge. Manganese with vitamin K supports blood clotting and hemostasis.[432]

Clinical Associations of Manganese

Change in CNS manganese tissue concentration may be accompanied by convulsions. Both high and low blood manganese has been associated with seizure disorders. In one reported case, a 3-year-old child presented with idiopathic seizure disorder. The only abnormal findings were elevated blood manganese and encephalopathy on EEG. The patient, who was non-responsive to antiepileptic medication, deteriorated to status epilepticus. Immediate resolution was attained upon administration of IV Ca-EDTA therapy. Exposure to welding done by her father over 1 month was the reason for the child's manganese burden.[433-435]

Increased activity in MnSOD with concurrent reduction in cytosolic SOD (which is copper and zinc dependent) was demonstrated in vitro after cellular gamma ray irradiation exposure, demonstrating that MnSOD assessment may be a biomarker of radiation sensitivity, as well as illustrating the import of MnSOD in radiation-induced tissue damage.[436]

Iron overload disorders such as Friedreich's ataxia (FA), sideroblastic anemia (SA) and hemochromatosis demonstrate reduced activity of MnSOD. Both FA and SA present with increased iron deposition in mitochondria. FA, a neurodegenerative and myocardial disease, is caused by decreased expression of the iron-regulating mitochondrial protein, frataxin. Low frataxin causes iron overload and manganese depletion, greatly reducing MnSOD activity. In a frataxin-deficient yeast model, manganese was shown to increase MnSOD, whereas a MnSOD mimetic showed little effect. These iron-overload conditions require increased antioxidative support as afforded by MnSOD.[437-441] Manganese may be a worthy treatment consideration in such disorders.

Notes:

High doses of N-acetylcysteine was shown to induce formation of manganese superoxide dismutase in vitro, thereby preserving its activity.[442]

Women demonstrate increased absorption of manganese and increased MnSOD activity mediated by estrogen, which may exert antioxidant effects by this mechanism. All forms of SOD are down-regulated in estrogen-deficient mice that also show increased vascular free radical activity. Progesterone has been shown to reduce SOD activity, and thus antagonize the vasoprotection induced by estrogen. These findings may in part explain why hormone replacement therapy with estrogen plus progesterone displayed no beneficial effect on cardiovascular event rates in prospective clinical trials.[443-445]

Frank manganese deficiency in humans to date has been studied only by chemically induced manganese depletion. However, individuals with low manganese intake have impaired growth, poor bone formation and skeletal defects, reduced fertility and birth defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism. Men experimentally placed on manganese-depleted diets developed a rash on their torsos, and women consuming < 1 mg manganese/d in their diet developed altered mood and increased pain during premenstruation.[432]

Arginine converts to either ornithine or citrulline, producing urea or nitric oxide (NO), respectively. Inhibition of arginase reduces conversion of arginine to ornithine and promotes conversion into citrulline, thereby increasing NO production (and decreasing urea production). Because manganese is the cofactor for arginase, lowered plasma manganese correlated with lower arginase activity and corresponding increased nitric oxide production in patients with childhood asthma.[446] Similarly, manganese deficiency in rats enhances endothelium-dependent vasorelaxation of aorta. Arginase inhibitors are being considered as potential interventions for increasing nitric oxide.[447-449]

Toxic effects of inhaled manganese in dust or aerosols have been reported from occupational exposure in welding or steel alloy production. Toxicity via ingestion, primarily from water sources, has also been reported. Total parenteral nutrition is a potential iatrogenic route of toxic exposure to manganese. Manganese is being considered as an additive for gasoline, as a lead replacement. Although it has been shown to greatly improve oil combustion, attention must be given to the potential

for increased exposure.[450-453] Vegetarianism may increase manganese body burden via increased dietary consumption and/or iron deficiency-induced increased manganese absorption. Soy beverages, including infant formula, have been shown to contain 100-fold greater amounts of manganese than human milk, and 10-fold greater amounts than bovine sources. Studies using soy-based formulas in primates show increased incidence of behavioral disorders.[432, 454, 455] However, soy is rich in phytates that inhibit absorption of manganese as well as other elements. Thus, vegetarians eating large amounts of soy may, paradoxically, develop manganese deficiency. Since bile is the main route of manganese elimination, individuals with liver disease frequently present with higher levels of manganese, and therefore are at greater risk of toxicity.[32, 456-458]

The organ most vulnerable to manganese toxicity is the brain. Manganese concentrates in areas with high iron, including the caudate-putamen, globus pallidus (GP), substantia nigra and subthalamic nuclei.[459] The neurotoxicity of manganese appears to be mediated by the oxidation of divalent to highly oxidative trivalent manganese via superoxide, inducing a cascade of oxidative mediators damaging cellular components, primarily in the mitochondria.[459] Chronic, low-level exposure to manganese has been implicated in neurologic changes, decreased learning ability in school-aged children, and increased propensity for violence in adults.[452]

Frank manganese toxicity, "manganese madness," presents similarly to schizophrenia. Symptoms include compulsive or violent behavior, emotional instability, hallucinations, fatigue and sexual dysfunction.[100] Mechanistically, this initial presentation is likely due to lesions in the GABAergic neurons of the globus pallidus. As the condition progresses, damage to the dopaminergic neurons in the substantia nigra causes a clinical presentation similar to parkinsonism, but differentiable by the presense of dystonia induced by GP lesions. Furthermore, manganese-induced dopaminergic neuronal oxidation caused general derangements in the hypothalamic-pituitary-adrenal (HPA) axis, including abnormal serum prolactin, TRH, FSH and LH. Additionally, excess manganese can inhibit astrocyte glutamate reuptake, thereby increasing glutamate's excitotoxic potential, and its time in the synapse.[432, 456, 458–460]

Clinical efficacy has been demonstrated for EDTA chelating therapy in a case of occupational parkinsonism due to manganese exposure. Improved clinical pattern due to reduction of heavy metal deposition in basal ganglia was confirmed by MRI. Occupational exposure to manganese compounds in this case resulted in high blood and urinary levels of the metal.[126]

Manganese enters the CNS and is absorbed along the length of the small intestine through the divalent metal transporter 1 (Figure 3.8). Maximal GI absorption is about 3%. Since iron shares the same transporter, increased manganese GI absorption and CNS delivery has been shown to occur in iron deficiency states, contributing to increased manganese burden.[100]

Conversely, manganese absorption is decreased in the presence of iron. Excretion is primarily via bile to feces, with minimal elimination in urine. Phytates may inhibit absorption, and reducing dietary manganese and increasing biliary elimination further decrease manganese in the body.[32, 100] Given the similarity manganese has with iron, it may be that similar counter-ions would increase GI bioavailability, including sulfate, gluconate and citrate.

Manganese is rapidly cleared from blood and stored in liver and other organs.[32] In plasma, manganese is largely bound to gamma-globulin and albumin, with a small fraction of trivalent manganese bound to the iron-carrying protein, transferrin.[432]

Assessing Manganese Status

Some review articles have concluded that there is no reliable index or biomarker for evaluating manganese insufficiency.[32] Others have concluded that of the direct biomarkers used, RBCs are best associated with long-term levels and are considered to be a good index of manganese status.[461] Manganese is frequently measured in profiles of trace elements in RBCs or whole blood where low levels are found in manganese-depleted individuals. A number of studies examining normal or deficient manganese in a variety of human populations have relied on erythrocyte measurements.[155, 462-464]

Such findings may be combined with other functional markers known to appear abnormal when manganese insufficiency is affecting metabolic activity. Altered plasma concentrations of ammonia and urea are found in association with decreased hepatic manganese concentration in young growing rats.[465] Thus, serum BUN and sensitive urinary markers of urea cycle activity (see Chapter 6, "Organic Acids") may be helpful along with demonstration of an elevated plasma arginine-ornithine ratio to achieve an assessment of low manganese effects.

Hair manganese is a valid indicator of toxicity in cases of manganese excess, but there is controversy over its use for deficiency states.[466,467] Inconsistent results have been reported from studies using plasma, serum or urine to evaluate manganese status.[32, 468-475]

Because of its strong paramagnetic quality and primary site of toxicity in the CNS, manganese toxic burden is readily assessed using T1-weighted MRI. RBC manganese demonstrates a high correlation with MRI ($r = 0.55$, $p = 0.02$) in manganese-exposed workers prior to onset clinical symptoms. Additionally, RBC manganese was shown to correlate specifically with CNS globus pallidus burden. RBC and MRI manganese assessment also correlated in liver cirrhosis patients.[468-470]

Manganese Repletion Dosing

Bioavailable forms of managnese include sulfate, lactate, succinate, gluconate and citrate. Dosing range is 5 to 13 mg/d for adults.

IODINE (I)

Adequacy assessment: Urinary iodine, TSH, TT4, TT3, FT4, FT3, RT3

Optimal forms: Potassium iodide, molecular iodine

Clinical indications of deficiency: Goiter, hypothyroidism, hyperthyroidism, fibrocystic breast disease

Food sources: Seaweed, shellfish, marine fish, iodized salt

Iodine (I) is necessary to make thyroid hormone, a regulator of energy metabolism that is vital for normal growth and development, maintenance of body temperature, and brain development. Thyroxine (T4) contains 4 iodine atoms and triiodothyronine (T3) contains 3 iodine atoms, so iodine constitutes 65% and 59% of T4 and T3, respectively.[476, 477] Dietary organic iodine (I_2) is converted to iodide (I^-) in the gut and absorbed throughout the gastrointestinal tract.[478] Iodide is 100% bioavailable. The term "iodine" may be used for the purposes of this discussion to denote all forms of the element.

Of the 20 to 30 mg total-body normal iodine content, 8 mg is in the thyroid gland.[33] As shown in Figure 3.13, follicular cells surround the gell-like colloid in the central lumen where thyroglobulin (Tgb) is synthesized and iodinated. Tgb is synthesized by follicular cell ribosomes and moves into the lumen (or colloid) of the follicle. Tgb is oxidized by peroxidases, iodinated, and coupled before it reenters the follicular cell for hydrolysis when T4, with lesser amounts of T3, are finally released into circulation. Thyroperoxidase (TPO) iodinates tyrosyl residues on thyroglobulin and is responsible for phenoxy-ester bond formation between the rings of monoiodo-L-tyrosine (MIT) and diiodo-L-tyrosine (DIT) to form T3 and T4 on Tgb. Afterwards, lysozome-mediated hydrolysis liberates the iodinated compounds from Tgb. MIT and DIT are recycled and T3 and T4 are released into the bloodstream. Peripheral conversion of T4 to the metabolically active T3, and subsequent breakdown of T3, requires the selenoproteins[476] iodothyronine deiodinases (D1 and D2).[32] Similarly, the deiodinases work inside the follicular cell to recycle tyrosine and iodine.

Clinical Associations of Iodine

Iodine deficiency is a worldwide problem. The major cause of iodine deficiency is inadequate intake due to low soil concentration, with resulting low concentration in crops.[27] The iodine content of plants grown in iodine-deficient soil may be as low as 10 µg/kg, compared with 1,000 µg/kg for plants grown on soils with high iodine content.[481]

Iodine deficiencies are the leading cause of preventable mental retardation worldwide.[27] Iodine deficiency is known to affect all stages of life. For the fetus, it can lead to abortions, stillbirth, congenital anomalies, increased perinatal mortality, endemic cretinism and deaf mutism. In the neonate, it can cause goiter, hypothyroidism, mental retardation and increased susceptibility of the thyroid gland to nuclear radiation. Neonatal screening can prevent neurological consequences of congenital hypothyroidism in infants after birth.[477] In children and adolescents, iodine deficiency can lead to goiter, subclinical hypothyroidism, subclinical hyperthyroidism, impaired mental function, retarded physical development and susceptibility of the thyroid gland to nuclear radiation. Finally, in the adult, the consequences of iodine deficiency are goiter, hypothyroidism, impaired mental function, spontaneous hyperthyroidism in the elderly, iodine-induced hyperthyroidism and increased vulnerability of the thyroid gland to nuclear radiation.[482-486] Iodine deficiency coupled with high goitrogen intake for a long time period can bring about goiter.[487,488] The combination of goitrogenic thiocyanides with selenium deficiency is a risk factor for endemic myxedematous cretinism.[489] For more discussion on the impact of goitrogens on hormone function see Chapter 10, "Hormones."

Iodine is essential not only for proper thyroid function, but it is also needed in mammary tissue. Iodine

can be used therapeutically in mammary dysplasia and fibrocystic breast disease, presumably due to the similar uptake and utilization of iodine in thyroid and mammary tissues.[490] In a review of 3 clinical studies of fibrocystic disease, between 40 and 74% of patients experienced improvement with oral supplementation of molecular iodine (I_2).[491] Molecular iodine has also been recommended as an adjuvant to breast cancer therapy, given its suppressive effect on tumors and its role in controlling mammary gland proliferation.[492] In animals, long-term iodine deficiency increases carcinomas of thyroid epithelial cells. Population studies have found

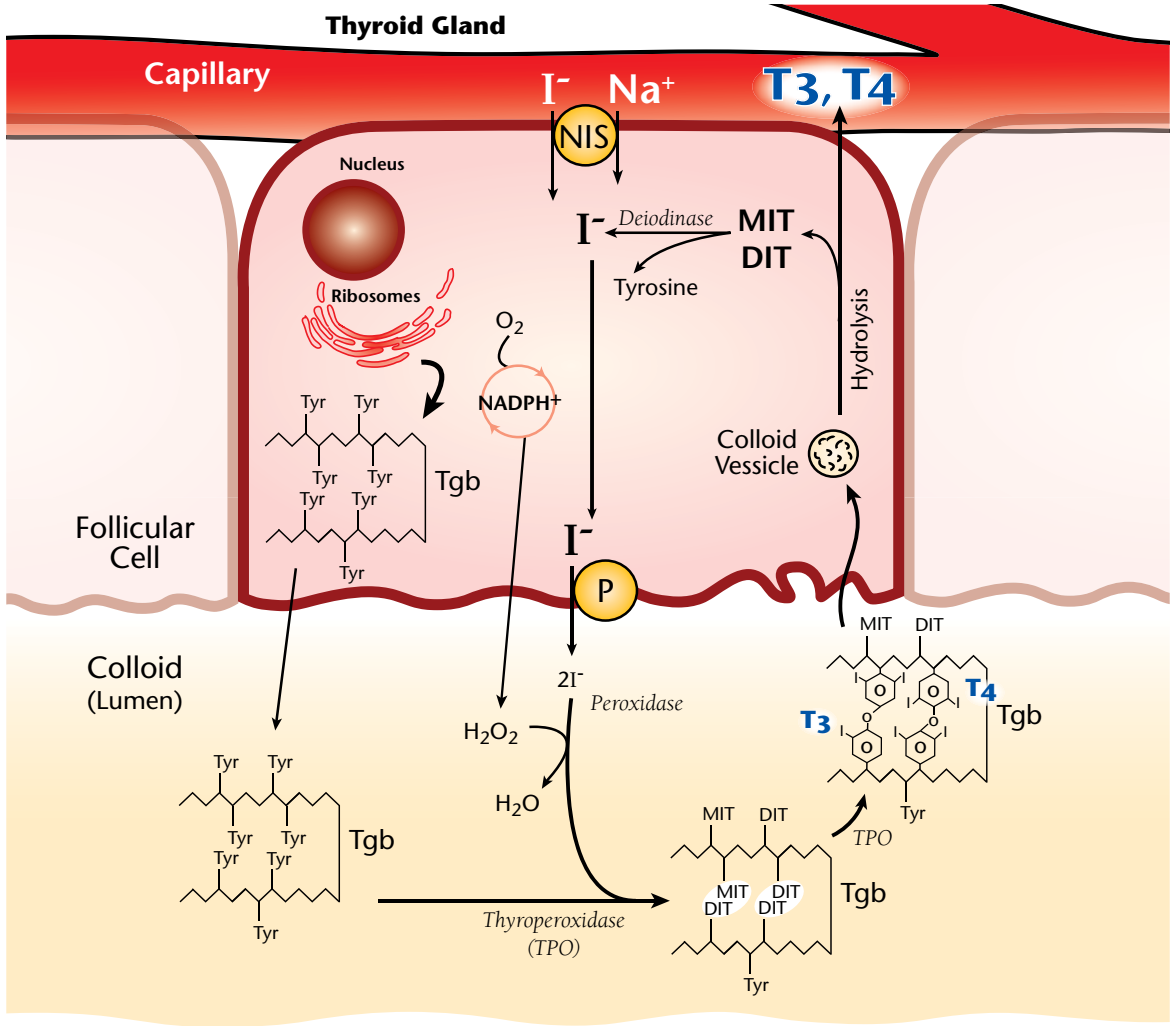


Figure 3.13 — Iodine Transport and Thyroid Hormone Synthesis in the Thyroid Follicular Cell

The thyroid gland contains many follicles composed of a central lumen (colloid) surrounded by follicular cells. Iodide enters the thyroid follicle via the sodium iodide symporter (NIS) and moves toward the lumen of the follicle. The pendrin symporter (P) exports iodide from the cell into the lumen, where it undergoes oxidation by thyroperoxidase (TPO) to yield iodate (I_2^-). Thyroglobulin (Tgb), synthesized in the follicular cells via TSH-regulated gene transcription, contains around 115 tyrosine residues available for iodination when it enters the lumen. Thyroperoxidase adds iodine to tyrosyl residues, creating MIT and DIT. Intramolecular coupling of MIT and DIT yields protein-bound triiodothyronine (T3) and tetraiodothyronine (T4 or L-thyroxin). Thyroglobulin bearing MIT and DIT (and residual T3 and T4) undergoes phagocytosis to re-enter the follicular cell, where it is hydrolyzed, releasing T3, T4, MIT, and DIT. Free T3 and T4 are then exported from the follicular cell. Free follicular cell MIT and DIT undergo deiodination (selenium dependent), thereby recycling iodine and tyrosine for further synthesis of thyroid hormone in the follicle.

higher iodine intake associated with lower incidence for some types of thyroid cancer, that is, fewer aggressive and more papillary carcinomas.[493] Potassium iodine, given to individuals exposed to radioactive iodine in the Chernobyl disaster, reduced thyroid cancer.[494]

Halogens other than iodine have been shown to impact thyroid function. Bromine can replace iodine in both T3 and T4, without loss of hormone activity. In experimental animals, high bromide intake causes decreased iodine in thyroid, skin, and in mammary glands and increased iodine excretion.[495] In low-birth-weight infants exposed to chlorine dioxide by-products, TSH was significantly higher than those in the reference group.[496]

Iodine Toxicity

Although up to 1,000 µg of iodine daily is considered safe, therapeutic doses of iodine are not agreed upon, making iodine testing very important in patient care. Iodine repletion doses range between 6 and 50 mg/d.[497] Excessive intake of iodine reduces organic binding of iodine, resulting in hypothyroidism and goiter,[494] thyroiditis,[498] and autonomous thyroid nodules.[499] Excessive iodine intake can also stimulate autoimmune thyroiditis in animals and humans.[478, 500] When the thyroid is damaged, down-regulation of iodine uptake with excess intake can be dysfunctional. In one study, 15 of 34 infants positive for congenital hypothyroidism were diagnosed with hyperthyrotropinemia due to overconsumption of iodine (820–3,200 µg/d) by the mothers during pregnancy.[501] Chronic intake of large amounts of iodine can limit thyroid hormone synthesis and release. Tolerable upper limits of intake are largely mediated by the degree of iodine deficiency prior to loading. For instance, in long-term mild iodine deficiency, iodine trapping is more effective than in those with normal intake, making a sudden high dose potentially detrimental.[478] Therefore, supplementation should be gradual. Iodides are also used to inhibit iodination and proteolysis of Tgb in thyrotoxicosis.[476, 502] These concerns make testing for iodine essential in proper management of a patient.

Assessing Iodine Status

Direct Iodine Measurements: Approximately 10 to 20 µg of iodine are lost daily in the feces, and 100 to 150 µg as urinary iodine (UI) in iodine-sufficient populations,[502] thus excretion is primarily through the urine,[33] and UI is believed to accurately reflect the mean iodine intake in population studies (see Table 3.7).[503-505] Iodine deficiency is indicated for individuals with UI concentrations < 50 µg/L.[486]

Although the United States and Canada were not previously considered to be at high risk for iodine deficiency, restricted salt intake from efforts to control hypertension are changing that opinion. Iodine deficiency in the United States has increased in the last few decades. Mean urinary iodine (UI) went from near 220 µg/L in the 1970s to 145 µg/L by 1994, and then rose slightly to 165 µg/L in 2001 and 2002.[506] Presumably due to decreased iodized salt intake, 12% of the US population had UI below 50 µg/L in one large survey.[502, 507] The highest UI levels have been found in children when compared to adults, while the lowest iodine levels have been found in adults age 45 to 59. Men have higher UI than females overall.[508] These differences complicate making conclusions about iodine adequacy from population data.

Attention to specimen timing is important for UI determination. In a study of 3,023 spot urines, UI variation displayed circadian rhythm that was independent of subject, age, gender and season.[509] Concentrations were lowest in the morning, so this specimen may show falsely low levels when compared with limits from other time intervals. Sampling casual urine samples from various times of day, testing over more than one day, and reporting as µg/mg creatinine (UI/Cr) improves accuracy of measuring individual iodine status.[510]

Twenty-four-hour UI is preferred for its reliability and assessing individual iodine status, especially in conditions where urinary creatinine should be interpreted with caution (i.e., elderly, malnourished, or with muscle wasting). Patients must take care in specimen collection to ensure accurate results.[511] More than one 24-hour UI is ideal. Normal values for 24-hour urine collection are 100 to 460 µg/d.[512] When 15 men were tracked for a year with multiple sample collections, 24-hour urine specimens were found to be more accurate than UI/Cr.[513] Population studies have used random spot UI concentration measured as µg/L,[508,514] however, the concentration of iodine in a spot urine specimen is influenced by recent iodine intake, so the test may not accurately reflect an individual's iodine status.

Although 24-hour UI/creatinine eliminates within-day and day-to-day variations in iodine excretion, creatinine is affected by poor nutrition, advancing age and sedentary lifestyle. Large inter- and intraindividual variations have been reported for creatinine, so the

Table 3.7 — Guidelines of Appropriate Limits for Spot Urine Iodine Testing

Median urinary iodine (µg/L)	Iodine Intake	Iodine Nutrition
< 20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Optimal
200–299	More than adequate	Risk of iodine-induced hyperthyroidism within 5–10 years following introduction
> 300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid diseases)

creatinine normalization method may give inaccurate results for iodine levels,[515] misrepresenting iodine deficiency as iodine sufficiency.[483, 516] Testing for iodine in pregnancy requires special consideration, as UI has been shown to elevate in pregnancy, and whether it is a pregnancy-related increase or masked deficiency is yet unknown.[483, 519]

Iodine may be measured in elemental profiling of plasma or serum, although the values provide only a measure of circulating thyroid hormone under most routine clinical conditions. Normal values are 40 to 92 µg/L.[512]

Indirect Iodine Measurements: Physical examination of thyroid size by palpitation or measurement by ultrasonography provides clinical evidence of dysfunction from improper iodine status.[486] The late-stage chronic deficiency effect of palpable goiter is consistently associated with lower urinary iodine, such as when 568 school children in remote areas of Turkey were examined.[517] These procedures are examining end effects; however, that may not appear for years, during which iodine insufficiency may be producing clinical effects. Thyroid hormone and TSH testing has long been used as a kind of functional assessment of iodine status, although the levels vary for many reasons other than iodine status.

As iodine becomes the limiting factor for thyroid hormone production, T3 is increased, at the expense of T4 in the thyroid gland and in plasma, and longer-term deficiency will cause a decline in both hormones.[477] Thus, a high T3/T4 ratio is consistent with early iodine deficiency. Primary hypothyroidism, the most common cause of low thyroid function, refers to a lesion within the thyroid gland itself. In an iodine-replete individual, autoimmunity is the most frequent cause of hypothyroidism. See Chapter 10, "Hormones," for further discussion of hypothyroidism.

TSH and serum thyroglobulin (Tgb) increase in iodine deficiency.[518] However, mild to moderate iodine deficiency may not be reflected in TSH levels.[519-521] Thus, clinically significant iodine deficiency could have detrimental mental and neurodevelopmental consequences without tell-tale high TSH and without clinical hypothyroidism. In rats, iodine deficiency resulted in a variety of adaptive mechanisms to ensure adequate T3 supply to skeletal muscle, heart, lung and ovary, whereas other tissues, such as the brain, were deprived of T3.[521] Due to increasing rates of peripheral conversion, T3 may increase as iodine deficiency worsens, while T4 is still within normal ranges.[345] Other nutrients that affect peripheral conversion to T3 are covered in Chapter 10, "Hormones." Analysis of NHANES data shows no correlation of urinary iodine (µg/L or µg/mg creatinine) with TSH and T4.[522] The combination of UI with TSH and T4 provides improved assessment.

Functional Iodine Tests: The iodine skin test is a traditional approximation method of checking iodine status by painting the skin with a solution of iodine

Notes:

and observing the time interval for fading of the color. Rapid fading of the iodine, compared with control, is understood to indicate a need for iodine.[523] There are no accepted norms for the time for fading, and the observation is complicated by dark skin color.

Measuring thyroglobulin in whole blood or dried blood spot specimens (BS Tgb) is a promising new approach.[524] Chronic iodine insufficiency results in increased uptake of Tgb by follicular cells in order to increase the release of thyroid hormone. The process also releases portions of intact Tgb into blood that can be detected by sensitive analytical methods. At birth, iodine availability determines the degree of Tgb iodination that is reflected by serum Tgb.[525] Goitrous patients have elevated basal serum Tgb levels that normalized after 30 months of therapy with iodized oil.[526, 527] Along with significantly lowered urinary iodine excretion, Danish women in late pregnancy were found to have serum Tgb levels twice those of controls.[528] In myxoedematous cretinism with thryroid involution, paradoxically low serum Tgb and elevated TSH levels were found in response to iodine treatment, suggesting a low thryroid reserve and a reduced amount of functional thyroid tissue.[529] In these patients the serum Tgb/TSH ratio has been proposed as a predictive index of thyroid reserve and of positive response to iodine administration.[529] The availability of a standard reference material greatly facilitates establishing the absolute accuracy and interlaboratory comparisons for the BS Tgb test. The test is sensitive to iodine status and specific to the thyroid response, while being uncomplicated by reliance on intestinal absorption or urinary excretion of iodine.[524, 530]

Iodine Loading Tests: When 570 mg of iodine was given to healthy adults as an oral bolus, 24-hour urinary iodine levels were elevated above baseline for up to 6 months.[533] Such observations show that there is a large capacity for ioding sequestration. If that capacity is unfilled, then higher amounts of iodine are retained. When healthy, euthyroid males were given 100 mg of oral KI containing 76 mg of iodine, they excreted approximately 90% of the iodine in the following 24 hours.[911] After 8 days of consuming iodine supplemented table salt, an average of 92% of the extra daily iodine appeared in 24-hour urine from 12 healthy adults.[531] Such evidence has been used to propose a standard test for iodine status in which 90% of a 50 mg oral iode load is expected to be recovered as urinary iodide in 24 hours. Recoveries of less than 90% may indicate poor iodine saturation and potential benefit from extra iodine intake.[532] High retention (low % recovery) may arise due to pregnancy,[519] and goitrogen intake can easily cause false positives because the iodine fails to be absorbed.

Notes:

REFER TO CASE ILLUSTRATION 3.7

Other Related Tests: Tyrosine and selenium may warrant testing in thyroid deficiency. Rising TSH in a patient with low plasma tyrosine may signal thyroid hormone restriction by substrate availability, especially in those who have slow conversion of phenylalanine to tyrosine. See Chapter 4, "Amino Acids," for discussion of these amino acid issues, and see Chapter 10, "Hormones," for further discussion of thyroid hormone.

Repletion Dosing of Iodine

Thyroid function should be monitored during aggressive dosing of iodine. Forms of iodine include Lugol's (a mixture of iodine, iodide and potassium iodide, of which approximately 77% is iodine), Iodorol (iodine or I_2), which is administered in long-acting dextrose capsules or dextrin compound, iodate salts and the antimicrobicide betadine (1% iodine). T3 and T4 have also been used in replacement studies.[534] A combination of iodine and iodide is recommended, and therapeutic doses range from 6 to 50 mg/d. If giving Lugol's solution, 1 drop has 2.5 mg iodine and 4 mg iodide, whereas Lugol's tablets contain 5 mg iodine and 7.5 mg iodide.[497] Iodized salt and iodized oil are used for widescale public health measures against iodine deficiency disorders.[535] In hypothyroid children with elevated TSH, oral doses of iodine (0.1 mL containing 48 mg) were used to regain euthyroid status.[529] Iodized oil as injections have been employed successfully to improve iodine status better than oral oil.[526, 527] One study showed that oral delivery of 1 mL of iodized oil containing 480 mg of iodine protected subjects from iodine deficiency for 1 year.[536]

Selenium (Se)

Adequacy assessment: RBC, whole blood, hair or serum selenium, plasma selenoprotein P, urinary selenosugar

Optimal form: Mixed selenocompounds including: selenocysteine, selenomethionine, Se-methylselenocysteine

Clinical indications of deficiency: Compromised immunity, male & female reproductive health, cardiovascular health, inflammation regulation in asthma and thyroid hormone metabolism

Food sources: Garlic, onions, broccoli, Brazil nuts, brewer's yeast

Total-body selenium (Se) is approximately 14 mg.[32] Among essential trace elements, selenium occupies a unique position regarding biochemical and physiological mechanisms. Because of its similar size and bonding geometries, selenium may replace the sulfur atom in cysteine, creating a selenocysteine residue. Selenocysteine (Figure 3.14) became the twenty-first amino acid recognized as part of the universal genetic code.[537] In a series of complex steps taking place in the endoplasmic reticulum, a selenocysteinyl-tRNA residue is formed from reaction of selenophosphate with the L-serine adduct, seryl-tRNA. Selenocysteine inclusion into a polypeptide is dictated by the mRNA codon, UGA (previously thought to be a nonsense or termination codon).

In selenoproteins, the selenocysteine residue is part of the polypeptide, with the selenium atom frequently at the catalytic site. Selenoproteins include the glutathione peroxidases (GPx), which are involved in the reduction of hydrogen peroxide and hydroperoxide,[538] and the thioredoxin reductases (TR) that reduce a variety of molecules, including ascorbic acid, lipoic acid, coenzyme Q_{10}, vitamin K and tumor suppressor protein p53. Through its ability to reduce ascorbic acid, thioredoxin reductase regenerates vitamin E.[539, 540] Selenocompounds including TR dictate the redox activity of metallothionein, and are therefore involved in the binding or release of intercellular zinc, copper or cadmium (see sections "Zinc" and "Copper" above, and "Cadmium" below). In a selenium-deficient state, oxidation of metallothionein may lead to the uncontrolled release of metals, especially copper and cadmium, thereby contributing to toxicity.[382, 384] These events can set up a feed-forward cycle of further oxidative challenge and metal toxicity.[382]

Normal functioning of the thyroid gland itself is dependent on the selenoproteins iodothyronine deiodinase (ID), GPx and TR, demonstrating the interrelated metabolic roles of selenoproteins. GPx and TR increase antioxidant activity in the gland. ID converts the thyroid hormone thyroxin (T4) to the metabolically active thyroid hormone triiodothyronine (T3). The type III isozyme of ID inactivates thyroid hormones by catalyzing inner ring deiodination of T4 to reverse triiodothyronine (rT3) and T3 to 3, 3′-diiodothyronine (T2), both of which are biologically inactive. Regulation of type III iodothyronine deiodinase appears to be tissue-specific, with higher amounts of the isozyme present in brain and skin of adult humans.[541-543]

Supplementation with selenium in patients with autoimmune thyroiditis has been shown to significantly reduce antibody production and oxidative stress, most likely through increased GPx and TR activity. Additionally, low serum selenium is associated with increased risk of thyroid cancer, again likely due to reduced activity of GPx and TR.[544, 545]

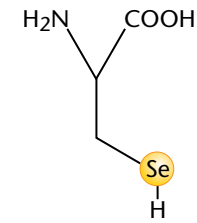


Figure 3.14 — **Selenocysteine**

Selenocysteine is similar to cysteine with a selenium atom instead of sulfur in the side chain.

Clinical Associations of Selenium

Given the diversity of selenoprotein activities, it is not surprising that selenium deficiency affects most physiologic systems, including endocrine and reproductive, hepatic, cardiovascular, immunological, gastrointestinal, and musculoskeletal.[538, 546-551] Selenium is widely recognized as a key nutrient in cancer prevention and treatment. As selenium intake decreases among study populations, there is significant increased risk of colon, prostate, breast, ovary, lung and hematopoetic cancers.[546] A relative cancer risk of 3.1 (95% confidence interval) was associated with serum selenium levels less than 45 µg/mL.[552] Mechanisms of action show decreased oxidized cytochromes, increased apoptosis and cell cycle arrest, decreased angiogenesis, inhibition of nuclear factor-kappa b, and in prostate cancer, selenium modulates androgen receptor and androgen receptor genes.[553, 554] Individuals with elevated body burden of toxic elements have greater difficulty maintaining sufficient selenium

status. Selenium is able to form seleno-glutathionyl arsinium ions that are excreted in bile as well as insoluble selenides with both arsenic[101, 555] and mercury.[556, 557] Although the formation of such compounds reduces toxic effects of the heavy metals by sequestering them as selenocompounds, depletion of the available selenium pool could lead to selenium deficiency.

Selenium levels in food vary greatly depending on soil levels. Severe deficiency associated with low soil selenium was first reported in the Keshan region of China. Keshan disease is a cardiomyopathy occurring primarily in women and children. Recent evidence shows Keshan disease is a selenium deficiency associated with a viral infection. Both influenza and coxsackie virus B3 demonstrated increased virulence when inoculated into selenium-deficient mice.[9] Similarly, Keshan-Beck osteoarthropathy, a degenerative joint disease caused by oxidative damage to cartilage, is associated with severe selenium deficiency due to soil depletion. The incidence of both conditions decreased with the addition of supplemental selenium.[558]

Frank selenium toxicity, called selenosis, is rare in humans. However, cases have been noted, primarily caused by contaminated soil. Symptoms of selenosis include garlic breath odor, thick brittle fingernails, dry brittle hair, red swollen skin of the hands and feet, and nervous system abnormalities including numbness, convulsions or paralysis. High-dose selenium ingestion or exposure was first recognized in the United States in South Dakota livestock ingesting "selenium accumulator" plants growing in selenium-rich soil. The rural families that lived in the area tested positive for high urine and hair levels of selenium.[558] Recently, selenosis-like symptoms were reported to occur in a small trial of men with prostate cancer taking doses as high as 3,200 µg/d for 1 year.[559]

Concern regarding carcinogenicity of certain selenocompounds, particularly selenomethionine, has existed for years.[32] Recent research validates that a number of selenocompounds are pro-oxidative in vitro, and teratogenicity due to high-dose selenomethionine exposure has been shown in animal studies.[555]

Selenium is efficiently absorbed in the GI, primarily as selenomethionine or selenocysteine, via methionine or cysteine amino acid active transport. GI absorption is enhanced in the presence of dietary proteins and vitamins E and A and inhibited with vitamin C, sulfur, arsenic and mercury.[32]

Selenium is metabolized via methylation and excreted as a number of different compounds, depending on selenium load. In toxicity states, trimethylselonium is released in urine, and dimethylselonium is exhaled from the lungs, producing the garlic odor of selenosis (Figure 3.15). Selenosugars are produced and excreted in urine at normal and therapeutic selenium intake. Selenosugars have gained attention as a way of assessing selenium repletion in cancer therapy.[553, 554, 560, 561]

Assessment of Selenium Status

Perhaps more than any other element, selenium has a narrow therapeutic window. Thus, evaluation of selenium status is essential for determining need for selenium therapy. Serum T4 elevation with T3 depression is a functional marker of selenium because the imbalance may be produced by depressed ID activity due to selenium deficiency.[543, 562]

Erythrocytes can be useful to establish selenium status, as selenium is present in high concentrations in erythrocyte glutathione peroxidase.[563-567] However, of all the selenoproteins, GPx has the lowest threshold for selenium saturation, thus erythrocyte selenium repletion may not reflect total-body status.[538] In other words, a patient with normal RBC selenium may still have insufficient selenium to supply other selenoprotein demands.

Blood selenium is found in many forms: 60% as selenoprotein P (SP), a glycoprotein containing about 10 selenocysteine residues, 20% as erythrocyte glutathione peroxidase, and 20% bound to albumin.[568] SP is the main selenium protein in the CNS as well as the blood. It is widely used in research as a marker for establishing total-body selenium status.[547, 569, 570] In selenium-deficient humans, serum GPx normalized more rapidly than SP, showing SP to be a preferred marker of the total-body selenium pool.[571]

In clinical settings, whole-blood selenium is widely used to assess selenium status.[32] Also, large studies evaluating selenium in cancer and other diseases have relied on plasma and serum levels.[554] Whole blood selenium may be more reflective of long term selenium status than plasma selenium.[572]

Urinary selenocompounds can also be useful in assessing selenium status. Selenometabolites such as monomethylselenium are excreted in urine as well as selenosugars, primarily as methylseleno-N-acetyl-D-galactosamine. As the selenium load increases, methylation activity increases, causing production of

trimethylselenonium (TMS). TMS is excreted in high quantities in selenium toxicity[553, 554, 560, 561] (Figure 3.15).

When exogenous selenium exposure, especially from shampoos, can be ruled-out, hair selenium may accurately reflect selenium status. Selenium is readily accumulated in the cysteine-rich residues of hair, and

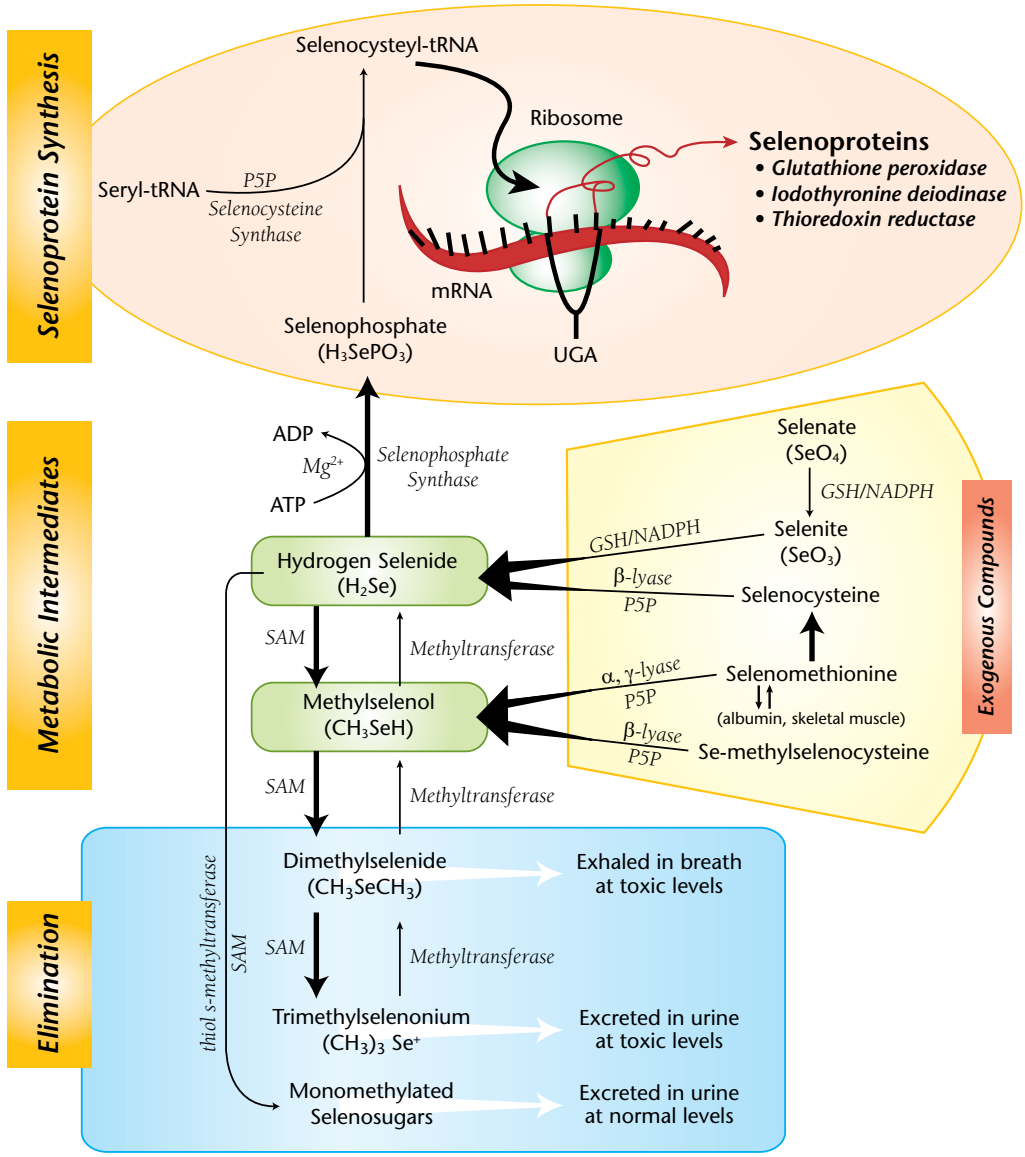


FIGURE 3.15 — Selenium Metabolism

Dietary selenocompounds may be converted to hydrogen selenide which generates selenophosphate for selenoprotein synthesis. Selenocysteyl-tRNA is incorporated into selenoproteins as dictated by the DNA codon UGA. The hydrogen selenide pool must be regulated since excess is toxic. Dietary selenomethionine and Se-methylselenocysteine also generate methyselenol which only slowly enters the hydrogen selenide pool. Methylselenol has been shown to be anticarcinogenic. In selenium excess, methylselenol is methylated with S-adenosylmethionine (SAM) to dimethylselenide and trimethylselenonium that are eliminated in breath and urine, respectively. Hydrogen selenide also generates monomethylated selenosugars that are eliminated in urine and may serve as markers of selenium status.

hair selenium has been shown to reflect dietary intake in both animal and human studies.[101]

Repletion Dosing of Selenium

Selenium supplementation is a complex topic because ingested selenocompounds can affect selenoprotein formation and enzymatic activity differently, and can be toxic.[573, 574] The most frequently used and best absorbed form of selenium is selenomethionine. Selenomethionine has been the main form of selenium used to date in cancer therapy, where it is shown to increase TR and glutathione-S-transferase activity in vivo.[553, 554, 573] However, it readily replaces the methionine residues in muscle proteins rather than directly contributing to other selenoproteins, and accumulation may lead to selenium toxicity. Certain methylated selenocompounds, such as Se-methylselenocysteine, have been shown to be less toxic and are being researched for their chemotherapeutic effect.[553] Se-methylselenocysteine activity is mechanistically different from that of selenoprotein activity, and it does not significantly enter the selenium pool that can contribute to selenoprotein status.[553, 573]

The RDA for selenium is 55 µg/d. Selenoprotein repletion doses of 60 to 100 µg,[571] and cancer prevention or therapy at 200 to 800 µg have been reported. Although the bulk of cancer research was conducted using selenomethionine, current evidence shows increased efficacy with lower toxicity using Se-methylselenocysteine and other methylated selenocompounds.[553, 554]

The forms most often used for supplementation are selenomethionine and selenocysteine. Optimal selenium supplementation may include a variety of selenocompounds, including Se-methylselenocysteine, selenocysteine and selenomethionine. Small amounts of inorganic selenium as selenite or selenate may also be used.[550, 573]

Notes:

Although yeast primarily contains selenomethionine, garlic, onions and broccoli are rich food sources of mixed selenocompounds. Of these, garlic contains the widest variety of selenocompounds, including low-dose inorganic selenium, in ratios that are safe and effective. However, selenium content of foods is highly variable, depending on soil content. Soil supplementation and organic farming can improve food selenium content.[10] Standardization of food extracts is needed.[575]

MOLYBDENUM (MO)

Adequacy assessment: Urinary sulfate, uric acid, elevated sulfite, xanthine, hypoxanthine. Direct assessment—hair

Optimal forms: All are equally well-absorbed

Clinical indications of deficiency: Sulfite intolerance, copper toxicity

Food sources: Beans, nuts, grains, green leafy vegetables

Total-body molybdenum (Mo) content is only about 7 mg,[32] meaning that concentration measurements of molybdenum are in parts per trillion.The word molybdenum comes from the Greek word *molybdos*, meaning "lead-like." Molybdenum chemistry is very unique in that certain molybdoenzymes are able to catalyze reactions utilizing molecular oxygen from either water, which is highly unusual, or O_2 with hydrogen peroxide as a reaction by-product. During the stress of transition from ischemia to reperfusion, the molybdoenzyme xanthine oxidase produces the reactive oxygen species

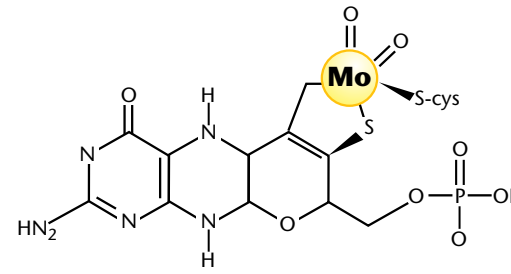


FIGURE 3.16— Molybdopterin

The molybdopterin (MPT) cofactor contains molybdenum at the catalytic site. The MPT shown above is specific for the sulfite oxidase of hepatic and intestinal detoxification systems. All MPTs have similar core structures of a pterin ring bound to a molybdenum atom. The formation of molybdopterin appears to involve copper, which occupies the molybdenum site before being displaced by molybdate (MoO_4^{2-}). Other cofactors involved in the creation of molybdopterin include ATP, magnesium and iron.

superoxide rather than hydrogen peroxide. The local oxidative stress is considered part of the pathogenesis of reperfusion injury. Therefore, adequate presence of endogenous antioxidant defenses including superoxide dismutase, catalase and glutathione peroxidase are necessary to minimize injury.[32, 200]

Molybdenum sits at catalytic sites in a complex structure called molybdopterin (MPT) (Figure 3.16), where it acts as a cofactor for enzymes such as xanthine oxidase and xanthine dehydrogenase, sulfite oxidase, and aldehyde dehydrogenase.[576-578] Multiple, potentially lethal, inborn errors of metabolism are associated with genetic derangements of MPT.[32] Molybdenum also appears likely to be involved in the formation of taurine from hypotaurine dehydrogenase.[579]

Clinical Associations of Molybdenum

Molybdenum supplementation has been shown to reduce sulfite sensitivity, a condition marked by asthma, shortness of breath, edema, dermatitis, and possible anaphylaxis by increasing sulfite oxidase activity, in patients with low blood molybdenum.[580-582] Molybdenum has been shown to prevent copper retention in tissues and increase urinary copper excretion. This effect is so strong that copper deficiency has been induced in animals exposed to excess molybdenum. Tetrathiomolybdate (TTM) is a powerful copper chelator commonly used in the treatment of Wilson's disease.[583-585] TTM is also being explored as an intervention for certain cancers by inhibiting copper-dependent angiogenesis.[586] TTM successfully inhibited tumorigenesis in *Her2/neu* mice genetically programmed to develop breast cancer.[415] TTM has also been shown to inhibit pulmonary and liver fibrosis and type 1 diabetes in animal models.[587] In special clinical situations, a molybdenum-induced copper depletion has therapeutic benefit.

Although molybdenum toxicity and deficiency may be rare, the fact that both states have been reported suggests that other less severe cases more commonly occur.[32, 588] Individuals with mild molybdenum cofactor genetic polymorphisms can survive into adulthood, but with profound disabilities.[589] Risk for gout increases for individuals with very high molybdenum intake.[590, 591] Frank deficiency states are largely relegated to those on total parenteral nutrition, with symptoms including mental disturbance and coma.[32]

About 90% of dietary molybdenum is absorbed in the stomach and small intestine, apparently by passive transport.[592] Transport occurs bound to alpha-2-macroglobulin.[593] Excretion is largely in urine, although some is found in bile and feces.

Assessing Molybdenum Status

Given the low total-body status of molybdenum, functional biomarkers may be more effective than direct measurement of molybdenum status. Biomarkers include decreased urinary levels of sulfate and uric acid with elevated sulfite, hypoxanthine and xanthine.[334] Blood and urine specimens have been used for direct molybdenum measurement, but they are mainly reflective of intake and have not been adequately evaluated.[345, 594] Given the cysteine-rich composition of hair, and molybdenum's ability to complex with sulfur, hair should concentrate molybdenum more effectively than other tissues. Indeed, a few studies show correlatation of hair molybdenum with disease conditions, one in particular demonstrating low molybdenum in patients with severe motor disabilites on enteral nutrition.[595-597] An additional study showed correlation with water and hair molybdenum levels.[177] Supplemental molybdenum is available chelated to metabolic acids such as picolinate (50 to 400 µg) and citrate. Ammonium or sodium molybdate are other commonly used forms. However, given its ready absorption, all forms are likely bioavailable.

Chromium (Cr)

Adequacy assessment: RBC, whole blood, urine, hair; Insulin, blood glucose

Optimal forms: Nicotinate, chloride, histidine or picolinate salts

Clinical indications of deficiency: Blood sugar dysregulatory conditions

Food sources: Whole grains, legumes, nuts, yeast, meats

Unlike most essential elements that have multiple metabolic functions, the only known role for chromium (Cr) is in potentiating insulin receptor tyrosine kinase (Figure 3.17).[598] This autoamplification allows chromium to exert broad influence on carbohydrate, lipid and protein metabolism. Total-body chromium concentration is only about 4 to 6 mg, and decreases with age. There are small chromium storage pools in the testes, kidneys and spleen. Trivalent chromium is the only oxidation state required in biological systems.[32] Hexavalent chromium is a well-known carcinogen that is particularly associated with lung tumor induction.[599]

Clinical Associations of Chromium

Chromium and insulin work in tandem. When insulin is released into circulation, chromium transport to insulin-sensitive cells is increased. Once inside the cell, chromium acts as an autoamplifier of the insulin receptor tyrosine kinase. However, chromium is a nutritional double jeopardy. It is known to be removed from some carbohydrates during the refinement process, making it less available during the insulin rise.[600, 601] It has also been demonstrated that increased urinary wasting of chromium occurs in conditions of elevated blood glucose and insulin.[598] Thus, consuming refined carbohydrates exacerbates losses of chromium and induces insulin resistance.

In the 1950s, rats on a chromium-deficient diet were found to have reduced ability to remove glucose from blood.[32, 602] Subsequent research demonstrated that chromium transport and cellular uptake is stimulated by the presence of insulin. Chromium is delivered to insulin-sensitive cells on the iron-binding transport protein transferrin.[603] In the cytosol, it is theorized that chromium complexes with apochromodulin, inducing a conformational change, which creates active chromodulin. Chromodulin is a protein that is rich in cysteine, glycine, glutamate and aspartate residues and tightly binds four ions of trivalent chromium. It is also referred to as low-molecular-weight chromium-binding substance (LMWCr), and is similar in structure to yeast glucose tolerance factor (GTF). Chromodulin dramatically increases the tyrosine kinase activity of the insulin receptor, thereby inducing downstream events stimulated by insulin. For example, chromium supplementation

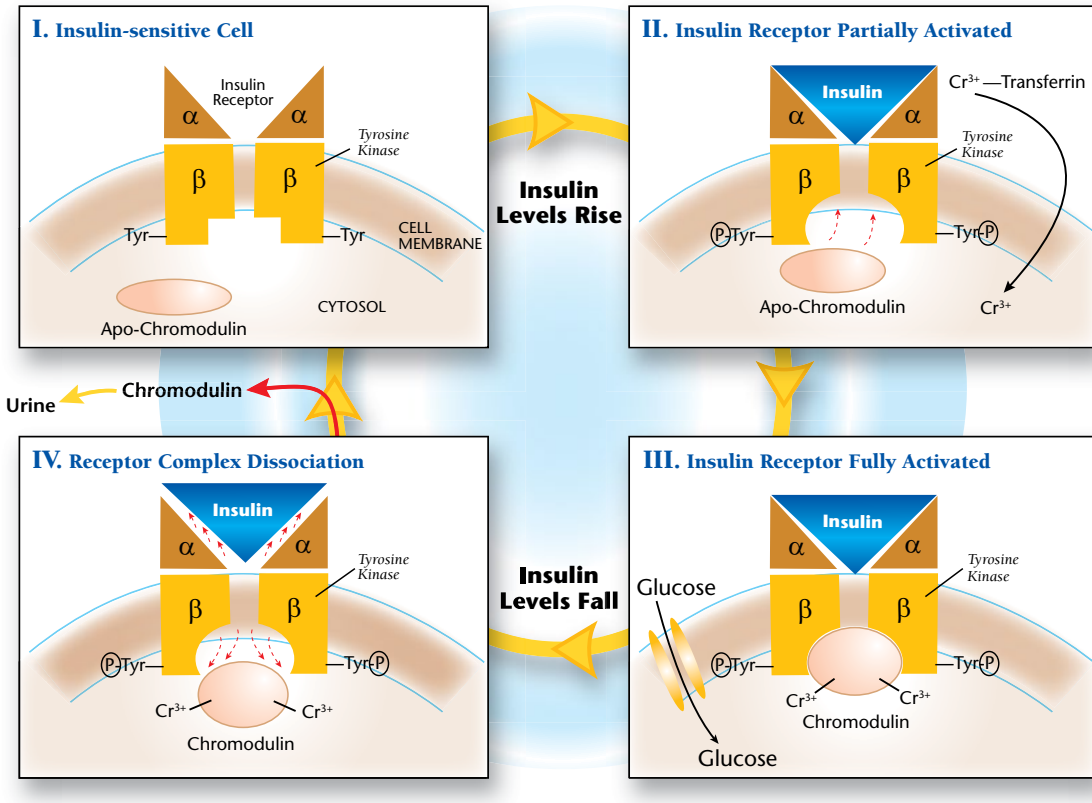


FIGURE 3.17 — Chromodulin at the Insulin Receptor Tyrosine Kinase

The rising and falling of blood insulin triggers actions mediated by chromium binding in insulin-sensitive cells. The inactive insulin receptor contains two α and two β subunits (I). Binding of insulin to the extracellular α subunits induces conformation changes in the cytosolic surfaces of the β subunits, activating their tyrosine kinase activity (II). The ensuing phosphorylation cascade activates chromium transport from plasma transferrin, resulting in the transformation of apo-chromodulin to chromodulin (III). The insulin receptor becomes fully active with chromodulin binding, inducing rapid transport of glucose and an ensuing fall of blood insulin concentrations. With the dissociation of insulin, the receptor ejects chromodulin (IV), a portion of which escapes the cell to appear in urine as an end product of the entire signaling cycle.

enhanced translocation to the plasma membrane of glucose transporter 4 protein in insulin-resistant animals.[603, 604] Once blood insulin levels drop, the insulin receptors undergo a conformational change that allows for the release of chromodulin, which is then apparently expelled from the cell and eliminated in urine. However, in cases of insulin resistance, with increased concentration of blood glucose and insulin, there is a paradoxical urinary wasting of chromium, most likely in the form of chromodulin.[598]

Since chromium is integral to insulin signaling, all insulin-mediated metabolic events improve with identification and correction of chromium insufficiency.[605, 606] Chromium-induced improvements have been demonstrated in type 2 diabetes, including improved lipid and carbohydrate metabolism, reduced blood insulin and glucose, and reduced body weight.[607] Chromium supplementation has shown efficacy with atypical depression, illustrating a link between blood sugar, insulin and mood.[608] Both gestational and steroid-induced diabetics have demonstrated positive response to chromium supplementation.[598] Chromium use in individuals exhibiting no blood sugar irregularities has no demonstrated beneficial effect.[598, 604]

Hexavalent chromium (Cr VI) is 1,000 times more toxic than trivalent chromium (Cr III). In addition to its carcinogenicity, topically, hexavalent chromium is an irritant, causing severe dermatitis. Cr VI is still widely used in industry, and is present in cigarette smoke, paint pigment, chrome plating, leather tanning, metal prostheses and copy-machine toner.[609] Microflora appear to participate in the reduction of hexavalent chromium to trivalent chromium, minimizing the effects of toxic exposure.[610] Contamination of ground water with hexavalent chromium, and the associated morbidity and mortality to residents of the area resulted in the largest settlement paid in a direct action lawsuit in US history and was the subject of the film *Erin Brockovich*.[611]

Jejunal absorption is inversely related to dietary intake, but is generally quite low, ranging from 0.5 to 2%.[601, 612] Similar to iron, chromium absorption is inhibited by phytates and enhanced by ascorbic acid. There appears to be competition by other elements, including iron, zinc, manganese and vanadium. Excretion is via both renal and fecal routes.[32] Chromium appears in urine primarily as chromodulin. There is a lag time between oral ingestion of chromium and appearance in urine, indicating incorporation into chromodulin prior to excretion. Elevation of glucose and insulin lead to increased excretion of chromium, as chromodulin.[598] The Fe-transport protein, transferrin, appears to maintain Cr^{3+} levels in the blood plasma and to transport Cr to tissues in an insulin-responsive manner.[598, 603]

Assessing Chromium Status

Total-body chromium is so low that analytical issues have limited accurate direct measures of the element.[598] However, instrumentation advances such as the addition of the dynamic reaction cell (DRC) filters for inductively coupled plasma mass spectrographic (ICP-MS) methods allow accurate detection of chromium in urine, serum and whole blood. The DRC adaptation removes interfering argon carrier gas atomic species, largely eliminating interferences that have compromised chromium measurements in the past.[423, 613-615]

While methodology has improved, chromium levels in states of insufficiency differ depending on matrix and physiological conditions, resulting in inconsistencies that appear to greatly complicate interpretation.[345, 598, 616, 617] Erythrocyte chromium has been used to assess excessive levels of exposure in workers exposed to chromate.[618] When exogenous chromium contamination is limited, hair continues to be a viable specimen option for establishing long-term chromium status.[619]

Given the difficulties with interpretation of direct chromium concentration measurements, functional evidence for dysglycemia, such as elevated blood glucose and insulin levels, or an abnormal glucose-insulin tolerance test can provide a functional assessment of chromium insufficiency.[345] Thus far, the reversal of symptoms with chromium supplementation is currently the only generally accepted indicator of chromium deficiency.[598, 616]

Since chromium is excreted as the insulin-stimulated metalloprotein chromodulin, urinary chromium presents a special situation, where levels may provide a type of functional assessment because of the high percentage that is excreted in the form of chromodulin. Further research into the interpretation of fasting and non-fasting urinary chromium levels is warranted. When exogenous chromium contamination is limited, hair continues to be a viable specimen option for establishing long-term chromium exposure.[619] An abnormal glucose-insulin tolerance test may provide a functional assessment of chromium insufficiency.[345]

Chromium Repletion Dosing

Chromium picolinate (200–1000 µg) is an effective supplementation for treating diabetes[620, 621] and weight gain from insulin insensitivity.[622] Chromium picolinate may function similarly to chromodulin.[604] The form derived from yeast called chromium-glucose tolerance factor (GTF), the glutathione-dinicontinate complex similar to the chromodulin complex, is not effectively absorbed.[32] The chromium-histidine complex appears to be highly bioavailable.[604]

COBALT (CO)

Dosage considerations: See B_{12} in Chapter 2, "Vitamins"
Clinical indications: B_{12} deficiency or pernicious anemia
Food sources: Internal organs, nuts, yeast extract

Cobalt (Co) is an essential trace element due to its well-known role in vitamin B_{12}, hence the name "cobalamin." One atom of cobalt is found at the center of the porphyrin-like corrin ring structure of B_{12}. Vitamin B_{12} is important in hematopoiesis and thyroid function.[32] The consequences of B_{12} deficiency are well known, including central nervous system complaints, pernicious anemia and potentially fatal macrocytic anemia (see Chapter 2, "Vitamins").

Animals are unable to incorporate cobalt into the vitamin, and therefore must eat the preformed vitamin. Since there is no RDA for cobalt, vitamin B_{12} status must serve as a surrogate marker. Intake of 1.5 µg/d of vitamin B_{12} supplies about 0.06 µg cobalt.[32]

Ingested cobalt is largely excreted in urine. Gastrointestinal absorption varies from 5 to 45%.[623] Factors such as iron status and the chemical form of cobalt appear to explain discrepancies reported for dietary absorption rates.[624, 625] Toxicity may occur at cobalt intakes above 300 mg/d, although even therapeutic doses at 29.5 mg/d have been associated with toxicity, including goiter, hypothyroidism and heart failure.[626] Use of cobalt salts in beer was banned because they may have caused cardiomyopathies.[627] Whole-blood cobalt was detected at approximately 0.17 µg/L in patients awaiting surgery.[628]

Notes:

ELEMENTS OF UNCERTAIN HUMAN REQUIREMENT

The elements in this section may be required for human function, but they have yet to gain general acceptance as nutritionally essential. A number of different criteria are used to establish essentiality. The simplest criteria require that the element be needed for optimal metabolic functioning, with deficiency resulting in suboptimal functioning. Accurate measurements of these compounds is possible with current laboratory instrumentation. In the proper forms, supplementation of these elements is reported to be of clinical benefit under various criteria, as discussed below.

BORON (B)

Dosage considerations: 12–20 mg/day
Clinical indications: Sterioid hormone modulation, bone health, prostate cancer
Food sources: Apples, soy, grapes, nuts

Boron (B) is gaining acceptance as an essential nutrient in humans, as it appears to fit the primary criteria of essentiality. Full recognition is likely forthcoming due to the discovery of an active boron-sodium co-transporter (NaBC-1) in kidney tubules and salivary gland acinar cells in mammals. Modest boron supplementation has been shown to stimulate mitogen-activated protein kinases (MAPK), inducing cell proliferation and growth. Aggressive boron supplementation has a paradoxical inhibitory effect on MAPK. MAPK activity has also been shown to be influenced by the presence or absence of NaBC-1 transporters, illustrating their regulatory role in boron homeostasis.[629-631]

Clinical Associations of Boron

One US epidemiologic study reported an inverse correlation between prostate cancer and boron intake.[629, 632] In animal studies, dietary boron supplementation reduced prostate tumor size and content of the tumor trophic factor IGF-1. It may be that the mechanism of IGF-1 reduction occurred via the reduction of prostate-specific antigen that is dramatically reduced by boron supplementation.[633]

In human and animal studies, boron has been shown to increase plasma steroid hormones, particularly 17-beta estradiol and testosterone. In vitamin

D-deficient animals, boron supplementation increases half-life of available vitamin D, thereby minimizing the onset of vitamin D deficiency symptoms. The putative mechanism behind both actions is inhibition of microsomal hydroxylation required for steroid hormone and vitamin D clearance.[634]

Although boron appears to have a regulatory role in 26 enzymes, including those involved in energy metabolism, none of them require boron as a cofactor.[635] Boron is a metalloid element with similar properties to silicon. Boron is best known for its role in bone health because of the effects on steroid hormones mentioned above. Boron markedly reduces urinary calcium and magnesium loss, as well as increases calcium absorption. In human studies, when compared with healthy bone, arthritic bone was associated with almost a 20-fold decrease in boron content.[636–637]

Boron toxicity resulted in testicular atrophy, decreased seminal volume, decreased sexual activity and stunted growth.[635, 638] Borax (meaning boron-containing compounds) and boric acid are well-known antimicrobials, and each has been used in laundry detergent and medicinals.[639]

Assessment of Boron Status

Serum and urine boron measurements have been employed to establish supplementation efficacy, rates of excretion and dietary intake.[635, 640-643] Hair has been used to screen for boron deficiency. However, as with any hair analysis, one must carefully consider external contamination, since many commonly used hygiene products contain boron, including shampoos.[177, 635, 640-645] Due to the poor understanding of boron biochemistry, and its lack of involvement as an enzymatic cofactor, functional markers have not yet been found.

Boron Supplementation

Boron is rapidly absorbed by the gut, likely as passive diffusion; boron is circulated systemically as free boric acid. Most is rapidly excreted in the urine. In mammals, there appears to be a small pool of storage boron, primarily in heart, spleen and bone. In human trials, boron has been used at supplementation levels of 1 to 11.61 mg/d, with 20 mg being the UL. Approximately 3 mg/d is the most commonly used dose. This level corresponds to the upper range of boron intake in food.[637, 646] Although modest boron intake is generally associated with improved clinical outcomes, high doses can produce stunted growth and impaired steroid hormone production.[635] Since boron is rapidly excreted in the urine, acute toxicity is rare, and doses up to 297 mg are tolerated.

The selenium deficiency disease known as Kashin-Bek is associated with severe lack of boron (and other elements such as molybdenum and germanium). Boron is suspected as an etiologic factor because Kashin-Bek disease produces a type of degenerative arthritis.[644, 645]

Nickel (Ni)

Dosage considerations: < 5 µg/day
Clinical indications: Unknown
Food sources: Foods cooked in stainless steel cookware

Total-body nickel (Ni) is 7 mg. In certain biological systems, nickel has been shown to occupy the catalytic site of superoxide dismutase, where it is involved in the reduction of the superoxide radical to hydrogen peroxide.[647, 648] Nickel is necessary for urease activity, a major virulence factor of the gastric pathogen *Helicobacter pylori*.[649] Considerable evidence exists to demonstrate the essentiality of this transition metal in animals. Deficiency of nickel is associated with poor growth and reproductive function, and has been shown to work in a cooperative way with calcium, iron and zinc. However, conclusive evidence that it is essential in humans is lacking.[650] A major reason for the difficulty is that nickel's function in man is not known. Food content of nickel is limited, although it can accumulate in foods, particularly those high in acid that are prepared in stainless steel utensils, due to the high nickel content of stainless steel. No RDA has been established, and required amounts are likely considerably less than 500 µg/kg body weight.[650] Average intake in the United States in dietary supplements is less than 5 µg/d. Nickel competes for GI absorption with iron,[32] and therefore may be regulated by the enterocyte divalent metal transporter protein. Nickel is transported in blood primarily bound to albumin, and excreted primarily in urine.

Chronic exposure to some forms of nickel via inhalation is carcinogenic. Mucosal tissue irritation associated with nickel includes asthma, rhinitis and sinusitis. Dermatitis is also associated with nickel worn as jewelry.[453, 651]

Assessing Nickel Status

Urine and plasma have been used for assessing nickel exposre, although only acute exposure is revealed because of the rapidity of nickel clearance from blood.[453] Hair nickel has been measured for assessment of nickel status, and may prove a useful biomarker for past exposure.[272, 652, 653] Hair nickel levels were shown to be elevated relative to controls in 71 nickel-sensitive women.[627]

LITHIUM (LI)

Dosage considerations: 400–1000 µg/day
Clinical indications: Psychosis, depression
Food sources: Vegetables, grains

Average daily dietary intake of lithium (Li) is 650 to 3,100 µg/d. Lithium stores are highest in the cerebellum, cerebrum and kidneys. Females generally concentrate 10 to 20% more than males. High-dose (average 900 mg) lithium carbonate has long been used to treat psychiatric disorders, most notably manic depression. In areas where water lithium levels are low, a concurrent increase in suicides, homicides and crime has been noted. Urine lithium levels showed significant inverse correlation with neurosis, schizophrenia, all psychiatric admissions and homicide, in decreasing order of magnitude. One study showed that 400 µg/d in former drug users caused an increase in total positive mood scores, whereas the placebo group showed no consistent change.[654] In lithium-deficient animals, behavioral and reproductive abnormalities were present. Lithium has been shown to impact several enzymes, including monoamine oxidase and tyrosine hydroxylase, as well as some hormones and vitamins, and has a putative role in modulating electrolyte distribution. Lithium increases GABA activity and modulates the serotonin indoleamine pathway, thus impacting serotonin and melatonin and modulating circadian rhythms. It has also been shown to increase the anti-inflammatory eicosanoid PGE_1. Lithium is important during fetal development, as evidenced by the high amount present in the embryo.[654] Lithium has also been shown to enhance bone formation and improve bone mass in mice through stimulation of the Wnt signaling pathway.[655, 656] A provisional limit of safe oral dosing for lithium of 1,000 µg/d was proposed by Schrauzer.[654]

At the supraphysiologic doses used to treat psychosis, lithium has a narrow therapeutic range, and toxicity may occur. Lithium toxicity may be life threatening, or result in persistent cognitive and neurological impairment. Long-term use of lithium carbonate for psychosis has also been associated with thyroid suppression and increased alopecia.[657] Serum lithium must therefore be closely monitored.[658]

Assessing Lithium Status

In healthy individuals, RBC lithium is greater than plasma lithium, with an RBC-plasma ratio of 1:57. However, in patients being treated with supraphysiologic doses of lithium, plasma levels are greater than in RBCs. Urine has been used to establish lithium deficiency. Hair lithium levels have been shown to correlate with total-body content. Numerous studies have shown that lower lithium values correlate with increased violent criminality, learning disabilities and heart disease. Students in high academic standing had greater hair lithium levels than average.[627, 659] Low hair lithium was demonstrated in young children with autism.[619]

VANADIUM (V)

Dosage considerations: 9–250 µg
Clinical indications of deficiency: Insulin insensitivity, abnormal blood lipids
Food sources: Buckwheat, parsley, soybeans, safflower oil

Approximately 2%, or 5 to 10 µg/d of dietary vanadium (V) is absorbed in the small intestine.[32] Absorbed free vanadium is rapidly excreted in the urine. Iron-binding proteins such as transferrin and ferritin may bind vanadium,[32] and other cellular binding sites are likely in the organs where vanadium is found: kidneys, bone, liver, spleen and bone.[135, 660] Neither RDA nor AI (adequate intake) has been established for vanadium because little is known about its role in human biochemistry. However, supplementation of vanadium has ranged from 9 µg in multielement formulas to 125 mg/d as vanadyl sulfate in diabetic patients.[32]

Clinical Associations of Vanadium

Vanadium deficiency has not been established in humans, but in animals it can lead to increased abortion, diminished lactation, poor growth, thyroid changes and changes in hepatic lipid profiles.[661] Some studies suggest vanadium has a role in glucose and lipid metabolism, red blood cell formation and thyroid function.[135]

Vanadium assessment and treatment may be indicated in cases of metabolic syndrome or non-insulin-dependent diabetes mellitus (NIDDM) due to its role in insulin sensitization.[662] Vanadium has been suggested to alter blood lipids in patients with high cholesterol.[135]Symptoms that appear to be associated with excessive vanadium are hypertension, decreased coenzymes A and Q_{10}, bipolar disorder,[663] and disruption of energy metabolism.[135] Exposure to vanadium in air or water is of concern for those working in or living near vanadium plants, and symptoms may include respiratory disorders,[664] green tongue, high urinary excretion of vanadium,[665] and reduced neurobehavioral abilities.[666]

Optimal forms of vanadium include sodium metavanadate[662] and vanadyl sulfate.[32]

Assessment of Vanadium Status

Vanadium is difficult to accurately measure due to its low concentration and poor reference materials.[667] Urine vanadium is traditionally used to assess occupational work exposure.[664, 668] An upper limit of 0.5 µg/L has been proposed for urinary vanadium.[667] Whole-blood vanadium has been shown to significantly differentiate children with vanadium exposure (median 0.078 µg/L) from non-exposed children (median 0.042 µg/L), but authors stated that hair levels of vanadium did not significantly correlate with vanadium exposure.[669] This may have to do with distinction of acute versus chronic exposure. Estimated normal values for blood and serum vanadium are 1 nmol/L.[667]

Strontium (Sr)

Dosage considerations: 125–680 mg
Clinical indications of deficiency: Osteopenia, osteoporosis
Food sources: Green leafy vegetables, brazil nuts

Total-body strontium (Sr) is about 320 mg, with 98% residing in bone. Daily dietary intake is about 1 to 5 mg. Strontium possesses a larger atomic mass than calcium, although the elements exhibit similar properties.

Clinical Associations of Strontium

Strontium incorporates into hydroxyl crystal lattice of bone, stimulates new cortical and cancellous bone formation, and decreases bone resorption by inhibiting osteoclastic activity. These effects are shown to occur during strontium supplementation only and decrease after the cessation of therapy.[670-672] The efficacy of strontium supplementation for improving bone status and reducing fracture risk in osteoporotic individuals has been demonstrated in many well-designed human and animal trials[670-681] using the synthetic chelate strontium ranelate. Animal studies using low-dose strontium demonstrated increased bone formation and volume, without adverse effects on bone matrix.[676] Although a detailed discussion extends beyond the scope of this text, Table 3.8 summarizes efficacy of strontium therapy as compared with common pharmaceutical interventions, including calcitonin, bisphosphonate, PTH and hormone replacement therapy on bone mineral density (BMD) and

Table 3.8 — Effectiveness of Strontium and Pharmaceutical Treatments for Osteoporosis[673] *

Intervention	BMD Vertebral	BMD Hip	Vertebral Fracture	Hip Fracture	Peripheral Fracture
Etidronate	↑	↑	↓	↓ or NS	NS
Risedronate	↑	↑	↓	↓	↓
Alendronate	↑	↑	↓	↓	↓
HRT	↑	↑	↓	↓	NS
Calcitonin	↑	↑	↓	NS	NS
Raloxifene	↑	↑	↓	↓ or NS	NS
PTH	↑↑	↓ (cortical bone)	↓	NS	↓
Fluoride	↑↑	NS	↓	NS	↑
Strontium	↑↑	↑	↓	↓	↓

** Up arrows indicate increased bone mineral density (BMD). Down arrows indicate decreased fracture rate. NS indicates that no significant change was found.*

Of Further Interest...

A few caveats regarding strontium therapy should be considered. Strontium competes with calcium for GI absorbtion.[677] Vitamin D will initially improve strontium absorption,[678] but high-dose strontium inhibits vitamin D formation and induces depletion of the vitamin D pool in animal studies.[682, 683] Therefore, human studies routinely add vitamin D and calcium to control and treatment groups. Whereas recent research has been focused on strontium ranelate, earlier research on natural strontium salts also demonstrated efficacy, including strontium gluconate, chloride, citrate, lactate and carbonate.[673] All forms of strontium have bioavailabilities in the 25 to 30% range, but gastric tolerance appears to be better with the ranelate and citrate forms.[673]

High doses or long-term therapy with strontium salts has definite risk of inducing nutrient depletions, as well as the potential for weakening the bone strength. Another caveat is that strontium will falsely attenuate bone mineral density when dual-energy x-ray absorptiometry scan is used to evaluate BMD during strontium therapy. Other assessment tools, including calcium and vitamin D assessment and bone resorption markers, should be concurrently employed.

fracture occurance.[670-681] There are many nutrients associated with improved bone density, including a number of elements discussed in this chapter, such as calcium, magnesium, copper, boron and possibly lithium. Further discussions can also be found in Chapter 2, "Vitamins" and Chapter 4, "Amino Acids."

Strontium Toxicity

There are a number of stable isotopes of strontium, including ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr. Radioactive strontium, ^{90}Sr, is a nuclear waste product and a human carcinogen. Cancer incidence (primarily bone and bone marrow) in children under age 10 years living near New York and New Jersey nuclear plants showed there is a short latency period between radiation exposure and cancer onset.[684] Strontium has also been shown to impact reproduction in animal studies. The half-life of strontium-90 is 29 years, during which it constantly emits potentially damaging radiation. During the decay process, strontium emits beta particles to become radioactive yttrium-90 that continues to emit radiation.[609]

Assessment of Strontium Status

Serum strontium levels have been evaluated during therapy to establish GI absorption.[670] Urinary calcium and serum vitamin D can be used to monitor effects on these nutrients. Hair strontium is commonly measured, although there is little evidence linking it to bone levels.[685-688] Strontium has been shown to concentrate in hair with increased environmental exposure.[689] Bone resorption tests such as deoxypyridinoline, pyridinoline, bone-specific alkaline phosphatase or C-telopeptide of type I collagen may be used to establish reduction in bone resorption due to strontium therapy.[672,679, 690] Interpretation of DEXA results must allow for the false attenuation of strontium.

Toxic Elements

Although the toxic elements, including cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg), have always been present in the crust of the earth, their use by humans has resulted in exposure levels above those compatible with optimal biological function. Specifically, industrialization over the past 200 years has increased emissions of toxic elements much greater than from natural sources. Due to the mobilization into the biosphere, human exposure to toxic metals in soil, air and water has increased. Entry into the food chain via accumulation by crop plants and fish are also common pathways for exposure.[691, 692] Other sources of exposure include medically approved mercury amalgams and the mercury-containing medicines such as the vaccine preservative Thimerosal.[693]

Although much has been done to successfully reduce lead exposure, recent research has demonstrated that blood lead levels much lower than the current CDC acceptable level of 10 µg/dL are associated with toxicity.[694] Cumulative effects from co-exposure to multiple toxic metals can amplify toxicity, such as the increased renal dysfunction produced by combined exposure to arsenic and cadmium, or the increased CNS deficits in the presence of lead and arsenic.[556, 695,696]

Essential element deficiencies can exacerbate toxic exposures through numerous mechanisms. Not only may the toxic metals be more readily absorbed via the essential element transport proteins (present in the gastrointestinal tract and CNS)—which are generally increased in numbers during deficiency states—enzymes that lack essential element cofactors are more vulnerable to toxic interference as well.[98] Essential element protection against toxic element effects, such as selenium's ability to bind and inhibit the toxicity of both mercury and arsenic, is reduced in deficiency states.[101, 556]

General Mechanisms of Metal Toxicity

An important difference between toxic organic compounds and toxic metals is that although organic compounds can be chemically destroyed by exposure to heat, UV light, or chemical or microbiological attack, toxic metals are virtually indestructible. The only recourse for the disposal of environmental toxic metals is to bind them to ligands that alter their toxicologic and environmental behavior, sequestering them from re-entry into the environment. In the human body, a similar, poignant point is that toxic metals tend to accumulate undetected in the body over time. The insidious rise in biological levels or body burdens of toxic metals eventually threaten normal body processes, and, in many individuals, become a clinically relevant issue. Stressful episodes can become triggers that bring latent toxic metabolic inhibitions or destructive challenges into active symptoms.

The toxicity of an element depends on its physiochemical properties. Those properties that increase the absorption and transport of a toxic metal (i.e., its bioavailability) will determine its ability to reach one or more target sites within the body. Targets include sites such as membrane or structural proteins, enzymes, membrane lipids or DNA. Once at the target site in sufficient concentration, the metal may substitute for or otherwise displace an essential element from its binding site. This substitution is called "molecular mimicry."[697] Yet, because it does not share the exact same chemical properties of the displaced mineral, the toxic metal fails to substitute for its function. A more sophisticated discussion of this "foreign-coin-jams-the-juke-box" paradigm is beyond the scope of this book. Nonetheless, it provides a conceptual framework for understanding an important underlying mechanism for metal toxicity.

Three other mechanisms of toxicity deserve special attention, particularly in the context of metals as neurotoxins: (1) inorganic metal transformation to an organic species, (2) sulfhydryl group binding, and (3) free radical generation. The first mechanism determines the abililty of a toxic metal to penetrate compartments in the body and is an important factor in determining bioavailability. Biological systems have the ability to add methyl groups to metals, which serves to make the metals more soluble in lipid environments. A lipid-soluble metal is many times more neurotoxic than aqueous forms because it can penetrate lipid membranes and concentrate in lipid-rich tissue such as the brain. The second mechanism revolves around structure-function changes that occur separately from molecular mimicry. Many toxic metals (in particular, lead, mercury and arsenic) avidly form covalent bonds with the sulfhydryl groups of cysteine amino acids belonging to proteins. The formation of such complexes can lead to structural changes to the detriment of the protein's function. The third mechanism, free radical generation, involves the formation of highly reactive oxygen species having unpaired electrons. Unchecked formation of free radicals leads to lipid peroxidation, cellular membrane degeneration and ultimately to cell death (see Chapter 8, "Toxicants and Detoxfication"). Many metals are potent catalysts of free radical reactions. Though cells have evolved defensive mechanisms to guard against free radical formation or to repair the damage caused by them, metals present in sufficient concentrations will promote formation of enough free radicals to saturate these defenses.

Notes:

TOXIC METAL ASSESSMENT

One of the first actions to consider when treating the toxic metal-exposed patient is to reduce exposure. Table 3.9 lists common sources of exposure beyond the obvious ones of living or working near industries where the metals are mined or processed. The clinician quick-reference table at the top of each section provides further assessment tools, including toxicity symptoms, body burden assessment, protective measures, suggested chelating agents and specific sources of exposures.[2]

Each section on toxic or potentially toxic metals includes a discussion on direct and functional markers for assessment of body burden. (The section "Element Status Assessment" at the beginning of this chapter is relevant to specimen choices for toxic element testing.) As with the essential elements, more than one assessment tool may be required to gauge both degree and impact of the toxic metal burden. For instance, as discussed in Chapter 8, "Toxicants and Detoxification," porphyrin abnormalities can establish whether a toxic element functional impairment is present, whereas measuring direct elemental concentrations in body fluids can identify which toxicant is present.

UNIT CONVERSIONS

Commonly Used Conversions in Toxic Metal Assessment

- **ppb = ng/ml**
- **ppm = µg/mL or mg/L**
- **ppb/10 = µg/dL (primarily used for lead assessment)**
- **1ppb = 0.001 ppm**

Notes:

ALUMINUM (AL)

Toxicity symptoms: Abnormal speech, myoclonic jerks, osteomalacia, progressive encephalopathy, Alzheimer's disease, Parkinson's disease

Assessment: Whole blood, serum, hair, urine

Biochemical marker: Total porphyrin elevation

Protective measures: Iron, calcium, phosphorus (lowers intestinal absorption)

Chelating agents: DFO

Common sources: Aluminum cookware, antacids, tobacco and cannabis smoke

After oxygen and silicon, aluminum (Al) is the most abundant element in the crust of the earth. Exposure to aluminum is ubiquitous via food, water, air and soil,[698] and aluminum is used to produce beverage cans, cooking pots, siding, roofing, aluminum foil and airplanes. Further, it is found in antacids,[699] buffered aspirin, food additives (especially in grains and cheeses),[700] astringents, vaccinations,[698] cat litter, antiperspirants, infant formula and baking soda. Aluminum has been detected in calcium, magnesium, and phosphate salts used in parenteral solutions,[701] and it has been found high in lung tissue as a result of tobacco and cannabis smoke inhalation.[702] When aluminum is used in water treatment facilities, concentration in community water supplies can reach 93 µmol/L (2,500 µg/L).[703]

Aluminum is absorbed in the acidic environment of the stomach and in regions of the proximal section of the intestine.[704] It is said that 95% of aluminum binds serum proteins, 70 to 90% of which binds transferrin.[295,704] Biliary excretion is normally the major pathway of aluminum elimination.

Aluminum absorption and its toxic effects can be mediated by diet and by other elements. Common dietary acids (such as citrate and malate) can increase aluminum absorption,[699] especially in the brain[705] and bone.[705] People with low calcium can have increased aluminum absorption, and calcium has been recommended to reduce aluminum exposure in post-menopausal women.[706] Urinary and plasma aluminum levels were found to increase significantly during supplementation with calcium citrate, apparently due to enhanced aluminum absorption.[707] Calcium deficiency in rats made them more vulnerable to aluminum transportation to the neurons and to aluminum neurotoxicity.[708]

Although trace levels of aluminum may not be of concern for most adults, aluminum exposure during infancy and adolescence is a different matter because of

Table 3.9 — Sources of Toxic Elements

Aluminum	Cadmium	Lead	Mercury	Arsenic
Aluminum cooking utensils	Drinking water	Some red lipsticks and painted toys	Dental amalgams	Rat poisons
Baking powder (Al sulfate)	Soft water (from galvanized pipes)	Leaded house paint	Broken thermometers and barometers	Automobile exhaust
Antacids (certain brands, see labels)	Soft drinks from dispensing devices with galvanized plumbing	Drinking water from lead plumbing	Grain seeds treated with methylmercury fungicide	Household detergents
Antiperspirants	Refined wheat flour	Vegetables grown in Pb-contaminated soil	Predator fish, certain lake fish	Wood preservatives
Aluminum cans	Canned evaporated milk	Canned fruit and juices, canned evaporated milk	Mercuric chloride Thimerosal (Ethylmercurithiosalicylic acid sodium salt)	Insecticide residues on fruits and vegetables
Drinking water (alum used as bactericide)	Processed foods	Certain chinese and aruveydic herbal preparations, boxed wines	Calomel (body powder, talc, laxatives)	Wine (if arsenic in pesticides used)
Milk and milk products (from equipment)	Oysters, kidney, liver	Milk from animals grazing on Pb-contaminated land	Cosmetics (check labels if possible)	Colored chalk
Alum in pickled foods (check labels)	Cigarette smoke, tobacco products	Bone meal	Latex and solvent-thinned paints	Wallpaper dye and plaster
Nasal spray	Superphosphate fertilizers	Organ meats such as liver	Hemorrhoid suppositories	Drinking water, seawater, well water
Toothpaste	Dental appliances	Lead-arsenate pesticides	Mercurochrome, merthiolate	Sewage disposal
Ceramics (made from Al 203 clay)	Ceramic glazes	Leaded caps on wine bottles	Fabric softeners	Seafood (source of non-toxic arsenic)
Dental amalgams	Paint pigments	Rainwater and snow	Floor waxes and polishes	Chicken
Cigarette filters and tobacco smoke	Electroplating	Improperly glazed pottery	Air conditioner filters	
Automotive exhausts	Silver polish	Painted glassware	Wood preservatives	
Pesticides	Polyvinyl plastics	Painted pencils	Certain batteries	
FD&C color additives	Rubber carpet backing	Toothpaste	Fungicides for lawns and shrubs	
Vanilla powder	Nickel-Cadmium batteries	Newsprint	Leather tanning products	
Table salt, seasonings	Rust-proofing materials	Colored printed materials	Thimerosal-preserved immunization fluids	
Bleached flour		Eating utensils	Adhesives	
American cheese		Curtain weights	Skin-lightening creams	
Medications containing kaolin (Al silicate)		Putty	Certain ointments to treat psoriasis	
Sutures with wound-healing coatings		Car batteries	Photoengraving	
Rat poisons		Cigarette ash, tobacco	Tattooing	
		Lead shot, firing ranges	Sewage sludge used as fertilizer	

its tendency to accumulate over time. Compared with breast milk, cow's milk and some infant formulas have 2- to 3-fold and up to 10-fold higher levels of aluminum, respectively.[709] Aluminum in vaccines may be a significant source of aluminum exposure in infants.[710]

Ingestion of aluminum from water and food sources is typically 5 to 10 mg/d for adults.[700] This amount of aluminum far exceeds the safety dose of lead, mercury, cadmium and arsenic. Although many people tolerate aluminum well, aluminum in high doses can cause health problems for susceptible individuals.

Clinical Associations of Aluminum Toxicity

The major tissue sites of aluminum toxicity are the nervous system, immune system, bone, liver, and red blood cells, and aluminum may interfere with heme (porphyrin) synthesis.[711] The greatest information on human aluminum toxicity comes from patients undergoing dialysis or total parenteral nutrition (TPN). Dialysis treatments for patients with kidney failure have led to intoxication due to aluminum-contaminated dialysis fluids,[712] and children treated with phosphate binders have developed aluminum intoxication.[713]

Consequences of aluminum toxicity are encephalopathy[701] and abnormal speech, myoclonic jerks, convulsions, and a predisposition to osteomalacic fractures. Aluminum interferes with enzymatic activity measured in blood, liver and kidney.[714] Aluminum toxicity is accompanied by anemia. Aluminum overload in dialysis patients caused microcytic anemia[715] and decreased serum ferritin and hemoglobin.[716] Interference in the porphyrin pathway enzymes is indicated by the development of porphyria cutanea tarda in hemodialysis patients.[717] Dermatitis after vaccinations has been attributed to aluminum-containing vaccines.

Occupational exposure to aluminum(III) increases the characteristic neurofibrillary formation leading to Parkinson's disease.[718] Increased exposure levels also have strong etiological implications for neurological disorders, particularly Alzheimer's disease. Although it is not proven that aluminum is the causative agent,[719] it is striking that aluminum accumulates in the neurofibrillary tangles of neurons that characterize the brains of Alzheimer's patients (as well as in patients with amyotrophic lateral sclerosis and Parkinson's and Huntington's diseases).[720] In vitro studies show that exposure of neurons to aluminum causes apoptosis[721,722] and disruption of mitochondrial transport to the synapse in neurons.[723]

High blood levels of aluminum in individuals without renal disease were associated with hypertension when compared with controls.[724] In patients with multiple sclerosis, urinary aluminum levels were found to be as high as if the patients were aluminum toxic and undergoing chelation.[725]

Aluminum exposure affects catecholamine metabolism and behavior. In a study of 33 workers exposed to aluminum and 40 controls, urinary aluminum was significantly higher (12.2 µg/L) than controls (5.78 µg/L), whereas serum aluminum was unchanged. The group with aluminum exposure had higher vanilmandelic and homovanillic acids in urine (see Chapter 6, "Organic Acids") and were significantly different from controls on neurobehavioral tests.[726] Aluminum administered to rats with diets low in copper, zinc or iron decreased brain levels of catecholamines.[727]

Aluminum replaces calcium in bone, disrupting normal osteoid formation and mineralization. In hyperaluminemic individuals (serum ≥ 5 µmol/L or 135 µg/L), 67% had either osteomalacia or aplastic bone lesions, and a subgroup (n = 21) of the individuals with high aluminum had significantly increased bone resorption and decreased bone formation.[728] Aluminum interrupts calcium exchange, preventing renal resorption of calcium. Aluminum effectively interferes with the normal physiologic control of parathyroid hormone, and therefore may impact the conversion of vitamin D to its active form.[729, 730] Dialyzed or TPN patients have decreased serum calcitriol. After aluminum removal, calcitriol concentrations returned to normal.[731] Aluminum may also lead to hypercalciuria and osteopenic bone disease in premature infants on TPN.[701]

Aluminum intake may negatively affect intestinal microbiota. Horses consuming high aluminum forage can develop histological similarities to Crohn's disease, leading to a fatal granulomatous enteritis, suggesting that exposure of intestinal bacteria to aluminum may lead to increased pathogenicity.[732]

Status of other elements appears to affect the vulnerability to neurotoxic effects of aluminum. Iron deficiency predisposes laboratory animals to higher aluminum absorption and, conversely, aluminum decreases absorption and uptake of iron. Administration of aluminum alone produces increased free erythrocyte protoporphyrins and decreased iron concentrations in RBC, spleen and blood. When iron was given with aluminum, serum aluminum decreased.[704] In pregnant rats fed oral

aluminum, urinary aluminum, manganese and copper were higher than controls. Aluminum concentration was found high in the liver and kidney and distribution of essential minerals, in tissues such as bone, brain, liver and kidney, were altered in aluminum-treated rats.[6] Therefore, assessing copper, zinc and iron status helps to determine a patient's vulnerability to the toxic effects of aluminum, and appropriate elemental treatments may help to overcome aluminum toxicity.[733]

Assessing Aluminum Body Burden

Because of the ubiquitous nature of aluminum on particles suspended in air, specimen contamination must be avoided by collecting and processing specimens with great care. Special evacuated blood collection tubes are required for testing aluminum in blood. Blood collected in the standard evacuated blood tubes may be contaminated by as much as 0.5 µg of aluminum from rubber stoppers containing aluminum silicate.

Problems with measuring erythrocyte aluminum include insuring an aluminum-free anticoagulant.[715] Erythrocyte aluminum has been measured,[715, 734] but serum aluminum levels above 5 µmol/L (135 µg/L) are predictive of aluminum toxicity.[735-737] In animals, 2 weeks of 100 mg/kg aluminum chloride administration did not affect serum aluminum levels.[704] Symptoms of anemia in dialyzed patients were found with serum aluminum levels over 60 µg/L prior to skeletal and neurological dysfunction.[738-740]

One study of dialysis patients and controls showed that RBC aluminum correlated with plasma aluminum. RBC and plasma aluminum were inversely correlated with mean red cell volume.[715] However, other authors have shown that high plasma aluminum precedes erythrocyte elevations.[741] Testing for anemia is indicated in patients with high RBC, plasma or serum aluminum. Higher plasma aluminum is seen in infants fed soy formula compared with breast-fed infants.[742]

Whole-blood aluminum has been used to evaluate aluminum levels,[743] and one study showed that serum and blood were comparable. Whole-blood concentrations of controls were 12.1 µg/L, and 79 µg/L in dialysis patients.[744] Very high aluminum levels corresponded in some patients to dialysis encephalopathy and/or osteodystrophy.[745]

Hemodialyzed patients (n = 39) were found to have hair aluminum of 226 nmol/g, compared with 126 nmol/g for controls (n = 49).[746] Aluminum injections into rabbits showed a dose-dependent relationship with hair aluminum concentration.[747]

Urinary aluminum can provide information about aluminum intake and has been used to monitor humans exposed to dangerous amounts of aluminum.[748-750] Aluminum is also eliminated by the fecal route. The porphyrin enzyme uroporphyrinogen decarboxylase was unaltered in aluminum-exposed workers, but other effects on the pathway were manifest as elevated total porphyrins in urine.[751, 752]

Management of the Aluminum-Toxic Patient

Desferrioxamine (DFO) is a chelator of aluminum and iron that has been used to treat acute aluminum toxicity. In fact, cases of aluminum toxicity are managed similarly to iron toxicity.[2] DFO decreased RBC and plasma aluminum and improved hemoglobin, hematocrit and mean cell volume in 13 patients.[715] Chelation treatment with desferrioxamine should be handled conservatively due to the risk of inadvertently mobilizing large amounts of aluminum to the brain, which may enhance encephalopathy or a chemical interaction.[295] The chelator, L1 (1,2-dimethyl-1,3-hydroxypyrid-4-one, deferiprone or DMHP) is also used to safely lower aluminum total-body burden.[2, 753] Ascorbate combined with DFO has been used to remove aluminum from human brain cells.[754] As discussed above, it is important to assure adequate status of essential elements (calcium, iron, copper, zinc). Silicon is an antagonist of aluminum.[725] Further testing of an aluminum-toxic patient might involve measurement of bone resorption, urinary catecholamines, oxidative stress, and even vitamin D.

Notes:

ARSENIC (AS)

Toxicity symptoms: Peripheral arteriosclerosis ("blackfoot disease") "rice-water" stools, proteinuria, hyperkeratosis, "milk and roses" hyperpigmentation, garlic breath odor, stomatitis

Body burden assessment: Urine, hair and nails, whole blood, urinary porphyrins

Protective measures: Selenium, *Emblica officinalis*, sulfur amino acids, glutathione

Chelating agents: DMSA, DMPS, DMPA

Common sources: Metal foundry, drinking water, seafood, glues, industrial exposure, contaminated wine, contaminated herbal supplements, cigarette smoke, arsenic-treated wood

Arsenic (As), infamous for its use in homicide by poisoning, exists in toxic and non-toxic forms. The toxic forms are the inorganic species of arsenic, As-III and As-V. Preserved "pressure-treated" wood contains appreciable amounts of the toxic forms of arsenic, as do many insecticides. The organoarsines arsenobetaine and arsenocholine are non-toxic forms of arsenic that are present in many foods, especially shellfish and other predators such as cod and haddock in the seafood chain. The EPA has set limits on arsenic levels in drinking water to 0.01 parts per million, whereas industrial exposure to workers is limited to 10 µg arsenic per cubic meter of air for an 8-hour work day.

Arsenic expresses its toxicity by: (1) binding with sulfhydryl groups, which causes distortion of protein structure and loss of enzyme activity; (2) tightly binding dihydrolipoic acid, a necessary cofactor for keto acid dehydrogenases; and (3) competing with phosphate for binding to ATP during its mitochondrial synthesis, decreasing phosphorylation of ADP.

Clinical Associations of Arsenic Toxicity

Lethal arsenic poisoning is characterized by toxic hepatitis and pancreatitis, neurological dysfunction, respiratory difficulty, renal failure and cardiovascular disturbance.[755] Exposure to arsenic-laden drinking water can induce symptoms of gastroenteritis and lead to cancer, diabetes,[756] and neurological and vascular dysfunction. Arsenic can cause skin neoplasm and dermatosis.[757] On the other hand, it has been proposed that too little arsenic is problematic; it depresses growth and interrupts normal reproduction in animals.[32, 758, 759]

Arsenic binds sulfhydryl groups and its oxidative effects deplete intracellular glutathione and may disrupt other sulfhydryl-containing enzymes and proteins.[760] Cysteine, glutathione and metallothionein are protective against arsenic exposure,[761] whereas low sulfur amino acid status (especially methionine) potentiates arsenic-induced cytotoxicity.[762]

Arsenic has been linked to increased risk of cancers of the skin, lung, bladder, liver, kidney and prostate. Oxidative damage induced by arsenic has been postulated to be a cause of carcinogenesis. In 28 patients with arsenic-induced skin neoplasm, 78% had significantly higher oxidative damage to DNA, measured by urinary 8-hydroxy-2′-deoxyguanosine (8OHdG) when compared with 11 controls.[763] There was a significant relationship between urinary arsenic species and 8OHdG in 134 people chronically exposed to well water high in arsenic (see Chapter 9 "Oxidant Stress and Aging").[757] Arsenic was shown to be a major cause of blackfoot disease and apparently antagonizes selenium and iron as the disease progresses.[764] In a study of apolipoprotein E-deficient mice fed drinking water with 20 to 100 µg arsenic/mL, the intimal area of the aorta had lesions, and interleukin-8 was one of many inflammatory mediators whose gene expression was upregulated.[765]

Assessing Arsenic Body Burden

Urinary arsenic is most commonly measured to screen for arsenic exposure because levels reflect intake from all sources.[766] Urine reflects arsenic exposure in the few days prior to specimen collection.[698] Urinary inorganic (toxic) arsenic peaks at about 10 hours and returns to normal 20 to 30 hours after ingestion. Non-toxic organic arsenic is completely excreted in 24 to 48 hours after ingestion. Patients should abstain from eating seafood for 48 hours prior to testing because of the high content of non-toxic organoarsines in most seafood. If unsure, the clinician should rule out ingestion of seafood in cases of borderline or moderate arsenic elevation before implementing aggressive therapy. Specialized laboratory methods of separation are required to differentiate non-toxic organic forms from the toxic inorganic forms of arsenic.[767]

Increased urinary arsenic levels were found in children living near copper smelters or due to arsenic in drinking water.[768, 769] Steel workers showed significantly higher urinary arsenic (and mercury and selenium) than unexposed controls.[770] Compared with controls, urinary arsenic was higher in children living with elevated water and dust levels (4.75 µg/100 mL vs. 1.17 µg/100 mL). High urinary arsenic correlated with tap water intake (as opposed to bottled water intake) and with distance from the copper smelter.[769]

Hair or nail arsenic represents several months of exposure prior to taking the specimen.[695] The concentration of arsenic in hair is typically greater than in other tissues because of its high affinity for keratin, which is rich in cysteine residues. Arsenic circulating in blood binds keratin in hair or nails, trapping it at the time of exposure. When arsenic exposure is removed, hair levels in new growth return to normal within weeks.[627] In one study, hair, nail and urinary arsenic levels were comparable.[771] Hair arsenic greater than 1 µg/g dry weight has been taken to indicate excessive exposure.[772] Arsenic ingestion is associated with arsenic content in hair,[773,774] but that has been disputed.[769]

Arsenic is rapidly cleared from the blood, as it combines readily with sulfhydryl groups of tissue proteins. Since elevated arsenic in the serum drops within 6 to 10 hours, serum arsenic is not a good specimen for determining chronic arsenic exposure. Whole blood is suitable for identifying acute exposure to arsenic, and high levels should be addressed immediately.[775] There is a paucity of information on erythrocyte arsenic in the scientific literature.

Refer to Case Illustration 3.8

Urinary porphyrin profiling provides functional markers of arsenic poisoning effects in the porphyrin pathway used for heme biosynthesis. Single-dose arsenic exposure of rats produces increases in protoporphyrin IX, coproporphyrin III and coproporphyrin I in tissues and urine.[776, 777] Mice exposed to arsenic in drinking water show a dose-response relationship with urinary coproporphyrin I after 12 months of exposure, along with less dramatic increases of coproporphyrin III.[778, 779] Fecal coproporphyrin I showed a specific positive response to arsenic exposure in geese.[780] Drinking-water arsenic exposure in rats produced multiple abnormalities in the porphyrin pathway. Delta-aminolevulinic acid dehydratase (ALAD) activity was inhibited along with increase of zinc protoporphyrin (ZPP) in blood.[781] Human population survey data confirm that both uroporphyrin III and coproporphyrin III increase in arsenic-exposed individuals.[782]

Management of the Arsenic-Toxic Patient

The arsenic antidotes DMPS and DMSA have been favored because they demonstrate relatively low toxicity with high therapeutic index, and they can be administered either orally or intravenously. However, intravenous DMPS can be quite toxic to the kidneys. Dimercaprol (BAL) appears to be useful in acute poisoning cases, but has been criticized for its low therapeutic index, painful administration, odor and redistribution of arsenic to brain and testes. DMSA and DMPS, on the other hand, are better for chronic arsenic poisoning.[783]

D-Penicillamine (DPA) is also used in arsenic chelation, whereas N-acetyl cysteine (NAC) and monoisoamyl DMSA (Mi-ADMS) are still in experimental stages.[2] N-(2,3-dimercaptopropyl)phthalamidic acid (DMPA) is reported to be effective.[784] In acute arsenic poisoning, intravenous and intraperitoneal DMSA (10–20 mg/kg/d) has been used as well as venovenous hemofiltration, hemodialysis and peritoneal dialysis.[755] In a patient who attempted suicide by consuming ~600 mg arsenic trioxide, DMPS treatment resulted in the first urine void containing 215 mg/L, which decreased 1,000-fold after 8 days of treatment, at which time the patient was released.[785]

Crude extract of ***Emblica officinalis*** decreased arsenic-induced cell damage in mice,[786] and oral supplementation of ***Centella asiatica*** (300 mg/kg/d) during exposure reduced some toxic effects of arsenic in rats.[781] Selenium can bind arsenic and render it non-toxic; however, selenium deficiency can occur in those cases, as described in the section "Selenium" above.

Arsenic is partially detoxified by methylation to mono- and dimethylarsine. Folate deficiency coupled with high arsenic exposure can decrease methylation of arsenic, resulting in greater risk of cancer and peripheral vascular disease. Folate administration to deficient individuals exposed to arsenic was shown to significantly increase methylation of arsenic when compared with controls.[787] Twice daily administration of spirulina extract (250 mg) and zinc (2 mg) to 41 people with toxic arsenic levels was useful in treatment of arsenic-induced melanosis and keratosis.[788] Carotenoids have also been found to be lower in arsenic-exposed workers, indicating that serum beta-carotene and vitamin A testing and supplementation, as necessary, may protect from further nutrient-mediated effects of exposure.[789]

When arsenic toxicity is suspected, other appropriate tests include testing arsenic levels in drinking water; assessing methylation capacity (homocysteine, B_{12}, folate, methylmalonic acid, FIGLU); measuring sulfur amino acids, metallothionein, markers of glutathione status, and oxidative damage markers (see Chapter 6, "Organic Acids"); as well as measuring essential elements.

CADMIUM (CD)

Toxicity symptoms: Femoral pain, lumbago, osteopenia, renal dysfunction, hypertension, vascular disease

Body burden assessment: Whole blood

Biochemical marker: Coproporphyrin I

Protective measures: Zinc, iron, antioxidants

Chelating agents: EDTA; DMSA and NAC (experimental)

Common sources: Industry, spray paint, tobacco smoke, car emissions, plants grown in cadmium-rich soil

One of the most common routes and sources of cadmium (Cd) exposure is inhalation of tobacco smoke, which contains from 1 to 2 µg per cigarette. About 10% of the cadmium in tobacco smoke enters systemic circulation. Indeed, certain laboratories measuring whole-blood cadmium maintain separate ranges for smokers, as smoking is expected to more than double levels.[453, 790] Cadmium is obtained as a by-product of zinc and lead smelting, and is used in industry for electroplating, in the production of rechargeable batteries, and as a paint pigment. The spray painting of organic-based paints without use of protective breathing apparatus is a common cause of chronic cadmium overexposure. Auto mechanics are susceptible to cadmium toxicity from automobile exhaust emissions. Foods susceptible to cadmium contamination include organ meats and shellfish, which can contain between 100 and 1,000 micrograms per kilogram weight. Plants are able to concentrate cadmium, and it has been found in irrigation waters and fertilizers. About 1 µg of cadmium is found in each liter of breast milk. However, with some exceptions, cadmium has poor gastrointestinal bioavailability.[609]

Cadmium is primarily concentrated in liver and kidney. Cadmium is absorbed and transported via metallothionein and DMT1.[791] Cadmium and lead absorption is increased in iron-deficiency states via up-regulated DMT1 expression, which may contribute to the associated neurotoxicity of both elements.[98, 282, 792]

Notes:

Iron deficiency anemia has been reported in chronic cadmium toxicity.[609]

Cadmium is very similar to zinc. Both are group 12 transition metals, and as such, cadmium competes with zinc at all cellular binding sites, which are generally rich in sulfhydryl groups. The displacement of zinc from an enzyme-active site results in loss of enzyme activity. This type of toxicity is sometimes referred to as enzyme poisoning.

Clinical Associations of Cadmium Toxicity

The principal organs most vulnerable to cadmium toxicity are kidney and lung. Environmental cadmium exposure is associated with renal tubular damage and high blood pressure.[793] Numerous studies have shown that acute inhalation exposure to cadmium can cause death in humans and animals. During exposure, symptoms are usually mild, but within days following the exposure, pulmonary edema and pneumonitis develop, leading to death due to respiratory failure. Cadmium inhaltation has also been shown to exacerbate emphysema in smokers.[609] Over time, breathing aerosols containing cadmium can lead to pulmonary congestion resembling chronic emphysema. Other toxic cadmium effects, including decreased testis size, impaired endocrine function (higher follicle-stimulating hormone and estrogen) in men have been reported.[794]

Chronic exposure typically results in the slow onset (over a period of years) of renal dysfunction with proteinuria. Excretion of cadmium is proportional to creatinine, except when renal damage has occurred. Renal damage due to cadmium exposure can be detected by increased cadmium excretion, due to the release of intrarenal cadmium and decreased tubular resorption. Renal damage also greatly reduces the activation of vitamin D and increases calcium excretion.[609]

Osteomalacia, osteoporosis and painful bone disorders have been reported in humans exposed to cadmium in food; however, multiple risk factors are most often present, including poor nutrition and multiparity. The degree of loss of bone density has been correlated with excretion of beta-2-microglobulin, a biomarker of renal injury.[453, 609]

Assessing Cadmium Body Burden

Whole-blood cadmium is indicative of recent exposure, and is therefore not reflective of total-body burden. Normal concentration of whole-blood cadmium is up to

1 µg/L for non-smokers, and up to 4 µg/L for smokers. Whole-blood levels of 10 µg/L have been associated with renal dysfunction.

Urinary cadmium may be reflective of total-body burden, although recent exposure will increase levels. Monitoring urinary output of cadmium is an excellent means of assessing exposure, given normal renal function. Daily output of cadmium of 2 to 4 µg per 24-hour urine indicates toxicity (approximately 1–3 µg/g creatinine).

Fecal cadmium has been used to assess recent dietary intake, as gastrointestinal absorbtion is so low. Hair levels of cadmium may be reflective of exposure, as cadmium, like most toxic metals, concentrates in sulfhydryl groups, which are found in great abundance in hair. However, as with most metals, care must be taken to rule out exogenous contamination. Elevated hair cadmium has been demonstrated in smokers.[453, 609]

There are currently no good markers for assessment of damage to lung parenchyma during acute inhalation exposure of cadmium.

Infant height has been shown to vary inversely with maternal blood cadmium measured at 30 to 32 gestational weeks in Japan.[795] A large environmental survey of toxic elements in German children has led to reference values of 0.5 µg/L in whole blood and 0.5 µg/L in urine.[796] Ukranian children who took zinc supplements had blood cadmium levels in the upper quartile for the study cohort, with significant difference compared with non-supplementing children (0.25 µg/L vs. 0.21; $p = 0.032$).[797] The third US National Health and Nutrition Examination Survey revealed graded positive association of urinary cadmium with serum gamma-glutamyltransferase (a glutathione synthesis marker) and negative associations with serum vitamin C, carotenoids and vitamin E (p for trends < 0.01). These associations were significant in subgroups based on race, age, alcohol consumption, smoking and body mass index, leading to a conclusion that oxidant stress should be considered in the pathogenesis of cadmium-related diseases in humans.

Mangaging the Cadmium-Toxic Patient

The toxic effects of cadmium may be reduced by specific dietary components or supplements such as vitamins E and C and carotenoids, as well as the botanical black cumin seeds,[798-800] and if indicated, vitamin D.[609] Repletion in iron and the amino acids comprising the glutathione tripeptide are also likely valuable, given that deficiency of the former and increased synthesis of the latter have been demonstrated in cadmium toxicity. Supplementation with zinc and selenium salts also can reverse inhibitory effects of cadmium on human peripheral blood mononuclear cell proliferation and cytokine release.[801] Chelating agents used in cadmium toxicity include EDTA, DMSA and N-acetylcysteine.[2]

Lead (Pb)

Toxicity symptoms: Microcytic hypochromic anemia, renal dysfunction, hypertension, anorexia, muscle discomfort, constipation, metallic taste, low IQ (children)

Body burden assessment: Whole blood, serum, hair, urinary porphyrins

Protective measures: Calcium (reduces intestinal absorption), alpha lipoic acid- protection against toxicity, iron (reduces intestinal absorption)

Chelating agents: Ca –EDTA

Common sources: Certain supplements, paint, contaminated soils (and plants grown in them), plumbing

Lead (Pb), a toxin known since antiquity, is widely distributed in the environment, largely due to human activity. Although leaded gasoline was phased out in the early 1980s, lead is still found in the soil around highways. Leaded paint was banned in 1972, but the soil around many older homes painted with lead-based paints is contaminated with lead. Indeed, lead-based paint and its remnants remain the most significant source of lead exposure in the United States among children living in older neighborhoods to this day. Remodeling older homes, which frees up lead dust even from outside window frames, poses a significant exposure (see Case Illustration 3.2). In this way, lead ingested by mothers can be passed on to infants via breast milk.[802–803] Other sources of exposure include water transported through old lead pipes or pipes joined by solders, both of which can carry significant amounts of lead. Lead is still found in paint products intended for non-domestic use and in artist pigments.

Uptake and accumulation by crops grown in soil contaminated with lead and other toxic metals provides another health-threatening exposure source.[691, 804, 805] Mobilization of lead-rich particles from highly contaminated soils in urban areas is an on-going health concern for many large cities. Lactating Greek women residing in urban areas demonstrated higher breast milk lead levels than their non-urban counterparts.[806] Even in

areas far removed from industrial emission sources, lead concentrations in the surface soil layers are far above their natural concentration range.[807] One study showed eggs produced by free-range hens raised on contaminated soil contained high concentrations of numerous toxic elements, including lead, mercury and thallium, as well as organochlorines, including dichlorodiphenyltricholroethane (DDT).[808]

Other sources of lead exposure include certain herbal medicines, notably from China[809] and India. An Ayurvedic supplement called Chandraprabhavati was found to contain 11 mg of lead per tablet. An individual using the product demonstrated acute lead toxicity, including a gingival lead line, anemia and basophilic stippling.[810] Calcium supplements have long been a potential source of lead, especially those of bone and dolomite origin.[811-813]

Exposure to lead can occur by ingestion, inhalation or dermal contact. As much as 300 µg of lead per day may be ingested in a normal diet. The majority of cases of lead poisoning are due to oral ingestion and absorption through the gut. Well-nourished adults absorb only about 1 to 10% of ingested lead, whereas children can absorb as much as 50% of their dietary intake. The absorption of lead is increased in patients with compromised gastrointestinal integrity and low dietary intakes of calcium,[814] magnesium,[815, 816] iron, or vitamins C[817] and D.[818] Absorbed lead is rapidly incorporated into bone and erythrocytes, though eventually, lead will distribute into all tissues. An inverse correlation between lead and calcium or magnesium in erythrocytes was demonstrated in pregnant women, where elevated lead was associated with increased incidence of pregnancy-induced hypertension and preeclampsia.[815]

Ingested lead is absorbed by the iron transport protein divalent metal transporter 1 (DMT1) (see the section "Iron" above). Low iron intake causes increased synthesis of DMT1 to facilitate absorption of the available intestinal iron. The elevated DMT1 increases uptake of lead (and cadmium), leading to greater rates of lead accumulation in iron deficiency. DMT1 is also present in the CNS, and in iron deficiency states may be implicated as part of the pathogenesis of lead neurotoxicity. Thus, iron repletion can protect against lead toxicity.[98, 282, 819]

Lead toxicity causes degeneration by several mechanisms. The tertiary structures of proteins depend on free sulfhydryl groups that are adversely affected by lead's affinity for cysteine residues, similar to the enzyme poisoning by cadmium mentioned earlier. Frequently, these sulfhydryl groups are located within enzymes associated with antioxidation activity, such as glutathione reductase and glutathione peroxidase. Loss of structure of some proteins invariably leads to their instability and loss of biological activity. Thus, increased oxidation causing increased production of reactive oxygen species is one by-product of lead toxicity.[820] Proteins contained within astrocytes of the nervous system and renal tubular cells are particularly susceptible to the structural changes imposed by lead.[821] Lead has also been demonstrated to interfere with a number of calcium-dependent ion channels, receptors, and enzymes, including calmodulin,[822] protein kinase C, L-type calcium- and nicotinic acetylcholine-gated channels, and magnesium-dependent cGMP phosphodiesterase.[823]

Clinical Associations of Lead Toxicity

Pathology associated with lead involves most major organ systems, including urinary, nervous, circulatory (cardiovascular, hematologic and immunologic), gastrointestinal, skeletal, endocrine and reproductive.[453, 820, 824] The CNS is particularly vulnerable to lead, as it readily crosses the blood-brain barrier.[825] Indeed, cognitive dysfunction, neurobehavioral disorders and neurological damage have been associated with lead exposure at blood levels previously considered normal.[820] Chronic lead exposure reduces nerve conduction velocity in peripheral nerves in adult subjects without obvious symptoms or signs of toxicity. Signs of hemopoietic system involvement include microcytic, hypochromic anemia with possible basophilic stippling of the erythrocytes. Although it is relatively rare in most populations, bullet fragments embedded in soft tissue can cause lead toxicity. Two years after a gunshot wound, a 14-year-old girl had an 18-month course of chronic abdominal pain, vomiting and anorexia that was traced to the lead toxicity from bullet fragments in her leg.[826]

Hyperactivity, anorexia, decreased play activity, low intelligence quotient and poor school performance have been observed in children with high lead levels.[827] In children without obvious signs of lead intoxication, bone lead content was related to subjective and nonspecific symptoms (e.g., fatigue, impaired concentration, short-term memory deficits, insomnia, anxiety and irritability) and reduced performance on visual intelligence and visual-motor coordination tests.[828] In a landmark meta-analysis of lead exposure effects in children,

lead concentrations were shown to be significantly and positively correlated with teacher-rated learning disabilities.[829]

Refer to Case Illustration 3.9

When not deposited in tissue, lead is excreted into bile or urine. The kidney is susceptible to damage by lead when it is exposed to high lead concentrations during chelation treatments for lowering elevated body burden. Renal competence should be assured by demonstration of normal creatinine clearance before beginning any intervention to remove lead (or other heavy metal contaminants).

Assessing Lead Body Burden

The use of urinary porphyrin profiling for detecting low level effects of toxicants originated with observations regarding lead. Lead-specific effects on porphyrin pathway intermediates have long been recognized in medical literature.[830, 831] Inhibition of heme synthesis by lead results in a rise in urinary coproporphyrin and erythrocyte protoporphyrin. A significant hallmark of lead toxicity is anemia due to the lack of heme.

Delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase, the second and last enzymes of the committed pathway of heme biosynthesis, are inhibited by lead (see Chapter 8, "Toxicants and Detoxification"). Porphyrin pathway products have been evaluated for their sensitivity to low-level lead exposure and their magnitude of change in response to levels of exposure in rats. In order of decreasing sensitivity and decreasing magnitude of change they rank: urinary coproporphyrin > zinc protoporphyrin (ZPP)/heme > ALAD.[132] Thus, urinary coproporphyrin testing rivals whole-blood lead for sensitivity, and it offers the advantage of showing functional toxic effects in addition to revealing exposure.

Hair keratin has a high affinity for lead owing to its high fraction of sulfhydyl-containing cysteine residues relative to other amino acids. Thus, hair lead is a sensitive measure of lead exposure. Populations living in rural, urban and smelter areas had median hair lead of 9.1, 15.3 and 48.5 ppm, respectively. For those in apparent steady-state lead balance, hair levels were well correlated with blood lead.[832] Normally, hair lead content is < 5 µg/g. Lead levels > 25 µg/g indicate severe lead exposure. A strong dose-response relationship was found for hair lead as a predictor of teacher rating among school children in Boston.[833] Although hair lead is one of the most sensitive indicators of exposure, ZPP is more closely correlated with intellectual impairment in lead-exposed adults because the porphyrin test reveals functional pathway impairment rather than only exposure.[834] Results such as these attest to the variability among individuals with regard to susceptibility to the toxic effects of lead.

Serum or plasma is not a very useful specimen for lead screening except in cases of very recent exposure. This is because serum lead returns to normal levels within 3 to 5 days of last exposure. Urinary lead concentrations increase with lead poisoning, although urinary elimination of lead is a process that occurs for many days after a single exposure. Treatment with EDTA or BAL (also used for chelating arsenic, gold and mercury) accelerates the urine excretion rate of lead; thus, this therapy is useful for revealing lead exposure.

Whole blood is concentrated about 75-fold greater than that of serum or plasma, and it has the highest correlation with toxicity. For this reason, whole-blood lead is defined by the Centers for Disease Control as the preferred test for detecting lead exposure. The World Health Organization has defined high whole-blood lead levels as > 20 µg/dL in adults and > 10 µg/dL in children. The first German Environmental Survey on Children has proposed lowering reference values for whole-blood lead from 6 to 5 µg/dL.[796] As of 2007, the CDC acceptable level of whole-blood lead was 10 µg/dL. Proposals have been made to reduce it to 5 µg/dL, although the safe threshold for blood lead levels in children and in utero may be even lower.[694, 835-838] Lead exposure can produce cognitive deficits at commonly encountered blood levels.[837] Although one study of prenatal lead exposure demonstrated a lack of threshold below which effects on IQ disappear,[839] the findings have been challenged as a statistical artifact.[819]

Notes:

Managing the Lead-Toxic Patient

Prevention continues to be the best route for reducing toxic effects of lead.[840, 841] Thus far, large trials such as the Treatment of Lead-Exposed Children (TLC) effort, employing chelation treatments with succimer or treating nutrient deficiencies with zinc and iron have failed to show improved cognitive performance. This work has been challenged on the grounds of poor chelator choice and inadequate supplement dosing and duration. When elevated lead levels are detected in individual patients, treatment needs to be immediate, since chronic exposure, particularly in children is associated with poor prognosis. That said, the chelation agent of choice for the lead-toxic patient remains intravenous calcium disodium EDTA. Research continues to demonstrate efficacy using EDTA in adults. In one study, long-term IV EDTA in individuals with non-diabetic chronic renal disease and blood lead > 60 µg/dL was shown to significantly slow the progression of renal insufficiency as compared with controls.[842]

Nutritional and adjunctive support for the lead-toxic patient includes promoting gastrointestinal integrity and supplementing with calcium,[814] magnesium,[815, 816] iron, and vitamins C[817] and D.[814] Carotenoids have also been found to be lower in lead-exposed workers.[789] Nutrients with demonstrated benefit when used with or without chelating agents include alpha lipoic acid, zinc, taurine, selenium—which is able to bind lead directly—and N-acetylcysteine.[820]

MERCURY (HG)

Toxicity symptoms: Mental symptoms (erethism, insomnia, fatigue, poor short-term memory), tremor, stomatitis, gingivitis, GI and renal disturbances, decreased immunity

Body burden assessment: Whole blood, erythrocyte, serum, hair, urine, urinary porphyrins

Protective measures: Selenium (protects against cellular toxic effects)

Chelating agent: DMSA, DMPS

Common sources: Dental amalgams, fish consumption, preservatives (esp. thimerosal), industrial relaease

Mercury (Hg) as a neurotoxin has an intriguing history. The phrase "mad as a hatter" has its origins with the seventeenth and eighteenth century hat makers who suffered from mercurialism due to their use of liquid mercury in the manufacture of the popular felt-brimmed hats. Sir Issac Newton, the famous seventeenth century physicist, experienced a year of dark moods and marked personality change that puzzled friends and close associates. Posthumous analysis of archived samples of Newton's hair revealed highly elevated concentrations of mercury, which is evidence that supports the historical hypothesis that Issac Newton's "madness" was a result of his exposure to the toxic metal while he was conducting experiments to study its properties.[843]

The human population is exposed daily to naturally occurring mercury. The earth's crust releases approximately 30,000 tons of mercury per year as a product of natural outgassing from rock. Mining, smelting and combustion of fossil fuels, particularly coal, are a primary source of anthropomorphic mercury exposure.[609] Approximately 6,000 tons/year of mercury are used in the manufacture of electrical switches, for electrolysis, and as a fungicide. Ninety tons of mercury are used each year for making dental amalgams. According to the CDC, mercury released from amalgams may comprise up to 75% of an individual's mercury exposure. The amount of mercury released from amalgams ranges between 1.2 to greater than 27 µg/d.[609] Toxicity associated with mercury amalgams continues to be a serious concern, particularly in regard to pregnant women, as mercury is a known neuroteratogen.[453]

In its elemental form, mercury (Hg^0) is non-toxic. However, once chemically or enzymatically altered to the ionized, inorganic form (Hg^{2+}), it becomes toxic. Thus, bioconversion of mercury to its organic alkyl forms renders some forms such as methyl mercury highly toxic with great avidity for the nervous system. Another commonly encountered organomercury compounds is ethylmercury that is released from thimerosal.[844] Microorganisms in the environment and in the human intestinal tract can bioconvert non-toxic elemental mercury to inorganic Hg^{2+} and organic mercurous alkyl compounds. Methylmercury is highly water soluble and readily enters aquatic food chains, accumulating at higher concentrations in the tissue as it moves up the food chain of marine organisms. Bioaccumulation of methylmercury in organisms at the top of the aquatic food chain is on the order of 10,000 to 100,000 times greater than concentration in the ambient waters.[845] Indeed, methylmercury from seafood is considered to be the most important source of non-occupational human mercury exposure.[845] Analysis of commercial fish in New Jersey markets found bioaccumulation to be the highest (in descending order of magnitude) in yellow fin tuna, Chilean sea bass, bluefish and snapper.[846]

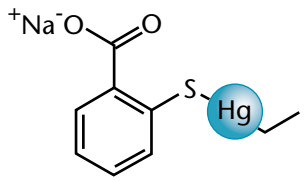


Figure 3.18 — Thimerosal

Thimerosal has been widely used to preserve vaccines used for immunizations. The mercury thioether structure of thimerosal can be metabolized or chemically degraded to release the much more toxic ethylmercury.

Although lack of data makes precise comparisons of safe levels of exposures to different forms of mercury difficult, we may gain insight about the potential for toxic effects from the available data on mercuric chloride, ethylmercury and methlmercury. Thimerosal, a mercury-containing preservative used in vaccines, has been a common source of mercury exposure for children. In the human body, thimerosal releases ethylmercury (see Figure 3.18). In 2002, it was demonstrated that the mean total mercury dose in vaccines received by 6-month-olds was 111.3 µg (range 87–175 µg).[847] In a 6.2 kg infant, 111.3 µg translates to 18 µg/kg/d or about 2.6 times the adult minimal risk level (MRL) of 7 µg/kg/d for acute mercuric chloride exposure.[609, 847, 848] The US Environmental Protection Agency (EPA) sets a reference dose (RfD) of 0.1 µg/kg body weight/d for chronic exposure to methylmercury, at which there are no recognized effects. Using the EPA's RfD, a vaccination containing 111.3 µg mercury would expose a 6.2 kg infant to 29 times the safe level for chronic methylmercury exposure.[849] Based on such inferences, governmental health authorities now advocate removal of thimerosal-containing childhood vaccines.[850]

Clinical Associations of Mercury Toxicity

There are three known ways by which toxic effects are produced by mercury: (1) It reacts with sulfhydryl groups impairing the activity of enzymes, (2) it generates protein adducts that are immunogenic, and (3) its highly lipophilic alkyl forms alter nerve membrane function.[453] Autoimmune glomerulonephritis in mercury-exposed individuals has suggested an association between exposure and autoimmunity in humans.[851, 852]

Mercury intoxication, in turn, can produce a triad of symptoms: (1) mental changes, (2) spontaneous tremor and deficits in psychomotor performance, and (3) stomatitis and gingivitis. The mental effects include erethism (excessive irritability, excitability or sensitivity to stimulation), depression, short-term memory loss, difficulty concentrating, insomnia and fatigue. Additional signs of neurotoxicity include loss of vision, hyperreflexia, sensory disturbances, imparement of speech and hearing, hyperhidrosis and muscular rigidity. Signs and symptoms of mercury intoxication involving other organ systems include renal and gastrointestinal disturbances, pain in joints and limbs, weight loss, metallic taste in the mouth and increased susceptibility to infections.[453, 845]

Mercury released from dental amalgams, which are composed of as much as 50% mercury, can have a negative impact on an individual's health. Although mercury-containing amalgams have been in use for over 100 years, their use was intensely debated at the turn of the century and again in the 1930s. Small yet measurable amounts of mercury are continuously released from the amalgam surface; the rate of release is accelerated by hot liquids and chewing. Normal bacterial flora converts a fraction of the released elemental mercury to its toxic forms, Hg^{2+} and alkyl mercury. A portion of the elemental mercury released from amalgams is unavoidably inhaled into the lungs, where it can be biotransformed to its toxic forms.[453] Studies have suggested that chronic mercury exposure in amounts released by amalgams

Notes:

provokes an increase in both mercury- and antibiotic-resistant strains of bacteria in the oral and intestinal flora. Such mercury-induced aquired resistance to antibiotics has been found worldwide in fish and soil bacteria.[853]

Epidemiologic data from the US EPA and CDC have led to estimates that more than 300,000 newborns each year may have increased risk of learning disabilities associated with in utero exposure to methylmercury.[858] Chinese children with both inattentive and combined attention deficit hyperactivity disorder (ADHD) have blood mercury levels higher than controls. Risk of ADHD was found to be nearly 10 times higher when blood mercury was above 29 nmol/L.[859]

With the pronounced rise in the incidence of autism over the last decades,[860] much debate continues regarding mercury's role in the pathogenesis of this

OF FURTHER INTEREST...

Toxic element accumulation is dependent on route and duration of exposure, form of toxic element and presence of protective measures. For example, rats maintained for 18 months on low-selenium diets and consuming drinking water containing 5.0 ppm of mercury as methylmercury had 10-fold higher mercury in brain compared with those given water with 0.5 ppm mercury.[854] However, brain mercury increased only slightly in similarly exposed rats fed diets with high selenium content (0.6 vs. 0.06 ppm), and no increase was seen at lower levels of exposure, showing the protective effect of dietary selenium.

Another important observation from these experiments was that mercury was higher in neonatal rats that also had lower retention of selenium, and blood and brain mercury levels fell with age as selenium levels stabilized. Such results raise timing issues and possible protective measures. Administration of vitamin C, glutathione or lipoic acid in combination with DMPS or DMSA to young rats for 7 days following a 7-day exposure to elemental mercury vapor had no effect on brain mercury.[855] Here, the toxic element form was elemental versus methyl mercury, and administration was by inhalation for 7 days rather than ingestion for 18 months. The protective measures were administered for only 7 days and only after exposure had occurred. Longer-term administration of the protective nutrients might produce quite different results, especially if tissue levels are raised before exposure.[856]

Kidney mercury in the rats exposed to mercury vapor was lowered by DMPS and DMSA, but no combination was found to affect levels in brain. These results provide insight about

differences in tissue distribution and ligand character. In metallothionein-rich kidney tissue, bound mercury is more dissociable than that bound to enzymes in the brain. Such differences among tissues in their sequestration tendencies leads to concern about potential redistribution induced by therapies that cause mobilization of toxic metals. Thus mercury released from extrahepatic tissues might transfer to brain as a result of chelation therapies. Very little is known about how much such redistribution actually occurs for any given chelator. Such effects may account for the suggestions that treatments of past mercury exposures with N-acetylcysteine or reduced glutathione may be counterproductive.[857]

For toxic elements other than mercury, the constant redistribution over time produces an accrual in bone, where they are bound in the hydroxyappetite matrix. These forms are of lower concern (and low contribution to laboratory element testing) until they are remobilized during bone resorption. Such issues complicate the evaluation and treatment of patients with toxic element effects.

neurodevelopmental condition.[847, 861] Although research does point to the eitiology of autism being multifactorial, numerous reports demonstrate that aspects of mercury toxicity appear similar to autism symptomatology.[862-864] In 2002, thimerosal was phased out of some vaccines, as recommended by the US Public Health Service and the American Academy of Pediatrics.[850, 861] Data from the Vaccine Adverse Event Reporting System (VAERS) reported a significant reduction in the proportion of neurodevelopmental disorders, including autism, mental retardation and speech disorders, as thimerosal was removed from childhood vaccines in the United States from mid-1999 onward.[865] As of the date of this writing, since regulations do not govern all sources, vaccines must still be verified as thimerosal free.

In addition to the previously discussed nutritional factors, gender and age can also influence mercury status and toxic consequences. In an Austrian population with generally low levels of mercury, values in males were influenced by fish intake, amalgan fillings, age and education level, whereas those for females varied only with dietary fish intake, indicating gender-specific effects.[866] In older Americans, visual memory ability declines as blood mercury levels rise, although other neurological tests such as finger tapping were uaffected.[867]

Assessing Mercury Body Burden and Toxic Effects

A primary function of the clinical laboratory is to assist clinicians in making decisions about when to treat a patient for heavy metal toxicity. However, there is considerable debate over how to establish reference limits for mercury (and other toxic metals) on clinical laboratory reports. The US EPA RfD for chronic mercury exposure of 0.1 µg/kg/d is equivalent to a total exposure of 7 µg/d in a 70 kg adult. If the amount of mercury absorbed from dental amalgams is combined with all other sources of mercury (e.g., fish, environmental, occupational and medicinal), the daily exposure to mercury is expected to exceed the RfD for some individuals. In populations such as occupationally exposed workers and the elderly, the percentage of mercury-threatened individuals can be much higher. Based on a study of normal, presumably healthy populations, mean whole-blood mercury concentration was found to be < 5 µg/L. About 1% of this population had whole-blood levels of mercury greater than 5 µg/L. Individuals with occupational exposure to mercury, such as dentists and dental technicians, may routinely have whole-blood mercury up to 15 µg/L. Significant exposure is evident when whole-blood alkyl mercury is greater than 50 µg/L, or when Hg^{2+} exposure is greater than 200 µg/L.

Based on the first German Environmental Survey on Children, lowering of reference values for whole-blood mercury from 1.5 to 1.0 µg/L has been proposed.[796] Consumption of large amounts of fish by pregnant women in Hong Kong results in prenatal exposure to moderately high levels of mercury shown by finding cord-blood mercury levels above 29 nmol/L (5.8 µg/L) in newborn infants.[868] A separate study found that, compared with the national average, women who ate fish were 3 times more likely to have elevated cord-blood levels. Of the 275 women who completed the study, 28.3% had cord-blood mercury above the 5.8 µg/L reference level set by the EPA.[869] In a random sample of 474 subjects in Baltimore, Maryland, 9% had blood mercury levels above the 5.8 µg/L limit.[870] However, elevated levels (> 5.8 µg/L) are found in 16.5 % of women in populations with high fish consumption.[871]

Blood mercury has revealed low level chronic and acute exposure from work environments,[872] whereas elevations of mercury have been reported as high as 16,000 µg/L in blood and 11,000 µg/L in urine.[873] At massive elevation levels, interpretation is straightforward, allowing assessment of patient exposure factors and clinical consequences. As with most tests performed on a broadly varying outpatient population, interpretation of results from measurements of mercury in blood or urine become more difficult as concentrations approach the population norms of 10 to 20 µg/L. Concurrent or follow-up testing of biomarkers that show toxic consequences, such as elevated porphyrins,

Notes:

beta-2-microglobulin or N-acetyl-beta-D-glucosamine can be very helpful.

The level of mercury in urine is a reliable way to assess exposure to inorganic mercury. Daily urinary levels greater than 50 µg indicate a Hg^{2+} overload. Hair levels of mercury greater than 1 µg/g also indicate mercury toxicity. The quantity of mercury assayed in blood and hair, but not urine, correlates with the severity of toxicity symptoms.

Erythrocyte mercury shows a strong relationship with erythrocyte selenium, suggesting a chemical linkage between the two elements.[563] Erythrocyte mercury was strongly correlated with plasma mercury, and both mercury and selenium levels were strongly correlated with fish intake.[563]

Hair has been a frequently used specimen by CDC and EPA for accurately assessing mercury exposure in selected populations.[845, 849, 858]A number of studies have shown positive associations between mercury concentrations in blood and hair. Hair to blood ratios ranging from 200 for maternal hair-cord blood to 360 for hair-blood values in 7-year-old children have been reported.[874] Populations of Brazilian communities showed a positive correlation of blood pressure with levels of hair mercury. At levels above 10 µg/g, the odds ratio for elevated systolic blood pressure was 2.9.[875] Both blood and hair mercury levels drop between the second and third trimesters of pregnancy. Maternal hair correlates with cord blood, both levels being related to fish intake.[876]

Measurement of mercury concentrations in body tissues or fluids provides evidence of exposure, but it does not answer the question of toxic effects that are dependent on many other factors. Variations in status of thiols such as glutathione, cysteine or lipoic acid shift the dynamics of mercury's effects, as do the levels of metallothionein, zinc and selenium, or even glutamine.[857] Specific patterns of urinary porphyrin abnormalities have been clearly associated with mercury, providing a convenient and sensitive biochemical marker of metabolic toxicity. See Chapter 8, "Toxicants and Detoxification," for further discussion of urinary porphyrin profiling for detecting clinically significant mercury exposure.

Notes:

Management of the Mercury-Toxic Patient

Removing the source and optimizing routes of mercury elimination should be the first treatment for mercury toxicity. Antioxidant intervention may be helpful for mitigating the oxidative damage caused by mercury toxicity. Some antioxidants such as N-acetylcysteine, alpha-lipoic acid and glutathione may posess chelative effects.[2, 856] Selenium has been demonstrated to effectively bind mercury, rendering the mercury ineffective (see the section "Selenium" above). More aggressive treatment for mercury toxicity calls for chelation therapy. Administration of BAL, penicillamine, EDTA, DMSA or DMPS will mobilize mercury and cause a rise in the daily urinary mercury excretion rate. The preferred chelation agents, based on their affinity for mercury and low toxicity, are DMSA or DMPS.[2] All of these agents should be used with monitoring of mercury metabolic toxicity, since they can mobilize relatively inert bound forms of mercury.[857] If porphyrin profile signs start to worsen, treatment may need to be suspended until newly mobilized mercury reaches equilibration with metallothionein and other routes of binding for excretion.

Removing brain accumulations of mercury is a challenge. DMSA and DMPS may not be effective agents for removing toxic metals found in the CNS, as they are very unlikely to cross the blood-brain barrier. It has been suggested that alpha-lipoic acid may cross the blood-brain barrier, and combinations of ascorbic acid and glutathione may help to allow mercury transport away from tissues by altering the ionic form. However, when combinations of these interventions were tested in mercury-exposed rats, no reduction in brain mercury was found.[855]

REFER TO CASE ILLUSTRATION 3.10

Elements of Potential Toxicity

Thallium (Th)

The position of thallium (Th) in the periodic table with aluminum and gallium and between lead and mercury suggests that it may be toxic to humans. Thallium is a tasteless toxin that has a long criminal history as a poison. Severe, painful neurological and gastrointestinal symptoms occur from thallium poisoning; alopecia is the most characteristic sign, as it is coupled with a black pigment at the hair root.[877] Thallium is present in cigarette smoke, and is readily taken up by plants grown in contaminated soil. Thallium alloyed with mercury is used in low-temperature thermometers. Thallium sulfate was banned as a rodenticide and pesticide in 1972, but poisoning by exposure to old treatment sites constitutes the main source of current exposure.[609] It has a medicinal history as treatment for ringworm, gout, tuberculosis and gonorrhea.

Thallium's mechanism of toxicity is based on substitution of potassium in Na/K pumps and interference sulfhydryl residues on enzymes, particularly in the mitochondrial electron transport chain. Specimens used for thallium detection include urine, which is the main route of excretion, and hair.[453, 627] Blood levels have been measured, but thallium is rapidly cleared, so exposure must be acute to achieve accurate results.[609]

The use of BAL, D-penicillamine or EDTA are contraindicated for treatment, as they have been shown to severely redistribute the toxicant. DMSA may be an appropriate alternative, although it does not effectively cross the blood-brain barrier. Prussian blue is currently used.[2] Treating the GI tract with activated charcoal may be effective for removal of ingested thallium. Potassium intravenous drips improve dieuresis of thallium.[453, 877]

Uranium (U)

Although uranium (U) has always been abundant on earth, its widespread use in military and industry uses, including nuclear power, has increased human exposure. Uranium can be ingested or inhaled and is cleared in urine rapidly, although some will pool in bone and kidney tissues.[609] High concern is placed on the toxicity of depleted uranium.[878] World War II studies on high-dose, short-term (24-hour) exposures to uranium that demonstrated effects on reproduction have been confirmed in subsequent animal studies.[453, 705, 879] Although minimal uranium is found in food and water,[453, 880] one study has demonstrated increased bone turnover in men exposed to naturally occuring uranium-rich drinking water.[881]

Uranium toxicity in humans leads to renal damage. Experimental studies demonstrate hyaline cast formation and necrosis of tubular cells. Additionally, pronounced deregulation of genes has been illustrated, particularly in relation to calcium transport and nephroblastoma-related genes. Lung cancer is commonly associated with inhaled uranium, as the particles are radioactive, and larger particles will localize to lung parenchyma.[878, 879]

Assessment of uranium is challenging, and may require assessment of proteins impacted by known genetic alterations.[878] Chromosomal aberrations consistent with uranium exposure have been shown to be effective biomarkers of strong past or current exposure.[882, 883] Uranium is rapidly cleared from the body (75% in 5 minutes and 95% in 5 hours post-exposure). The element is able to bind to transferrin and albumin in blood, and uranium phosphate has been found in bone, suggesting an ability to replace calcium. Urine can be a sensitive specimen for uranium exposure, but assessment should be undertaken promptly.[884]

Uranium exposure has been treated by IV infusion of 1.4% sodium bicarbonate. Although no chelating agents are US FDA-approved, inositol hexaphosphate demonstrated efficacy in animal studies.[878]

Tin (Sn)

Tin (Sn) is used in PVC, glass coverings, silicone and wood preservative, paints, biocides and pesticides,[885] and medicines, including Ayurvedic[886, 887] treatments. The most common route of exposure may be as stannous (tin) fluoride in dentrifices. Detailed reviews have been done of tin-containing antitumor agents[888, 889] and tin in wastewater.[890] Carcinogenicity has been demonstrated in a limited way in human and animal studies. Lipophilic organic trimethyltin compounds display immunotoxic effects. Neonatal rats exposed to tin displayed stunted growth and thymic atrophy. Tin has been shown to be neurotoxic, specifically in the hippocampal region, although the mechanism has yet to be elucidated.[890]

Specimens used for assessment of tin exposure include urine and blood, which may be useful for both acute and chronic exposure.[609]

Although scant evidence exists for treatment of trimethyltin, compounds such as BAL and DMSA have demonstrated limited efficacy toward tributyltin.[891, 892] Selenium was protective against the high incidence of lung cancer in tin miners shown to be selenium deficient.[893]

ANTIMONY (SB)

Antimony (Sb) is a potentially toxic element with no known biological function. Although it is less toxic than arsenic,[453] antimony is a similar group V metalloid with the capacity to interrupt enzyme sulfhydryl groups.[894] Antimony-based compounds are used as a flame retardant material in draperies, wall coverings and carpets. Antimony is present in cosmetic products. Other materials containing antimony include alloys, ceramics, glass, plastic and synthetic fabrics.[895] Antimony is present in incinerators, smelters and fossil-fuel combustion.

Since antimony is known to be absorbed through the dermal layer in animal studies, it has been speculated that antimony toxicity may play a role in sudden infant death syndrome (SIDS), where children are sleeping on antimony-containing fibers. A number of studies have associated significantly elevated antimony in both hair and liver samples of children who died from SIDS,[896, 897] although other work has challenged the validity of the findings.[890]

Antimony preparations are first-line interventions for parasite exposure, mainly schistosomiasis and leishmaniasis, although antimony-resistant species of leishmaniasis are increasing in frequency.[898] Antimony toxicity presents with nausea, vomiting, abdominal pain, hematuria, hemolytic anemia and renal failure. Subclinical exposure could result in increased reactive oxygen species and increased apoptosis. In one study, the presence of urinary antimony was closely associated with peripheral arterial disease.[899]

Because whole-blood antimony is cleared from the blood slowly over months, it best reflects chronic exposure.[900] Urine has shown good correlation with acute antimony exposure.[901]

Antimony is excreted via bile and urine as a glutathione conjugate. Treatment for antimony exposure is the chelating agent BAL[453] or DMSA. N-acetylcysteine may be a beneficial adjunctive therapy to support glutathione synthesis.

TITANIUM (TI)

Few human toxicological studies have been conducted for titanium (Ti) compounds. Inhaled titanium was associated with increased lung fibrosis.[902] Titanium alloys used in surgical implants have not demonstrated any carcinogenic potential. Some animal studies have demonstrated carcinogenic potential, primarily with intramuscular injections of pure titanium, titanocene or titanium dioxide compounds.[453, 902] A higher presence of inflammatory markers such as nitric oxide synthase (NOS) in tissue was observed with titanium versus zirconium dental implants.[903] Evidence for titanium biomarkers is limited, although hair and serum specimens have been shown to correlate with exposure.[685, 904]

CONCLUSIONS

Most individuals consuming a standard western diet have significant risk of one or more essential element deficiencies. Populations such as the elderly, pregnant women, small children and immunocompromised patients are particularly vulnerable to essential element deficiencies. Hypertension, eczema, neurological abnormalities and pain are only a few of the clinical ramifications of a deficiency of essential elements or toxic metal effects. Most of the top causes of mortality in the United States may be associated with essential element deficiencies. Symptoms of deficiencies may be vague and difficult to diagnose.

The impact of toxic element exposure is exacerbated by nutrient element deficiencies via a variety of mechanisms. The synergy of multiple toxic element exposures can result in clinical effects that supersede those of a single toxic element exposure. As a result, defininitions of safe levels for exposure to toxic elements continue to be lowered.

Treatments for toxic element-burdened patients include minimizing sources of exposure, normalizing routes of elimination, and implementing metabolic support and antioxidant repletion. In addition, most patients can be guided to dietary and lifestyle practices that will improve their tolerance to toxic elements. Clinical interventions can help raise tolerance by focused corrections of nutritional insufficiencies, especially regarding status of glutathione and amino acids, plus cofactors that can assist the production of metallothionein. In those cases where toxic element exposure leads to metabolic

compromise, utilization of appropriate chelating agents may also be required.

Accurate assessment of essential and toxic element status followed by appropriate treatment is required. Laboratory evaluations provide both direct concentration information and functional markers for assessing element status. Elements can be measured in whole blood, erythrocytes, or challenged or unchallenged urine or hair. Urinary porphyrin profiling can show metabolic toxicity. Single specimen profiles can reveal potential weaknesses and exposures, but testing of multiple specimen types is the preferred evaluation for reaching firm clinical conclusions. Each element has unique physiological properties, so the most sensitive and specific tests for evaluating a patient vary according to the element of focused therapy.

Continued improvements in laboratory methods may provide comprehensive element evaluations that include multiple specimens (RBC, urine, hair and stool) and functional markers. The use of appropriately dosed essential element dietary supplements based on need demonstrated by laboratory results can significantly improve the safety and efficacy of interventions. Once an abnormality in element status is identified in a patient, follow-up testing of elements in appropriate specimen types can provide evidence to the clinician that imbalances in nutrient and toxic elements have been resolved.

Notes:

Case Illustrations

Case Illustration 3.1 — General Pattern of Element Deficiencies

First Profile: 2004

As discussed in the introduction to this chapter, single-element deficiencies are infrequently encountered. Most often, laboratory evaluation will reveal two or more deficiencies together, with magnesium often being one of them. Multiple deficiencies occur for a variety of reasons, including soil depletion, poor diet and poor digestion/GI function. (See Figure 3.1).

A 60-year-old female presented with weight gain, poor eating habits, fatigue and menopausal symptoms. Her RBC element profile shows values below the first quintile cutoff for most essential elements. Impaired enzyme activities due to these cofactor insufficiencies were likely contributing to her presenting complaints.

Element - Erythrocyte (RBC) *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

Essential Elements	Results ppm packed cells		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
1 Chromium	0.19	L		0.25 - 0.80
2 Copper	0.73			0.52 - 2.00
3 Magnesium	28	L		40 - 80
4 Manganese	0.19	L		0.25 - 0.80
5 Potassium	782	L		1,000 - 2,500
6 Selenium	0.08	L		0.12 - 0.40
7 Vanadium	0.07	L		0.10 - 0.28
8 Zinc	4.7	L		6.0 - 11.0

Second Profile: 2005

With intervention using oral chelated elements, improved diet and digestive support, the 2005 follow-up study demonstrates improvement. Clinically, her chief complaints also improved. However, closer inspection of the RBC profile reveals that a number of elements are in the lower range of normal, indicating to the prudent clinician that continued supplementation with those elements is needed. Achieving total body tissue saturation after long-term elemental deficiency status is one of the most difficult areas of clinical nutrition. Multiple factors of diet, lifestyle, gastric physiology and intestinal health come to bear on a patient's ability to assimilate and retain major and trace elements. Other nutrient corrections (esp. amino acids) can help to restore levels of storage and transport protein that bear upon elemental physiology.

Element - Erythrocyte (RBC) *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

Essential Elements	Results ppm packed cells	Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
1 Chromium	0.37		0.25 - 0.80
2 Copper	0.57		0.52 - 2.00
3 Magnesium	41		40 - 80
4 Manganese	0.35		0.25 - 0.80
5 Potassium	1,383		1,000 - 2,500
6 Selenium	0.14		0.12 - 0.40
7 Vanadium	0.13		0.10 - 0.28
8 Zinc	9.9		6.0 - 11.0

Third Profile: 2006

The 2006 RBC element profile shows the elements hovering around their levels of a year ago, with magnesium again below the reference limit. The patient continues to enjoy much symptom relief, demonstrating a lag time between erythrocyte levels of elements and clinical picture, but a relapse is likely if these elements continue to drop. The patient admits that she has been lax with both supplementation and digestive aids, but resolves to resume her protocol. Thus, the laboratory data is useful for guiding the clinician and patient regarding the use of major and trace element supplements in both the treatment and long-term prevention of metabolic disorders. ❖

Element - Erythrocyte (RBC) *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

	Essential Elements	Results ppm packed cells		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
1	Chromium	0.28			0.25 - 0.80
2	Copper	0.54			0.52 - 2.00
3	Magnesium	38	L		40 - 80
4	Manganese	0.32			0.25 - 0.80
5	Potassium	1,367			1,000 - 2,500
6	Selenium	0.13			0.12 - 0.40
7	Vanadium	0.10			0.10 - 0.28
8	Zinc	7.6			6.0 - 11.0

Case Illustration 3.2 — A 49-Year-Old Female Undergoing Chelation with Multiple Lab Assessments

Diagnosis: Lead Poisoning

History of Present Illness: Pam, a 49-year-old female, had struggled with neurological problems for over two years. Symptoms included muscle spasms, twitching, stiffness, and pain. She could not walk short distances comfortably and had problems getting out of chairs and balancing. She was very fatigued and went to bed at 7 P.M. every night.

She visited the Mayo clinic and received a diagnosis of cramp fasciculation syndrome, also called stiff person syndrome. She then visited Johns Hopkins where she was diagnosed with Parkinson's disease. She had been tested for toxic metals with a 24 h urine test previously and arsenic was high-normal, but the clinician did not treat for heavy metal toxicities. She had undergone physical therapy but reached a plateau with some improvement of her symptoms.

Past Medical History: She had an osteosarcoma removed from her arm.

Social/Work/Environmental History: Pam is a registered nurse who lived for 20 years in a house that had lead paint. The lead exposure was verified by measurements taken by the local Board of Health. She had the paint on her windows removed and reglazed and she cleaned up the

Continued on following page…

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paint dust. Her work is one mile from a coal incinerator and downstream of a waste incinerator.

Family History: Pam's parents are alive and healthy without neuromuscular complaints.

Treatment and Results: Careful elemental status assessment led to the diagnosis of chronic metal toxicity. The directing physician tested Pam's erythrocyte elements (panels 1A and 1B) and whole blood toxic elements (panels 2A and 2B) on 2/20/06, 5/17/06, and on 8/17/06. Further, he tested urinary elements after provocation with EDTA on 3/20/06, with DMPS on 5/11/06, and with EDTA on 8/11/06 (panels 3A and 3B). Pam was given weekly intravenous chelation treatments and intravenous B vitamins, essential elements, and vitamin C. The patient also took oral nutritional supplements. The most recent urine challenge test shows somewhat higher lead and aluminum levels than previously found (results not shown).

Erythrocyte toxic elements were within normal limits throughout, although lead levels show systematic decreases to undetectable levels at the end of treatments. The chelation treatments caused lowering of essential elements in the RBC between 2/20/06 and 5/17/06, at which time the patient had low status of many nutrient elements. On 8/17/06 levels had plateaued or had increased, indicating more aggressiveness in supplementation of nutrient elements. These data illustrate the benefit from evaluating essential element status during chelation treatment to assure their protection against toxic effects of mobilized heavy metals.

Levels of arsenic and aluminum were initially above the reference limit (panels 2A and 2B) and they showed progressive decreases over the three time-points. In August aluminum increased slightly, suggesting ongoing exposure or mobilization of aluminum sequestered in bone. There was no significant change in whole blood cadmium, lead or mercury. Levels in whole blood are affected by recent exposure and chelation treatments that mobilize elements into circulation before excretion.

Urinary excretion of arsenic gradually increased with treatment (panels 3A and 3B). Cadmium, lead, and mercury showed similarly elevated urinary excretion on 3/20/06 and on 8/11/06, following the use of EDTA for mobilization challenge. However, the use of the much more specific mercury binding agent, DMPS, on 5/11/06 produced elevated mercury excretion with normal values for cadmium and lead. During successful chelation therapy, urinary excretion of toxic elements is expected to be initially high, falling to normal levels after the course of treatments has extended for sufficient time to allow clearance from various body tissues. The consistent presence of various elevated levels among the group of toxic elements, however, indicates that continued efforts to lower body burdens is needed.

Pam says she is, "doing really, really, well." She says that her tremors have stopped and she has doubled her strength. She is jogging now for the first time in 2.5 years. She can walk now for three miles and is biking also. She says, "I feel like I've been given my life back. I thought I would be in a wheelchair for the rest of my life." She can now get out of chairs without losing her balance.

This case shows the interplay between essential and toxic element concentrations in erythrocytes, whole blood and urine as a patient with elevated toxic element body burden is given intravenous chelation treatments. At the biochemical level, many interactions between the various elements make predictions of clinical outcomes from laboratory data alone impossible. Patient responses must guide treatment with the expectation that, with sufficient time, the treatment will lower toxic element levels throughout the body and measured levels will consistently drop within normal limits. ❖

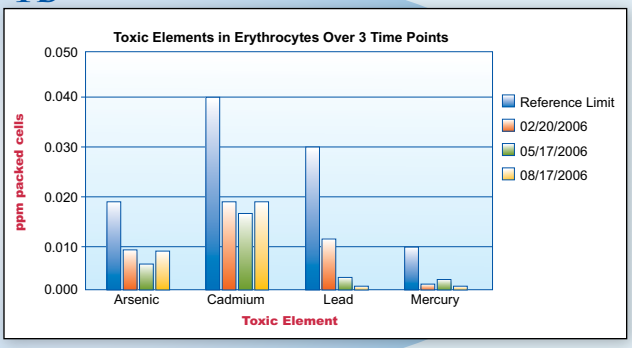
Notes:

1A

Element - Erythrocyte (RBC) *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

		Results ppm packed cells 02/20/2006		05/17/2006		08/17/2006		95% Reference Interval
Primarily Intracellular Elements Related to Nutritional Status								
1	Chromium	0.25		0.23	L	0.23	L	0.25 - 0.80
2	Copper	0.53		0.47	L	0.52		0.52 - 2.00
3	Magnesium	33	L	26	L	31	L	40 - 80
4	Manganese	0.27		0.30		0.37		0.25 - 0.80
5	Potassium	1,296		968	L	1,168		1,000 - 2,500
6	Selenium	0.16		0.13		0.14		0.12 - 0.40
7	Vanadium	0.11		0.16		0.11		0.10 - 0.28
8	Zinc	6.9		6.2		6.3		6.0 - 11.0
Toxic Elements		**02/20/2006**		**05/17/2006**		**08/17/2006**		
10	Aluminum	1.8		1.8		2.0		<= 3.0
11	Arsenic	0.009		0.006		0.009		<= 0.018
12	Cadmium	0.018		0.015		0.018		<= 0.040
13	Lead	0.013		0.005		<0.005		<= 0.030
14	Mercury	0.003		0.004		0.002		<= 0.010

1B

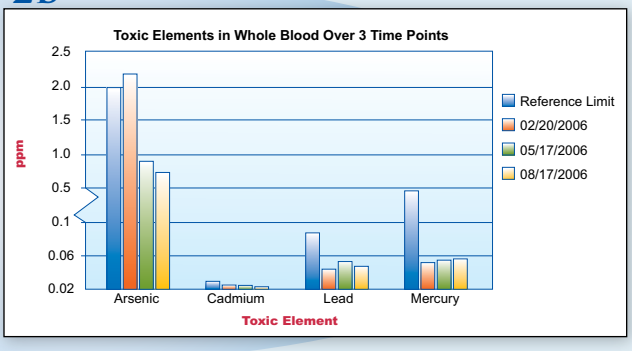


2A

Element - Whole Blood (Toxics) *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

		Results ppm packed cells 02/20/2006		05/17/2006		08/17/2006		95% Reference Interval
Highly Toxic Heavy Metals								
11	Arsenic	2.114	H	0.815		0.684		<=2.000
12	Cadmium	0.003		0.003		0.001		<=0.010
13	Lead	0.059		0.073		0.066		<=0.100
14	Mercury	0.162		0.210		0.269		<=0.500
Potentially Toxic Elements								
10	Aluminum	1.8	H	1.8	H	2.0	H	<=0.400

2B



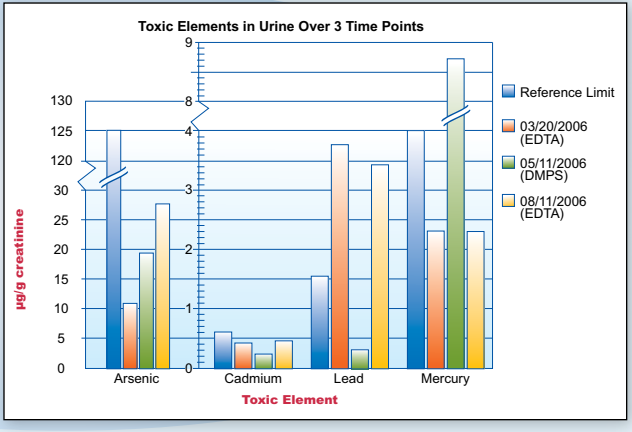
3A

Toxic Elements - 6-8 Hour Urine *Methodology: Inductively Coupled Plasma/Mass Spectroscopy*

Results ug/gm creatinine

		3/20/06 EDTA Challenge		05/11/06 DMPS Challenge		08/11/06 EDTA Challenge		95% Reference Interval
Highly Toxic Heavy Metals								
1	Arsenic	10.5		18.2		27.0		<= 125
2	Cadmium	0.469		0.31		0.496		<= 0.62
3	Lead	3.79	H	0.30		3.47	H	<= 1.58
4	Mercury	2.3		8.7	H	2.3		<= 4.0
Potentially Toxic Elements								
5	Aluminium	41.4		<3		45.3		<= 69
6	Nickel	8.70		3.48		12.6	H	<= 9.5

3B



Multiple lab assessments were used to evaluate Pam's chelation treatment over a 6 month time frame. Nutrient and toxic elements in erythrocytes are shown in 1A. The dates of collection, 2/20/2006, 5/17/2006 and 8/17/2006 are listed above each column of results. Quantitative results for toxic elements over 6 months are depicted in the bar graph (1B). The blue bar denotes reference limits. Similarly, panel 2A shows results for whole blood toxic elements, while 2B represents those changes graphically over 3 time-points. Panel 3A shows results from testing of 6 to 8 hour urinary excretion of toxic elements upon provocation, and 3B shows the bar chart representation of those changes. The values shown in shaded boxes are above 5th quintile limits, but less than 95% interval high limits.

Case Illustration 3.3 — Osteoporosis and Hair Analysis

The hair element profile shown below is from a 37-year-old woman who has high blood pressure and poor stress tolerance. She reports sensations of stomach burning after meals and she has been on hydrochlorothiazide for water retention for the past year.

The pattern of elevated calcium, magnesium, barium and strontium indicates negative calcium balance. The paradoxical elevation of hair calcium in response to negative calcium balance apparently is a response of the hair follicular cells to elevated parathyroid hormone. The other three elements that typically become elevated, as found here, share with calcium the physiological responses to parathyroid hormone. Relatively small amounts of the elements mobilized from bone are passed into the highly metabolically active follicular cells, where they become sequestered in the extruded hair shaft. However, the high capacity of hair for divalent element binding can causes strong elevation of the concentrations in hair as seen here.

The clinician in this case was aware of the patterns' interpretation, so a serum vitamin D test was ordered. The result of 23 nmol/L (32–100) was quite low, providing further explanation for the poor calcium status. The presenting signs may be related to various ionic calcium effects. Hair element profiling has a unique sensitivity for indicating the early signs of chronic negative calcium balance that is the forerunner of osteoporosis. In a 37-year-old woman such a finding before bone loss is detectable can be of great value in preventing the disease by nutrient supplementation (esp. vitamin D, calcium and magnesium) and by encouraging appropriate changes in diet and exercise. ❖

0020 Element Analysis - Hair

Methodology: Inductively Coupled Plasma/Mass Spectroscopy

	Essential Elements	Results mcg/gm		Percentile Ranking by Quintile (1st 2nd 3rd 4th 5th; 20% 40% 60% 80%)	95% Reference Interval
1	Calcium	5,650	H	1000	200 - 1,000
2	Cobalt	15.86	H	0.70	0.05 - 0.70
3	Copper	29		40	7 - 40
4	Chromium	0.71	L	3.00	0.20 - 3.00
5	Iron	32	L	200	20 - 200
6	Magnesium	1,007	H	100	28 - 100
7	Manganese	2.60		4.00	0.20 - 4.00
8	Phosphorus	269		350	89 - 350
9	Strontium	18.8	H	3.0	<= 3.0
10	Barium	24.6	H	10.0	<= 10.0

Case Illustration 3.4 — Ventricular Tachycardia

A 51-year-old female presented with hypertension and paroxysmal supervenventricular tachychardia (PSVT). Her history is significant for years of strict vegetarianism. Her other pertinent labs include a plasma taurine of 10 (range 26–103).

This patient's RBC magnesium level is well below the reference range. Magnesium is well-known for it's cardio-modulating effects, and hypomagnesmia may be associated with PSVT and hypertension. Taurine, a constituent primarily found in animal protein, is frequently low in strict vegetarians. Taurine is present in high concentrations in cardiac tissue and is involved in normal cardiac functioning, presumably modulating intra- and extracellular electrolyte movement. As discussed in the copper section in this chapter, low copper levels may also impact cardiac function, although less directly. As expected, supplementation of magnesium and taurine resulted in decreased PSVT in this individual. ❖

Element - Erythrocyte (RBC)

Methodology: Inductively Coupled Plasma/Mass Spectroscopy

	Essential Elements	Results mcg/gm		Percentile Ranking by Quintile (1st 2nd 3rd 4th 5th; 20% 40% 60% 80%)	95% Reference Interval
1	Chromium	0.25		.70	0.25 - 0.80
2	Copper	0.45	L	1.85	0.52 - 2.00
3	Magnesium	29	L	70	40 - 80

Case Illustration 3.5 — Hemochromatosis and Toxic Elements

Part 1

Chief Complaints: Jennifer is a 58-year-old female who complains of leg pain, fatigue that is worse in the afternoon, nausea, and feels light-headed when standing too long. She has intolerance to cold and experiences occasional heart flutters.

History of Present Illness: She has chronic leg varicosities, which have been stripped and ligated twice, at ages 25 and 57.

Social History: She occasionally consumes excess wine. She is a non-smoker. She works as a real estate agent.

Family History: The patient's father died at 87-years-old, but was generally well. Her mother died of skin cancer at 83-years-old. She has 10 siblings, one of which died from complications relating to multiple sclerosis at 52-years-old.

On Examination: Blood pressure was 150/100 and pulse was 84 (regular). She had trophic changes in her lower legs, with few varicosities. Fingers and toes had a bluish color. Pulses diminished in her feet and her feet were cool to the touch. Capillary refill test was delayed in feet and hands.

Laboratory Testing: Jennifer's standard chemistries were within normal limits. Iron indices are routinely tested by the ordering physician at which time Jennifer's serum ferritin and transferrin saturation were high (see table). She was tested for the hemochromatosis gene *C282Y* and found to be positive and homozygous.

Due to Jennifer's cardiovascular symptoms, which are associated with lead burden, the clinician decided to give her an EDTA urinary challenge test, which demonstrated elevations in cadmium, lead, mercury, aluminum and nickel.

Treatment and Results: Weekly intravenous EDTA chelation therapy and phlebotomy were initiated. After six EDTA treatments, ferritin dropped from 482 µg/L to to 323 µg/L. After three phlebotomies, hemoglobin dropped from 143 g/L to 113 g/L. Phlebotomies were then reduced to every other week. After 47 phlebotomies, blood pressure normalized to 130/78. Patient reports increased sense of well-being and improved energy; leg pain is resolved.

Plan: Continue weekly EDTA chelation and phlebotomy every two months (per hematologist). Run a urinary toxic element assessment after 20 EDTA treatments, then continue with monthly maintenance treatments.

Family Implications: All family members were screened. Of Jennifer's 10 siblings, one is homozygous and four are heterozygous for the hemochromatosis gene. Jennifer's children are heterozygous for the *C282Y* gene. Jennifer's deceased sister's son is homozygous. Given the family genetic

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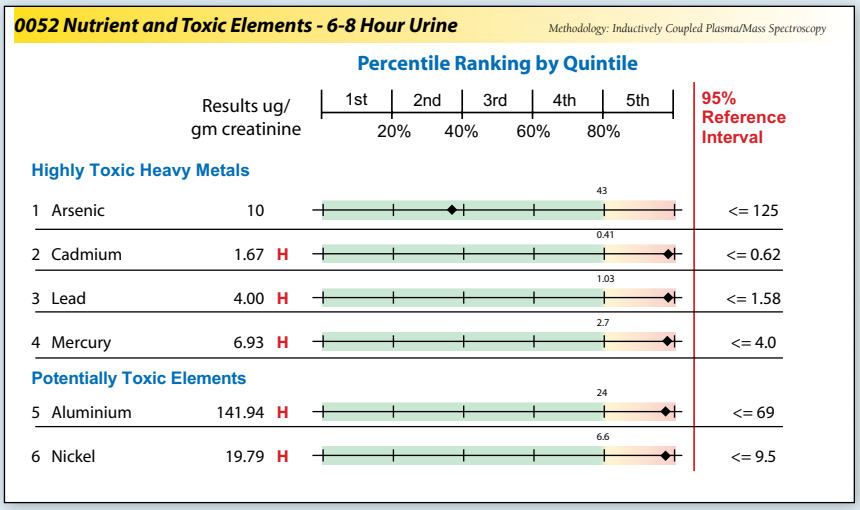


	Pre-Treatment Results		Post-Treatment Results		Normal Range
Hemoglobin	143 g/L	Normal	113 g/L	Low	120–150
Serum Ferritin	482 µg/L	High	16 µg/L	Low	40–200
Iron	28 µmol/L	Normal	24 µmol/L	Normal	10–30
TIBC	41 µmol/L	Low	43 µmol/L	Low	45–75
Transferrin Saturation	68%	High	56%	High	14–50

presentation, mother and father must have been heterozygous carriers of the hemochromatosis gene mutation.

Discussion: Hereditary hemochromatosis is an autosomal recessive trait. The most prevalent form of hereditary hemochromatosis is a mutation of the *HFE* gene, either *C282Y* or *H63D*. Hemochromatosis is characterized by severe fatigue (74%), arthralgia (44%), and hyperpigmentation or skin bronzing (70%). The result of this genetic mutation is high iron stores and high absorption of iron resulting in accumulation of iron in the liver, leading to liver cancer.[905] Excessive consumption of alcohol is often associated with hemochromatosis for unknown reasons. Jennifer's consumption of alcohol probably increased her absorption of iron. Free metal ions in the tissues will increase the rate of free radical generation. The oxidative damage from both iron overload and toxic element overload could be responsible for inflammation, presenting as varicose veins in Jennifer's case.

Treatment with phlebotomy and toxic metal chelation resolved fatigue and leg pain. This case illustrates the importance of using multiple profiles and specimen types to detect imbalances in total-body status for an element. At initial evaluation, serum ferritin and transferrin saturation were the only frankly elevated markers seen in Jennifer while TIBC was low. Transferrin saturation is considered to be the best marker for detecting hemochromatosis and this is evident in this case by its continued elevation even after phlebotomy. Her post-treatment results show low Hb and low serum ferritin, yet she is not a candidate for iron therapy.

This case is courtesy of John Cline, MD, BSc

Part 2

A male welder in his 30s was diagnosed with liver cancer. Previously, diagnostic work-up revealed hemochromatosis, but he did not undergo treatment. Cirrhosis, which can progress to liver cancer, is one of the most common diseases resulting from hemochromatosis-induced tissue damage and the most common cause of death in cases of hereditary hemochromatosis.[905] Results show that ferritin is extremely high. Zinc, cadmium and lead are also elevated in erythrocyte specimen. The cancer is likely related with iron deposition in the liver, occupational heavy metal exposure, and the great oxidative damage brought about by overload of multiple toxic metals.

Chronic Inflammatory Markers

Ferritin	713	H	28 - 397	ng/mL
Fibrinogen	139	L	175 - 400	mg/dL
c-Reactive Protein (HS)	3.6	H	<= 3.0	mg/L

Element - Erythrocyte (RBC) Methodology: Inductively Coupled Plasma/Mass Spectroscopy

	Essential Elements	Results ppm packed cells		Percentile Ranking by Quintile (1st / 2nd / 3rd / 4th / 5th; 20% / 40% / 60% / 80%)	95% Reference Interval
1	Chromium	0.45		0.70	0.25 - 0.80
2	Copper	0.92		1.73	0.52 - 2.00
3	Magnesium	72		72	40 - 80
4	Manganese	0.62		0.70	0.25 - 0.80
5	Potassium	1,266		2,375	1,000 - 2,500
6	Selenium	0.24		0.30	0.12 - 0.40
7	Vanadium	0.15		0.24	0.10 - 0.28
8	Zinc	11.7	H	10.0	6.0 - 11.0
	Toxic Elements				
10	Aluminum	0.6		2.5	<= 3.0
11	Arsenic	0.007		0.014	<= 0.018
12	Cadmium	0.045	H	0.030	<= 0.040
	Repeated and Confirmed				
13	Lead	0.045	H	0.021	<= 0.030
14	Mercury	0.002		0.008	<= 0.010

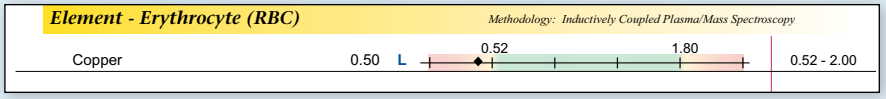
These two cases show adults with both hemochromatosis and toxic element overload. Iron overload disorders have been characterized by abnormalities in proteins that dictate iron regulation such as transferrin receptors, hepcidin, DMT-1 transporters, hemojuvelin and ferroportin.[905–910] Disruption in normal iron homeostatic mechanisms caused by these various mutations may explain accumulation of iron and toxic elements in parallel. Because many patients have a toxic metal body burden, the heavy metal burden in these two cases of hemochromatosis may be only coincidental. Nonetheless, management of a patient with iron overload warrants evaluation of toxic metal burden and treatment. ❖

Case Illustration 3.6 — Fatigue and Copper Deficiency

A 39-year-old female presented with mild fatigue. She was interested in a general wellness evaluation and denied any other complaints. Blood chemistries, adrenal stress, sex and thyroid hormone laboratory studies were unremarkable.

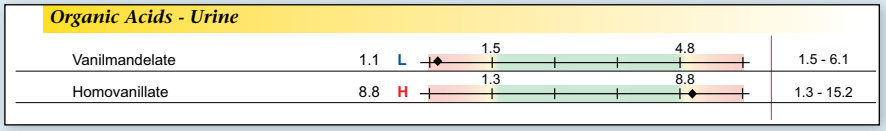
Three different types of laboratory findings using both direct and functional markers demonstrated a modest copper insufficiency in this case. **(1)** Direct assessment of RBCs demonstrates low copper.

1



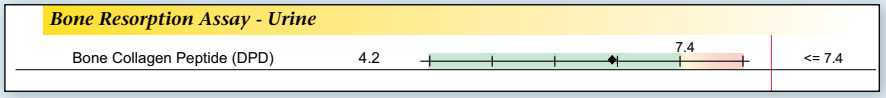
(2) Functional evaluation of copper's deficiency is positive, as evidenced by the elevated homovanillate to vanilmadelate ratio (HVA/VMA). Homovanilate is the main catabolite of dopamine, and vanilmadelate is the main catabolite of norepinephrine and epinephrine. An elevated ratio may demonstrate low copper availability impacting activity of the cupro-enzyme dopamine beta-hydroxylase, which converts dopamine to norepinephrine. Poor catecholamine availability is likely contributing to this individual's fatigue.

2



(3) Copper deficiency has also been shown to cause abnormal bone resorption via reduced activity of the cupro-enzyme lysyl oxidase. Copper deficiency can, thus, cause lowered levels of bone resorption markers, giving false negative results. Bone loss may be occurring due to impaired rates of formation, even though the rate of resorption is normal. From these data showing various manifestations related to copper status we may deduce that although the HVA/VMA and RBC copper show copper deficiency, the normal bone collagen peptide marker (deoxyperidinoline DPD) demonstrates that the copper deficiency has not yet impacted bone metabolic activity. The more copper-sensitive catecholamine pathway may be normalized by improving copper status which also reduces risk of bone loss. ❖

3



Notes:

Case Illustration 3.7 —
Iodine Deficiency and Thyroid Goiter

This 53-year-old white female had a partial thyroidectomy (right lobe) 10 years ago. Several aspirations from the right lobe had been done over the three years prior to actual thyroid surgery. The aspiration needle biopsies always showed "a benign colloid lesion." An ultrasound 13 years ago described the right lobe as a "large, complex, predominantly cystic mass which statistically most likely reflects a benign nodule but the sonographic appearance is nonspecific and additional etiologies cannot be excluded." Her TSH levels during that time remained within normal limits. A total T4 level was in the lower normal range prior to surgery.

Eight years after her partial thyroidectomy, while on Armour Thyroid, she had a TSH level of 0.5 uIU/mL (0.35–5.50 normal limits). A 24 hour urine iodine loading test and an overnight urinary iodine were measured. The loading test included an oral dose of 50 mg of potassium iodide and collection of urine for the next 24 hours. The premise of this test is that if the patient is deficient in iodine, a large portion of the iodine bolus will be retained, resulting in low urinary iodine excretion. In an iodine-replete patient, more than 90% of the bolus will be excreted.

The overnight urinary iodide concentration was low, consistent with iodine deficiency. Only 19 mg of the 50 mg oral bolus was excreted in 24 hours. The 37% excretion/24 hr result further indicated iodine deficiency. The patient was treated with 25 to 50 mg potassium iodine/iodide daily for 6 months and retested.

Urinary Iodine - 6-8 Hour and 24 Hour Urine

	Results		Reference Limits
Before treatment			
Spot Iodide	0.11		0.1 - 0.15 mg/L
24 Hr, Urine Loading Iodine/Iodide	19	L	> 44 mg/24 h
% Excretion/24 h	37%	L	> 90%
After treatment			
Spot Iodide	3.74	H	0.1 - 0.15 mg/L
24 Hr, Urine Loading Iodine/Iodide	40	L	> 44 mg/24 h
% Excretion/24 h	79%	L	> 90%

After treatment, the patient felt more energy than she had for years. She reported warmer body temperature, thicker hair and nails, resolution of fluid retention and her normalized body weight. She was able to decrease her dose of Armour Thyroid from 90 mg BID to 45 mg BID, and, if she misses an afternoon dose she feels fine. The patient continues to take thyroid hormone to avoid risk of recurrence of goiter in the remaining lobe, although there is no current evidence of goiter. The frank iodine deficiency found in this case brings into question the need for thyroidectomy without thorough iodine status evaluation. ❖

This case is courtesy of Carolyn R. Walker, MSN, ARNP.

Notes:

Case Illustration 3.8 — Toxic Elements in Urine of Patients Living in Western Massachusetts

Berkshire County is in rural western Massachusetts. The area drew early European immigrant settlement because of the powerful Housatonic River that runs southward through the county. In addition to industrial facilities such as the General Electric Plastics Processing Plant that poured non-biodegradable organic compounds such as PCBs into the Housatonic, heavy agricultural activities in the river basin have resulted in heavy metals from pesticides and fertilizers in water and soil. The three cases shown below have resided all or most of their lives in Berkshire County. The data are from 24-hour urine specimens obtained after DMSA provocation. They show elevated body burdens of lead and, in one case, simultaneous very high arsenic exposure. In addition to treatment for toxic metal burden, a thorough investigation of the potential sources for these exposures, including but not limited to water, water pipes and house paint is indicated for all three of the patients. ❖

Case 1

Case 2

Case 3

24 Hour Urinary Toxic Elements

	Highly Toxic Heavy Metals	Results ug/day		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
Case 1					
1	Arsenic	13		48	<= 104
2	Cadmium	0.20		0.49	<= 0.69
3	Lead	1.7	H	1.1	<= 1.6
4	Mercury	< 0.5		1.1	<= 5.0
Case 2					
1	Arsenic	71		48	<= 104
2	Cadmium	0.66		0.49	<= 0.69
3	Lead	9.3	H	1.1	<= 1.6
4	Mercury	< 0.5		1.1	<= 5.0
Case 3					
1	Arsenic	556	H	48	<= 104
2	Cadmium	0.58		0.49	<= 0.69
3	Lead	2.3	H	1.1	<= 1.6
4	Mercury	< 0.5		1.1	<= 5.0

Notes:

Case Illustration 3.9 — Neurologic Effects of Lead Poisoning

A 16-year-old female presented with episodic ataxia, severe fatigue, muscle twitching and difficultly concentrating. Due to the severity of the symptoms, the patient stopped attending high school. She has a past medical history that includes a diagnosis of Lyme disease in 2002. Her mother is a physician who has regularly assessed her daughter for nutrient and toxicant levels as a part of a general wellness protocol **(A)**. Toxic metal screenings were normal until 2005, when the onset of the symptoms symptoms noted above. An elemental profile ordered on a urine specimen taken after intravenous EDTA challenge revealed profoundly elevated lead. **(B)**. The exposure source was determined to be a single water fountain at school from which the patient and her friends regularly drank for three years. Her friends who drank from the same fountain also reported experiencing similar symptoms, most commonly associated with compromised memory, concentration and fatigue.

Water testing revealed that their drinking fountain lead levels greatly exceeded the EPA acceptable limit of 15 ppb **(C)**. An investigation discovered that the water fountain had been recalled by the manufacturer due to a faulty refrigeration holding tank that was leaching lead into the water.

The patient's lead levels have slowly dropped over ensuing years **(D,E,F)**, with whole blood lead returning to a normal level in July 2007. Symptomatically, the patient continued to struggled with her chief complaints through early 2007. The fragility of her condition, which appears to be exacerbated by the Lyme disease, has made aggressive interventions intolerable, and therefore only very low doses of IV EDTA have been used. However, in the summer of 2007, symptoms abated in this patient enough to allow her to plan on a return to high school, with expectations of a fall graduation. ❖

	Result		95% Reference Interval
A. *2002 RBC Lead Levels (pre-exposure)*			
Lead	0.04		<= 0.10
B. *2005 24-hour Urine Lead Levels (with EDTA Provaction)*			
Lead	42 ug/24 hour	H	< 5 ug/24 hour
C. *2005 Water Lead Levels*			
Lead	205 ppb	H	< 15 ppb
D. *RBC Lead 2006*			
Lead	0.117	H	<= 0.030
E. *Whole Blood Lead 2006*			
Lead	12	H	< 10 ug/dl
F. *Whole Blood Lead 2007*			
Lead	1.9 ug/dl		< 10 ug/dl

Notes:

Case Illustration 3.10 —
Toxic Metals In Dementia

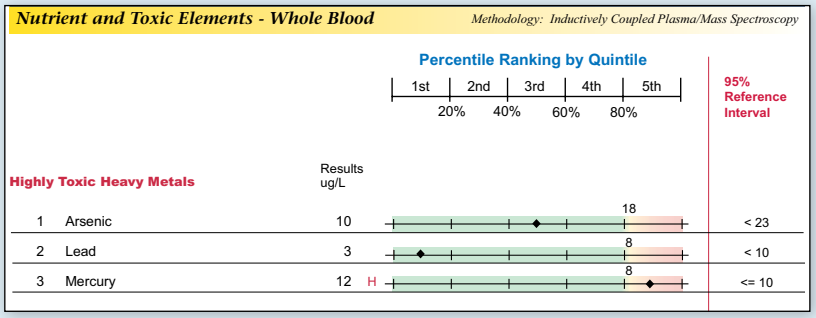
An 82-year-old female presented with dementia and an essential tremor for 10 years. Mercury amalgam removal had been performed five years ago. Element profiles in blood and urine were ordered simultaneously. The urine specimen was collected after challenge with a single gram oral dose of DMSA.

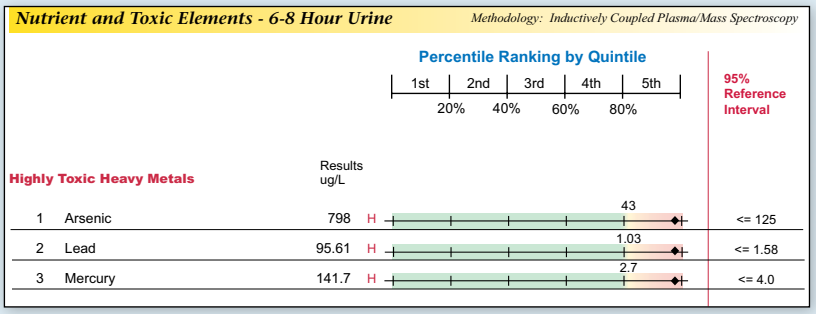
Discussion: The positive whole-blood mercury demonstrates continued elevated body burden or ongoing exposure despite amalgam removal five years ago. The urine mercury elevation corroborates the blood findings. Lead has a half-life in whole blood of about 36 days; thus, the normal whole-blood lead level coupled with an elevated chelated urinary specimen is indicative of past exposure. with current loading of renal metallothionein. Similarly, past exposure to arsenic that has been cleared from blood, accumulating in metallothionein and other proteins is indicated by the normal blood and strongly elevated challenged urine results. Arsenic exposure may be from seafood, which can contain high levels of organoarsines.

Plan: A thorough exposure history is indicated for this patient, including evaluation of exposure sources, although the dementia will likely compromise patient reliability in providing an accurate narrative. Other investigations such as a hematological work-up for anemia may be useful to assess the extent of toxic affects.

Treatment: Given the pronounced elevation of toxic metals in this individual, a detoxification protocol is needed. However, potential benefits must be weighed against this 82-year-old patient's ability to tolerate the treatments.

This case illustrates the need for an exposure history and toxic metal screening to be incorporated into general preventative medical guidelines. It is likely that the toxic metal burden has contributed to this individual's conditions, although such a late-stage discovery can minimize the efficacy of treatment. ❖





Notes:

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Chapter 4

Amino Acids

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CONTENTS

Notes:

TABLE 4.1 — SUMMARY OF NUTRIENT-RELATED AMINO ACID ABNORMALITIES

Amino Acid	Low Result	High Result
Anabolic/Catabolic Responses and Tissue pH Regulation		
Glutamic Acid (Glu)		α-KG, 600 mg TID
Glutamine (Gln)	*	α-KG, 600 mg BID; B_6, 100 mg
The Urea Cycle and Nitrogen Management		
Arginine (Arg)	* (Arg, 500 mg)	Mn, 15 mg, Excessive L-lysine supplementation
Asymmetric dimethylarginine		Arg, 3–6 g
Citrulline		Mg, 200 mg BID; α-KG, 600 mg BID
Ornithine (Orn)		Mg, 200 mg BID; α-KG, 600 mg BID, Excessive L-lysine supplementation
Aspartic Acid (Asp)	α-KG, 600 mg TID	Mg, 200 mg BID; Zn, 25 mg
Asparagine (Asn)	Mg, 200 mg BID	α-KG, 600 mg BID; B_6, 100 mg
Essential Amino Acids for Proteins and Energy		
Isoleucine (Ile)	*	B_6, 100 mg; Check for insulin insensitivity
Leucine (Leu)		
Valine (Val)		
Threonine (Thr)	*	B_6, 100 mg
Histidine (His)	Folate, 800 µg; His, 500 mg TID	B_6, 100 mg
Lysine (Lys)	Carnitine, 1–2 g Associated with increased stress response Check for excessive Arg intake	Vitamin C, 1 g BID; niacin, 50 mg; B_6, 100 mg; Fe, 15 mg (confirm with ferritin). Supplementation tends to decrease arginine. Excessive lysine can contribute to kidney pathology
α-Aminoadipic acid		B_6, 100 mg; α-KG, 300 mg TID
Neurotransmitters and Precursors		
Phenylalanine (Phe)	*	Fe, 30 mg; Tetrahydrobiopterin; Vit. C, 300 mg/d; Low-Phe diet
Tyrosine (Tyr)	Fe, 30 mg, Tyr, 500 mg TID	Cu, 3 mg; Fe, 30 mg; Vitamin C, 1 g TID
Tryptophan (Trp)	5-HTP, 50 mg TID	Niacin, 50 mg; B_6, 100 mg BID B_6, 100 mg; Fe, 30 mg; Cu, 3 mg
α-Amino-N-butyric acid	α-KG, 300 mg TID; B_6, 100 mg	
γ-Aminobutyric acid (GABA)		α-KG, 600 mg BID; B_6, 50 mg
Sulfur-Containing Amino Acids for Methylation and Glutathione Synthesis		
Methionine (Met)	*	B_6, 200 mg; α-KG, 600 mg BID
Cystine (Cys)	NAC, 500 mg BID	
Homocystine (HCys)		B_6, 100 mg; Folate, 800 µg; B_{12}, 1,000 µg; Betaine, 1,000 mg
Cystathionine		B_6, 100 mg
Taurine (Tau)	B_6, 100 mg	**
Precursors of Heme, Nucleotides and Cell Membranes		
Glycine (Gly)	Gly , 1,000 mg TID	Folate, 800 µg; B_6, 100 mg; B_2, 50 mg; B_5, 500 mg
Serine (Ser)	* B_6, 100 mg; Folate, 800 µg, Mn, 15 mg	Thr, 500 mg BID; BCAAs, 2 g TID
Sarcosine		B_2, 50 mg

Table 4.1 continued on following page...

Table 4.1 continued from previous page...		
Amino Acid	**Low Result**	**High Result**
Precursors of Heme, Nucleotides and Cell Membranes *(continued)*		
Alanine (Ala)	*	B_6, 100 mg
Ethanolamine		Mg, 200 mg BID
Phosphoethanolamine		S-Adenosylmethionine, 200 mg BID
Phosphoserine		Mg, 200 mg BID
Bone Collagen-Specific Amino Acids		
Proline (Pro)	α-KG, 600 mg BID; His, 300 mg	Vitamin C, 1,000 mg TID; Niacin, 50 mg
Hydroxyproline (HPro)		Vitamin C, 1,000 mg TID; Fe, 15 mg
Hydroxylysine (HLys)		Vitamin C, 1,000 mg TID; Fe, 15 mg
β-Amino Acids		
β-Alanine		*Lactobacillus* and *Bifidobacteria* sp.; B_6, 100 mg
β-Aminoisobutyric acid		B_6, 50 mg; B_{12}, 100 mg
Anserine		Zn, 30 mg
Carnosine		Zn, 30 mg
The Methylhistidines		
1-Methylhistidine		Vitamin E, 400 IU
3-Methylhistidine		BCAA,**6–9 g (if evident muscle wasting)

** Low levels are generally best normalized with balanced or custom mixtures of essential amino acids*
*** Multiple antioxidants: vitamin E, 800 IU; vitamin C, 1 g TID; β-carotene, 25,000 IU; coenzyme Q_{10}, 100 mg; lipoic acid, 300 mg*

Table 4.2 — Salient Features of Amino Acid Metabolism

Amino Acid	Unique Metabolic Feature	Physiological Effects
Glutamine	SN1-SN2 transporter modulated by pH Major gluconeogenic precursor Major amine group supplier	Principal tissue pH buffering system Principal amino acid blood supply of carbon for gluconeogenesis Principal blood supply of nitrogen
BCAA	Branched-chain ketoacid dehydrogenase complex (BCKDC)	Regulates rate of BCAA degradation
Glycine	Glycine Clearing System – Major metabolic fate of glycine – Stimulated by glucagon – cAMP mediated	Conditions such as fasting and low-carbohydrate diets that call for glucagon-stimulated glucose mobilization can cause high plasma glycine
Histidine	Transported via SN1 and SN2 membrane system shared by glutamic and aspartic acids Dietary deficiency has blunted effect on levels in plasma and urine due to release from hemoglobin and sarcosine Binds zinc and copper	Defects of transporter system proteins will produce elevated plasma histidine Anemia from increased hemoglobin degradation Excessive intake causes low metallothionein and loss of zinc and copper
Lysine	Not catabolized by initial transamination Stimulates cholesterol biosynthesis Blocks 5-HT4 serotonin receptors	High plasma lysine and glycine and low arginine and BCAAs are associated with hypercholesterolemia Explains favorable effect of lysine supplementation on stress-induced diarrhea and IBS
α-AAA	Product of lysine via pipecolic acid	Elevation reflects B_6 deficiency or hepatic peroxisome insufficiency
Tryptophan	Plasma level related to serotonin synthesis	Proposed as cause of fibromyalgia
Tyrosine	Phenylalanine hydroxylase – not produced in PKU – dependent on iron (vitamin C), tetrahydrobiopterin and NAD	Low – hypothyroidism, catecholamine deficit Low – need for iron, folic acid, vitamin B_3 Watch for high Phe/Tyr ratio indicating milder forms of PKU, some of which respond to tetrahydrobiopterin

AMINO ACIDS IN HUMAN HEALTH

Amino acids are central to virtually every function of the human body. Of the 20 amino acids required for synthesis of proteins, nine must be derived from dietary protein because they cannot be produced in human tissues. The elaborate gene expression mechanisms of all cells result in the assembling of amino acids into thousands of specific proteins that make up the structures and catalyze the metabolic reactions necessary for life. In addition to protein synthesis, individual amino acids flow into pathways that produce hormones and neurotransmitters, detoxify thousands of chemicals, supply antioxidant protection and build bile acids for digestion.

In spite of their universal demand for life functions, free amino acids are present in very low amounts per total body content, most occurring in the circulating blood. When demand for amino acids increases, they must be supplied by dietary sources or from turnover of body proteins. This situation means that the pool of amino acids is highly dynamic, changing moment by moment by shifting the flow though dozens of metabolic pathways in response to multiple physiological signals. In perfused liver, removal of amino acids from the perfusate causes dramatic and almost instantaneous increase of proteolysis.[1] Because so many processes are affected by amino acids, knowing when and how to implement amino acid therapies has great clinical value.[2] The full clinical potential of nutritional, hormonal and detoxifying therapies can be achieved only when amino acid supply for tissue restoration is assured.

The assessment of amino acid status is particularly challenging when the object is to identify restrictions that might contribute to suboptimal function and the loss of organ reserve in chronic diseases. This chapter presents considerable detail about each of the amino acids commonly measured by laboratory testing of body fluids. The overall outlook is that patterns observed by measurements of amino acids in whole blood, plasma, or urine may be used to identify patients who are candidates for amino acid therapy. The information about individual amino acids may then be used to adjust the therapeutic emphasis and to explain clinical responses to therapy. A patient who cannot sustain normal levels of essential amino acids in fasting plasma or whole blood may be a candidate for individualized amino acid supplementation to assure optimization of total body function. The question of how "normal" is defined in this context must be addressed.

Notes:

The initial clinical use of amino acid testing was for detection of neonatal inborn errors of metabolism (IEM) where only abnormally high levels are of interest. Low limits frequently fall at (or even below) zero when they were calculated as the mean minus two times the standard deviation.[3] To meet the need of identifying patients who may not have frank metabolic diseases of genetic origin, yet still have need for amino acid therapy, it is useful to use the first quintile (lower 20% of reference population) as a cutoff point. In other words, when 80% of the reference population shows essential amino acid levels higher than those in a patient with chronic disease, then that patient may be considered a candidate for essential amino acid enhancements.

Ingested dietary protein is digested to release amino acids, which are absorbed into the bloodstream in the small intestine. The efficiency of protein digestion depends on adequate residence time in the stomach where food must be mixed with large amounts of hydrochloric acid to initiate protein denaturation and to stimulate pancreaticobiliary fluid flow. Partially digested chyme, leaving the stomach in small portions, elicits bicarbonate-rich pancreatic secretion along with bile from the gall bladder to assist protein unfolding and digestive enzyme activation. Various classes of amino acids are absorbed most actively in discrete regions of the jejunum and upper ileum by active transport, resulting in their passage through enterocytes and into capillaries. Blood drains from the small intestines into the portal circulation that delivers amino acids to the liver. The liver possesses numerous enzyme systems for metabolism of amino acids and during sleep the liver carries out net synthesis of some amino acids to sustain blood (plasma)

levels for detoxification and biosynthetic processes active at that time. The physiological stresses of digestion result in significant daily losses of protein (digestive enzymes, desquamated cells and mucin) and account for a major part of dietary amino acid requirements.[4]

The main objective of this chapter is to explain how laboratory testing can identify patients in need of therapeutic supplementation of essential amino acid mixtures, individual amino acids, or other therapies to correct abnormal amino acid status. Abnormally low or high amino acid levels in plasma or urine can be caused by dietary deficiencies of protein or micronutrients, variations in metabolic demands elicited by hormones, or toxic factors that increase losses or decrease utilization rates. Insufficient amino acid status can result from digestive impairments due to toxicants or lifestyle factors or from pathologies of the stomach, pancreas, liver, or small intestine.

Amino Acids Classifications

Since only L-amino acids are utilized in human metabolism, the stereoisomer is presumed to be L- and the designation will be dropped for simplicity. Therefore, all undesignated references such as "alanine" refer to "L-alanine." The nine essential amino acids (EAA) are sometimes referred to as indispensable amino acids because they must be obtained from food intake. Transfers of amino groups to organic acids that are produced in other metabolic pathways can form the other 11 amino acids used for protein synthesis. These 11 are sometimes called dispensable or non-essential amino acids (NEAA). Because of limitations in the rates of their formation some amino acids that are technically non-essential become essential to maintain optimal wellness under specific conditions of disease or genetic and environmental factors. These are called conditionally essential amino acids (CEAA). Other amino acids, such as hydroxyproline, are formed by reactions that add chemical groups after proteins are synthesized. Such amino acid derivatives are released into body fluids at cell death or during protein turnover in viable cells. Hydroxyproline and hydroxylysine are also released from turnover of the extracellular matrix that is largely composed of collagen. Biochemical transformations in detoxification or neurotransmitter pathways lead to the formation of still other amino acids and simple peptides. All of the amino acids and derivatives listed in Figure 4.1 may be measured in profiles of amino acids in plasma or urine.

Protein synthesis requires a basic set of 20 amino acids. Under conditions where all other essential nutrients are available, human adults can usually produce the 11 NEAA at rates adequate to sustain growth and reproduction. Arginine is essential during the first several months after birth, because infants are unable to produce sufficient amounts to sustain growth. Dietary or supplemental arginine also is beneficial for adults under a variety of conditions that create special demands. Amino acids that exhibit such clinical characteristics may be called conditionally essential. Glycine, glutamine and tyrosine are included in this category because they can help overcome conditions where their biosynthesis is inhibited or their physiological demands are increased.

Essential Amino Acids
Histidine
Isoleucine
Leucine
Lysine
Methionine
Phenylalanine
Threonine
Tryptophan
Valine

Conditionally Essential Amino Acids
Arginine
Glutamine
Glycine
Taurine

Non-Essential Amino Acids of Proteins
Alanine
Asparagine
Aspartic acid
Cysteine
Glutamic acid
Proline

Amino Acid Derivatives
α-Alanine
α-Aminobutyrate
α-Aminoisobutyric acid
Asymmetric-dimethylarginine
Citrulline
Cystine
Cystathionine
Homocysteine
Homocystine
Hydroxyproline
Hydroxylysine
1-Methylhistidine
3-Methylhistidine
Methionine sulfoxide
Nitrotyrosine
Ornithine
Phophoethanolamine
Phosphoserine

Figure 4.1 — Amino Acids Classifications

In this categorization, amino acids that do not meet the strict definition of nutritional essentiality, but may be beneficial under special circumstances are grouped as conditionally essential.

AMINO ACID REQUIREMENTS FOR PROTEIN AND POLYPEPTIDE SYNTHESIS

Protein and polypeptide synthesis produces the major flux of amino acids supplied by blood flow. The 20 peptide-forming essential and non-essential amino acids are universally required for enzymes, structural and transport proteins and cell-signaling polypeptides. This flux of amino acids to load tRNA for protein synthesis is the universal background for discussions of amino acid status. In addition, the carbon skeletons from amino acids flow into gluconeogenesis or fatty acid synthesis as demands for energy dictate. Amino acid nitrogen flows into pathways of nucleotide, neurotransmitter and polyamine biosynthesis. From studies in rats, we have learned that prenatal amino acid deficiency produces offspring with preference for energy-dense foodstuffs and hyperphagia, along with tendencies for greater insulin resistance, glucose intolerance, adiposity and hypertension.[5]

Amino acid catabolism increases to support gluconeogenesis during starvation. Glucocorticoids and glucagon up-regulate, while insulin down-regulates, the gene expression of many amino acid-catabolizing enzymes.[6] The rate of catabolism of most amino acids increases when dietary protein exceeds the body's requirements. Gene transcription rates are adjusted to achieve regulation of the catabolic sequences. When amino acids are catabolized in energy-yielding pathways

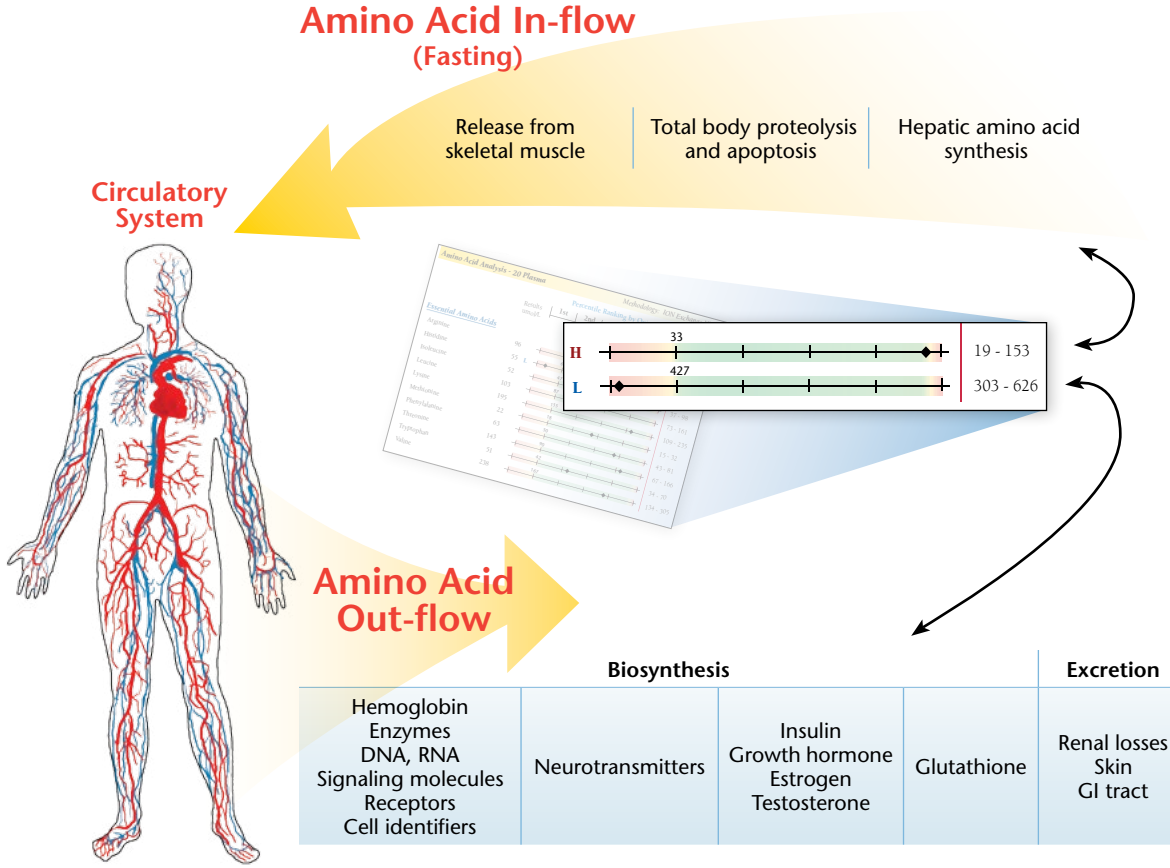


FIGURE 4.2 — Sources and Destinations of Plasma Amino Acids

The amount of amino acids in the free form in plasma is very small compared with plasma protein concentrations. In humans, biosynthesis cannot yield EAA and in the fasting state portal supply is absent. Concentrations of EAA measured in fasting plasma, therefore, are determined by the flux into the blood from tissue protein degradation balanced with flux out of the blood for protein synthesis, excretion, oxidation and formation of non-protein products.

Table 4.3 — Special Metabolic Functions of Amino Acids

Function »»	Glucose Synthesis	Urea Cycle	Glutathione Synthesis	Neurotransmitters	Creatine	DNA & RNA
Amino Acid						
Alanine	√√					
Arginine	√	√			√	
Aspartic acid	√	√				√√
Asparagine	√					
Cysteine	√		√√			
Glutamic acid	√	√	√	√		
Glutamine	√	√√				√
Glycine	√√		√		√	√
Histidine	√			√		
Methionine	√		√			
Phenylalanine	√			√		
Serine	√√			√		
Threonine	√					
Tryptophan	√			√		
Tyrosine	√			√		
Valine	√					

The carbon skeletons of most amino acids are able to enter into the biosynthesis of glucose. Those with multiple check marks are most easily transformed into glucogenic compounds and they play important roles in the maintenance of blood glucose during intervals of fasting. A smaller number of amino acids are involved in each of the other special functions.

or in skeletal muscle during strenuous exertion, toxic ammonia that is formed from their amino groups must be removed. In the liver, amino acids are used to produce transport proteins such as albumin, prealbumin, ferritin and transferrin. In the pituitary gland, all of the releasing hormones are polypeptides requiring the 20 protein-forming amino acids.

Since the process of polypeptide biosynthesis is relatively constant, it is the other amino acid-specific pathways that generally control the concentrations of individual amino acids measured in plasma or other body fluids. Figure 4.2 presents a diagram of the amino acid flux into the various pathways that determine plasma concentrations of amino acids. Most can serve as carbon sources for glucose synthesis and several serve demanding roles in synthesis of critical molecules like DNA, RNA and glutathione (Table 4.3). Neurons in the central and peripheral nervous system depend on the supply of amino acids for synthesis of neurotransmitters and for control of neuronal activation.[7,8] Intermediates formed by catabolic breakdown regulate cell activity. For example, arginine forms nitric oxide, the polyamines form putrescence and spermine and tryptophan forms quinolinic acid. Some of the symptoms that are frequently associated with impairment of various non-protein functions of individual amino acids are summarized in Table 4.4.

Notes:

Table 4.4 — Symptoms Associated with Impairment of Non-Protein Functions of Amino Acids

Amino Acid	Function	Symptoms of Insufficiency
Arginine	Ammonia removal[9]	Lethargy, irritability, nausea[10]
	Nitric oxide precursor	Hypertension[11]
	Creatine precursor[12]	Weakness
Histidine	Histamine[13]	Anemia,[14] sleep disorders[15,16]
Methionine & Cysteine	Glutathione, taurine, sulfate precursor[17]	Chemical sensivity
Glycine & Serine	Heme,[18] creatine, bile acids, glutathione, nucleic acid bases, methyl group metabolism, collagen precursor[19]	Anemia, depression,[20,21] poor detoxification[22]
Lysine	Carnitine precursor	Hypoglycemia, fatigue, CVD[23]
Tryptophan	Serotonin, melatonin, nicotinic acid precursor	Depression,[24] insomnia[25]
Phenylalanine & Tyrosine	Catecholamines, thyroid hormones, melanin precursor	Hypothyroid, lassitude, mental/emotional disorders,[26,27] schizophrenia[28]

Fasting Plasma Amino Acids

The cells of the liver, intestines and kidneys deliver amino acids to blood, supplemented by the release of non-essential amino acids from skeletal muscle to supply the demands of other tissues during the overnight fasting state. In this state, the influence of dietary protein is reduced so the primary factors influencing amino acid concentrations in blood plasma are the output from muscle and uptake for hepatic protein synthesis offset by hepatic release of amino acids from proteolysis (Figure 4.2).

Utilization of amino acids is highly tissue and time dependent. During sleep, the rate of brain melatonin synthesis increases and many tissues are more actively removing amino acids from the blood for tissue repair, neuronal plasticity and detoxification. In the morning, cortisol rises and amino acids are broken down from skeletal muscle and oxidized for energy. The demand for amino acids in individual tissues determines the rates of uptake. For example, postprandial gastric mucosal cells and mast cells during allergy seasons have a large demand for histidine to supply the formation of histamine.[29–31] Tryptophan demand by the cells in the small intestines is increased during episodes of diarrhea and serotonergic cells in the brain increase their utilization of tryptophan when SSRI medications are being used. The reason for the SSRI effect is the increased catabolism of serotonin that is maintained in the synapse under the influence of the drug.

The amount of an essential amino acid in plasma determines the rate of any dependent process in the tissues. For example, low plasma tryptophan results in reduced formation of serotonin in the brain.[32] The gut is an even larger consumer of tryptophan for serotonin synthesis, though this GI mucosal process is diminished in inflammation.[33] Low histidine reduces the potential for increasing flow of histamine. High plasma essential amino acid levels show either increased release due to catabolic states (see "The Methylhistidines" section later in this chapter) or decreased utilization as is seen when hepatic activity is inadequate to clear portal amino acid flow.[34]

Differences in Amino Acid Status Between Individuals

Inherited Metabolic Diseases Effects

Amino acid testing is widely used for neonatal screening and confirmation of genetic abnormalities in amino acid metabolism. The United States public health programs are estimated to screen 4.1 million infants annually.[35] Recently 14 aminoacidopathies were recommended for inclusion in all state screening programs: phenylketonuria, maple syrup urine disease, homocystinuria, citrullinemia, argininosuccinic acidemia, tyrosinemia type I, benign hyperphenylalaninemia, tyrosinemia type II, defects of biopterin cofactor biosynthesis, argininemia, tyrosinemia type III, defects of biopterin cofactor regeneration, hypermethioninemia and citrullinemia type II.[36] Phenotypes of genetic diseases produce effects that range from severe to mild.[37,38] The total

number of potential genetic polymorphisms that can affect amino acid status is too large to estimate. When phenotypic expression is mild, metabolic abnormalities may go undetected since overt pathology may not appear until later in life. Quantitative data on amino acid levels are therefore useful for early identification of mild phenotypes. Simple nutritional interventions can be effective for reducing morbidity and mortality.

Life-Cycle Effects

Effects of altered amino acid status may be seen very early in life as alterations of cell differentiation in utero that result in difficulties with blood pressure regulation after birth.[39] The plasma concentrations of some amino acids have been shown to be age specific in healthy children.[3,40] Rapid decreases during the first year of life are followed by gradually increasing concentrations that stabilize at 14 to 16 years of age. The age-related changes found for 18 amino acids are clustered within 15% of adult levels. Lysine and tryptophan concentrations, however, change approximately 30% from birth to adolescence. Comparison of North American and Taiwan Chinese populations show that plasma amino acid concentrations vary with age similarly in both groups, independent of race and diet.[41]

Dietary Protein Intake

Estimates of average dietary protein intake required to sustain amino acid status in healthy adult populations are of very limited usefulness in assessing the amino acid need of individual patients. In part because of new methods offered by research in proteomics and nutrigenomics, the question of adequate levels of amino acid intake has been the subject of numerous recent reports. Not only can low levels of catabolic products be measured in body fluids, but also individual differences in specific enzymes, such as the cytochrome P450s, can predict individuals who may have difficulty sustaining amino acid concentrations.[42–46] Conclusions regarding the amino acid needs for adults have been based primarily on nitrogen balance studies. Needs for infants and children are predicted from the smallest amounts compatible with normal growth. Even within a single study, widely ranging variability of individual needs are found to cluster around the average adult intake of 105 mg protein nitrogen (0.74 g protein) per kg per day required to sustain nitrogen balance. Some individuals were in nitrogen balance at 50 mg N (0.35 g protein)/kg/d, whereas others required 150 mg N (1.06 g protein)/kg/d.[47,48]

When dietary changes cause limitations of the essential amino acid pool, enzyme activities change to conserve tissue supply of individual amino acids.[49] For example, during nutritional stress, cell division rate slows. The return to normal growth rate when amino acids become available is critically dependent on responses such as expression of the CAT-1 (cationic amino acid transport) gene for the utilization of arginine and lysine, which is essential for cell survival.[50] In experimental animals when dietary protein was lowered from 21% to 6% of caloric intake, essential amino acids were reduced by an average of 36% (Figure 4.3). The branched chain amino acids correlated with dietary protein decline, but the non-essential amino acids increased as protein intake decreased because there was increased mobilization of amino acids from muscle to meet physiological demands. When these investigators examined hormonal

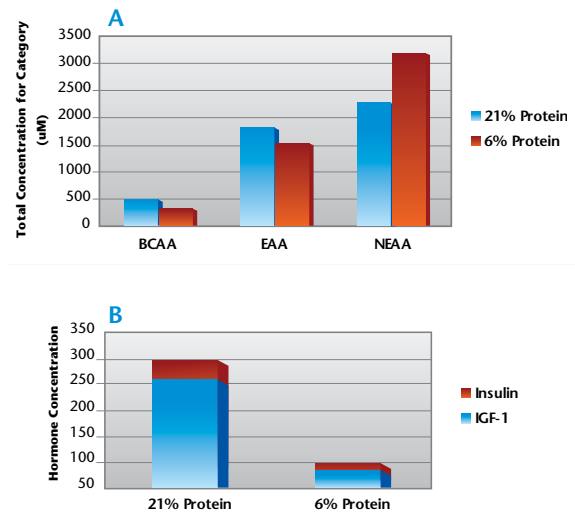


FIGURE 4.3 — **Responses to Dietary Protein Restriction**

Although branched-chain and other essential amino acids show the expected fall when dietary intake is restricted, plasma levels of non-essential amino acids tend to rise (A). Glutamine is the major contributor to this rise, as periveinous hepatocytes increase output to supply nitrogen to critical tissues for protein synthesis. Chronic low intake of protein induces hormonal responses (B). Insulin and especially insulin-like growth factor-I (IGF-I) fall in order to decrease the flow of amino acids into tissues so that plasma levels can be maintained for critical functions.

responses, insulin and insulin-like growth factor-I (IGF-I) were shown to fall dramatically with lowered protein intake.[51] By shifting signals to lower metabolic demands, this response assures that plasma amino acid levels do not drop so low as to prevent critical functions like neurotransmitter formation in brain and cardiovascular tissues. When dietary protein that has been imbalanced by depleting histidine or threonine is fed to rats, a single meal causes increased uptake of limiting amino acids with concurrent decreases of plasma amino acid concentrations.[52] Such rapid transport response mechanisms serve to maintain constant tissue flux of amino acids as intake patterns shift. They also have obvious bearing on interpretation of plasma amino acid results for patients on unbalanced diets.

Protein Digestion and Amino Acid Absorption

The suppression of stomach acid can strongly inhibit digestion of dietary protein in the stomach and the effects can extend to the small intestine by affecting pancreatic function.[53–57] Impaired digestion and assimilation account for some of the individual variability of protein requirements because high digestive efficiency means that lower protein intake levels can supply adequate delivery of amino acids for tissue functions. If emotional stress or use of antacid medications decrease stomach acid below the level needed to initiate the digestive processes, then a larger fraction of dietary protein remains undigested; amino acids are not released and the peptide residues pass into the lower gut where growth rates of bacterial populations are stimulated. During periods of acute stress, protein breakdown increases by as much as 20% with concurrent increased amino acid demands.[58]

Lowered status of essential amino acids may result from inadequate secretion of pancreatic protease enzyme activity. Because zinc is required as a cofactor in several pancreatic proteases, a deficiency of this element can affect plasma amino acids.[59,60] Lower serum levels of free amino acids due to malabsorption are found in patients with spleen deficiency syndrome where intestinal malabsorption is a typical feature of the condition.[61] Protein digestion inadequacy is commonly dealt with by administration of betaine hydrochloride and pancreatic enzymes at mealtime and daily use of zinc supplements to restore body pools of the trace element.

Diseases

Infection increases immune system demand for amino acids. The chronic viral infection of HIV-positive infants results in growth suppression. Uninfected infants use 42% of their leucine intake to synthesize new protein, but the HIV-infected group used only 19%.[62] Infection by the virus elicits the same changes in whole-body protein metabolism as do other conditions of stress. Depletion of glutathione by HIV infection is accompanied by 67%, 52% and 32% lowering of plasma cystine, tryptophan and methionine, respectively.[63] In hepatic encephalopathy, plasma concentrations of the aromatic amino acids are high (phenylalanine and tyrosine), whereas those of the branched-chain amino acids (leucine, isoleucine and valine) are low.[64]

Although the main emphasis of this chapter is the assessment of conditions that may respond favorably to increased amino acid intake, excessive protein intake as a risk factor for multiple diseases must be examined as well. There is strong evidence that excessive protein intake is a strong risk factor for many degenerative diseases, including heart disease, cancer, diabetes and Alzheimer's disease.[65] Multiple mechanisms have been investigated that can account for high dietary protein induction of diseases. Xenobiotic hepatotoxicity has been shown to be specifically increased by raising dietary protein, associated with altered activities of xenobiotic biotransformation in rats.[66] High protein can increase alflatoxin carcinogenesis by raising mixed function oxidase activities.[67] Such evidence calls for increased focus on use of biomarkers that signal excessive total protein intake. Urinary total nitrogen (by Kjeldahl analysis) is a simple, though laborious, measurement that is directly related to protein intake.[68] Urinary urea-nitrogen,

Notes:

ammonia, allantoin and uric acid also rise in direct response to dietary protein intake.[69] Quantitative evaluation of dietary records, of course, provides direct information about protein intake, but there are significant drawbacks to this approach. Inaccurate patient recall and data entry can produce large errors and the process of evaluation can be quite laborious. Simple, routine laboratory measures can provide direct, quantitative evidence of excessive protein intake and prompt clinical attention. Adoption of a plant-based diet is an even more simple prescription that is advocated for optimizing protein intake.[65]

Exercise and Injury

Protein supplements are frequently used by individuals attempting body- and strength-building routines, although studies in athletes have found insignificant effects on nitrogen retention[70] or optimal growth and strength gain.[71] However, essential amino acid intake has an immediate effect of reducing muscle protein breakdown and release of muscle amino acids into plasma.[72] Compared with controls given a placebo drink, intake of 100 g of carbohydrates 1 hour after resistance exercise decreased release of amino acids into blood. The effect extended for 3 hours after ingestion of the maltodextrin beverage, indicating an effect of insulin on muscle metabolism.[73] Because of the strongly fluctuating nature of total-body protein dynamics following strenuous exercise, specimens for analysis of amino acids should be taken only after an overnight rest period.

Injury is characterized by a unique pattern of elevated plasma branched-chain and aromatic amino acids as well as methionine.[74] The non-essential amino acids in muscle tend to decline with injury, glutamine having the most marked change. Following elective surgical procedures, oral glutamine supplementation helps to correct the drop of muscle glutamine and enhances net skeletal muscle protein synthesis.[75]

Amino Acid Transport

Intracellular and plasma levels of amino acids are influenced by transporters. Amino acid transporters reside in the plasma membrane and typically exchange sodium ions (active transport) and/or amino acids (passive transport). The transporters are classified as System A (SA) or System N (SN) and the SN class is further differentiated as SN1 and SN2 based on their differing responses to physiological stimuli.

The concentration of a given amino acid transporter in cell membranes has been shown to be tissue specific, that is, in the gut and kidney, effectively regulating small intestinal absorption and renal resorption of amino acids.[76–78] The transporters are coded by single genes or a number of separate genes.[76] Defective amino acid transporters have been implicated in genetic disease.[79] Similarly, they can be up- or down-regulated in response to exogenous signals such as nitric oxide or insulin,[76,80,81] thereby affecting the influx of a given amino acid into the cell.

The physiological substrates for the SN1 and SN2 amino acid transporters are Gln, Asn and His, whereas SN2 also transports Ser, Ala and Gly.[77] The amino acid transporter for taurine and β-alanine is appropriately named Taurine T (TAUT).[76] Both of these transporter systems will be discussed later in the chapter. Recent research has revealed that enzyme activities and membrane transporters (number and density in tissues) are altered by amino acid availability.

The significance of amino acid transport systems for interpretation of laboratory results is that genetic polymorphic variations in any of the several proteins of the transport systems will have effects on observed levels of amino acids. A case illustrating such an apparent effect for the SN1 and SN2 system is presented in the Histidine (His) section, later in this chapter.

Amino Acid Profiling in Clinical Practice

Identifying Candidates for Amino Acid Profiling

A primary reason for performing amino acid testing is to identify patients who cannot maintain normal plasma levels of essential and conditionally essential amino acids. The finding of low levels in fasting plasma or whole blood demonstrates that the patient is unable to sustain postprandial concentrations adequate for optimal cellular function for the reasons discussed above. Because of genetic, environmental, or iatrogenic factors that decrease stomach acid and pancreaticobiliary secretions, low levels in plasma do not necessarily coincide with low dietary protein intake.

High intestinal transit rates also can decrease protein digestion efficiency. [82] Low blood levels of amino

acids are generally treated with balanced formulas of free-form EAAs, not with individual amino acids, because they are convenient and do not induce further imbalances.

Analysis of amino acids has been used to advance knowledge of the molecular origins of diseases such as depression,[24] to monitor treatment in diabetic ketoacidosis,[83] and to predict responses to therapeutics such as antidepressants.[84,85] Abnormalities in plasma amino acid levels have been reported in clinical research of diabetes, Parkinson's disease, Alzheimer's disease,[86,87] metabolic acidosis, muscle wasting associated with acute uremia,[88] and pre- and postoperative morbidity.[89,90] Orally and tube-fed elderly patients with dementia have significantly lower levels of methionine and branched-chain amino acids than healthy controls.[91]

Diets high in arginine and glycine are associated with decreased serum cholesterol levels, whereas lysine and branched-chain amino acids are associated with increased levels.[92] The association of these changes with lower insulin/glucagon ratios has led to the hypothesis that plasma amino acid levels exert primary control over cholesterol synthesis.[93] Increasing sulfur amino acid composition of dietary protein in rats results in lower cholesterol levels.[94] In the chick, lysine-induced hypercholesterolemia is not mediated by the antagonistic effect of lysine on arginine or by the effect of lysine on food intake. Rather, a direct stimulatory effect on cholesterol biosynthesis occurs.[92] These factors may explain not only cholesterol modulation, but also other favorable clinical responses to amino acid supplementation regimens that achieve an improved balance between individual amino acids.

Parkinson's disease patients show significant alterations of plasma amino acids (higher phosphoserine, threonine, methionine, tyrosine, sarcosine and alpha-aminoadipic acid and lower valine, leucine and tryptophan) compared with controls.[95] Characteristic and significant amino acid patterns in plasma have also been found in patients with Alzheimer's disease.[96] 20 patients with chronic fatigue syndrome displayed abnormally low plasma amino acids and symptoms improved in 75% of the subjects with concurrent increase of plasma amino acid levels.[97] Some common clinical presentations that suggest assessment of amino acids are shown in Table 4.5.

In assessing chronically ill patients, it is important to keep in mind both general protein synthesis and special roles of amino acids. Table 4.8 shows commonly used groupings of amino acids based on shared characteristics. A patient with a severely degraded intestinal mucosal epithelium must replace many hundreds of grams of tissue en route to restoration of full wellness. Meanwhile, their most disturbing symptoms may indicate special roles of amino acids. The downstream, neurotransmitter metabolites of tryptophan are important to consider in patients with insomnia. Urea cycle stimulation by arginine may be relevant for ammonia toxemia. Relative to a nulliparous woman with no digestive disturbances, a pregnant woman who uses proton-pump inhibitors for heart burn is especially at risk of amino acid insufficiency.

TABLE 4.5 — COMMON CONDITIONS SUGGESTING AMINO ACID ASSESSMENT

Condition	Reason
Aging	Decreased stomach acid output
Cancer	Cachexia
Chronic fatigue	Mitochondrial inefficiency
Depression	Impaired neurotransmitter or receptor synthesis
Dietary restrictions	Poor protein intake
Medications to block acid release	Insufficient stomach acid
Pregnancy	Increased requirements

SELECTING A TEST: FASTING PLASMA, WHOLE BLOOD, OR URINE

Blood plasma has been the predominant specimen in published studies on amino acids. To determine whether a patient is in need of essential or conditionally essential amino acids, either fasting plasma or fasting whole-blood amino acid testing is the preferred test to avoid recent dietary influences. Fasting blood plasma also has by far the greatest validation of scientific studies and provides higher levels of reliability for showing chronic stresses that shift individual amino acid demands. Plasma from blood drawn at any given moment will reflect the state of the dynamic flux of amino acids leaving sites such as skeletal muscle and flowing into sites of utilization in liver, brain and other tissues.

To detect metabolic disorders due to genetic polymorphisms, micronutrient deficiencies or toxicant abnormalities, all of which tend to interfere with amino acid utilization, urinary amino acid testing is the preferred test. Transient strong elevations during post

Table 4.6 — Specimen Preference According to Critical Information

Desired Information	Specimen
Status of essential amino acids	Plasma
Leu, Ile, Val, Phe, Lys, Thr, Met, Trp	
Status of conditionally essential amino acids	Plasma
Arg, Tyr, His, Gly, Ser	
Markers of neurological dysfunction	Plasma
Trp, Tyr, Phe, Met, GABA	
Markers of vitamin and mineral deficiency His, Phe, Anserine, Carnosine	Plasma or Urine
Markers of bone loss or muscle wasting	Urine
HPro, HLys, Cys, 3-MeHis	
Markers of intestinal dysbiosis	Urine
β-Ala	

prandial periods tend to cause spillage into urine rather than accumulation in plasma. These situations present different clinical issues than those posed by essential amino acid insufficiency that is detected by plasma testing and that may be correctable by supplemental amino acids or digestive aids. The combination of amino acids in plasma or urine with organic acids in urine provides a more complete picture of amino acid abnormalities.

Technological improvements have allowed the introduction of amino acid profiling of blood spots obtained from finger-stick blood dried on filter paper.[98] This specimen provides a lower cost, non-invasive way to view concentrations of amino acids in whole blood (plasma and blood cells). Most essential amino acid concentrations are similar in the two specimens as shown in Table 4.7. Taurine is a notable exception, being much higher in whole blood due to high concentration in erythrocytes.[99]

A 24-hour urine amino acid analysis reveals amino acid metabolism throughout the period of daily activity, but has some limitations. Data from 24-hour urine is of particular value for evaluating those amino acids that primarily reveal tissue degradation. Hydroxylysine and hydroxyproline, for example, are released from collagen of connective tissue and bone. Reports of urinary amino acid studies are virtually always based on data from subjects in metabolic wards where 24-hour urine collection is done under the direction of medical staff. Collection of urine for 24 hours and reporting the total volume by free-living individuals are subject to frequent error that systematically shifts the results.[100] Details about certain amino acids could be obscured in a 24-hour urine due to decreased renal filtrate when blood concentrations fall below the renal threshold. This makes it difficult to determine deficiency states from urine amino acid data. A properly obtained 24-hour urine, however, provides valuable clinical information and is the preferred specimen for detecting abnormalities of some non-essential amino acid derivatives. Reporting amino acid concentrations in overnight urine normalized to creatinine offers an alternative that is free of most patient-dependent variability. Correction for dilution variation by creatinine is inherently prone to error in the very young and very old.

Interpretation of Amino Acid Abnormalities: The data from amino acid testing can reveal general and specific patterns of elevated and decreased amino acid concentrations. In general, if many amino acids are low, then supplementation of essential amino acids is indicated. If many amino acids are high, then there is a cofactor inadequacy or improper hormonal control. Single amino acids that are high or low must be considered on an individual basis. Each amino acid will be addressed in the discussions that follow in order to provide the background for understanding the full range of nutrient, cell regulatory and genetic issues that bear on interpretation. With such knowledge the clinician can determine when individual amino acids may be needed to affect specific

Table 4.7 — Adult Amino Acid Reference Limits for Plasma and Blood Spot Specimens

Amino Acid	Plasma (μM)	Blood Spot (μM)
Arginine	42–130	18–101
Histidine	53–101	19–102
Isoleucine	37–98	33–118
Leucine	73–161	62–186
Lysine	109–235	72–232
Methionine	15–32	8–27
Phenylalanine	43–81	38–97
Threonine	67–166	51–203
Tryptophan	34–70	21–59
Valine	134–305	110–329
Taurine	26–103	138–355

Amino acid values from plasma are determined by high-performance liquid chromatography with pre-column o-phthalaldehyde derivatization and fluorescence detection. Values from blood spot are determined by high-performance liquid chromatography with tandem mass spectrometric detection.

TABLE 4.8 — AMINO ACIDS GROUPED BY SHARED CHARACTERISTICS

Group	Members	Application
Branched-chain amino acids (BCAA)	Valine, Leucine, Isoleucine	Released from skeletal muscle during strenuous exercise
Large, neutral amino acids (LNAA)	Tryptophan, Tyrosine, Phenylalanine, Leucine, Isoleucine, Methionine	Compete for intestinal absorption and for transport at the blood-brain barrier
Basic amino acids (positively charged at neutral pH)	Histidine, Lysine, Arginine	Abundant in histones that bind to negatively charged DNA

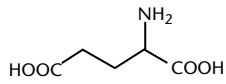
conditions. Abnormalities may also suggest other testing to investigate metabolic impacts and needs for other classes of nutrients or detoxification procedures.

The organization scheme used in Table 4.1 is followed throughout the chapter, but some amino acids with multiple roles are mentioned in more than one location. Several EAAs, which were discussed at the beginning of this chapter, are in the category "Amino Acids for Polypeptides and Energy." Glycine, aspartic acid and taurine are CNS-active compounds that might be included under "Neurotransmitters and Precursors," but instead they are grouped according to their unique metabolic functions such as detoxification.

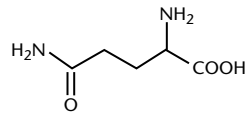
Anabolic/Catabolic Responses and Tissue pH Regulation

A Class of Their Own for the Most Versatile Amino Acids

Glutamate (Glu)



Glutamine (Gln)



Glutamine is the dominant free amino acid in fasting plasma. Glutamate and glutamine are critical in human physiology for the following reasons: tissue pH buffering; carbon supply for gluconeogenesis, particularly in the kidney; hepatic ammonia removal; genesis of glutathione; and neurotransmitter function (Figure 4.4). Each of the four major fates illustrated constitute a large flux of glutamine from plasma. The abbreviation "Glu and Gln" will be used to denote the pairwise participation of glutamate and glutamine because they are so intimately linked in their metabolic functions. Glu and Gln play a crucial role in maintaining acid-base homeostasis and whole-body nitrogen balance.

In the central nervous system, glutamic acid is the major excitatory neurotransmitter and source of the neurotransmitter γ-aminobutyric acid, an inhibitory neurotransmitter. Synthesis of glutamine from glutamic acid is an important part of the detoxification of ammonia in the liver and brain and glutamine synthesis in astrocytes is critical for glutamic acid regulation to protect the brain from glutamate excitotoxicity.

Glutamine functions include protein synthesis; the provision of nitrogen for the synthesis of amino acids, purines, pyrimidines and nucleotides; and the transport of amino-nitrogen to the intestines, liver and kidney. Glutamine plays roles as both precursor and inhibitor of nitric oxide production.[101,102]

A large percentage of ingested glutamine may be used for energy production by enterocytes.[103] This role has advantages for the enterocytes, but it means that glutamine supplementation may be ineffective for raising plasma glutamine levels. Endogenous formation of glutamine may be assisted by supplementation with balanced formulas of essential amino acids.

The first goal in this section is to explain the principles underlying the interpretation of abnormalities in laboratory evaluations where Glu and Gln are measured, mainly plasma amino acid profiling. A secondary goal is to provide understanding of the benefits and caveats regarding the dietary supplementation of glutamate and glutamine as individual amino acids.

Specimen collection and handling is critical for accurate measurement of Glu and Gln. At room temperature, enzymes in plasma can degrade glutamine to

glutamate. Therefore, specimens at room temperature for extended periods of time can have increased glutamic acid concentration with proportionally lower glutamine levels.

The distribution of fasting plasma values in a large, diverse patient population from outpatient clinics (Figure 4.5) reveals several useful points. First, one can see that average glutamine is about sixfold higher than glutamate. Overall, it is extremely rare to find glutamate levels above 200 µM, while glutamine varies from below 100 to nearly 1,200 µM. Individuals in the abnormal regions, labeled "A" and "B," are considered to be in a state of metabolic distress that is reflected by either low or high ratios of glutamine to glutamate. The specimen degradation issue mentioned at the beginning of the Glu and Gln section may contribute to some points in "A," so metabolic distress interpretation depends on knowledge of specimen history. For those specimens that were handled properly, the indiviudals are in danger of the various sequelae of inadequate glutamine described in this section.

Ureagenesis and pH Modulation

When amino acids are degraded in the liver,

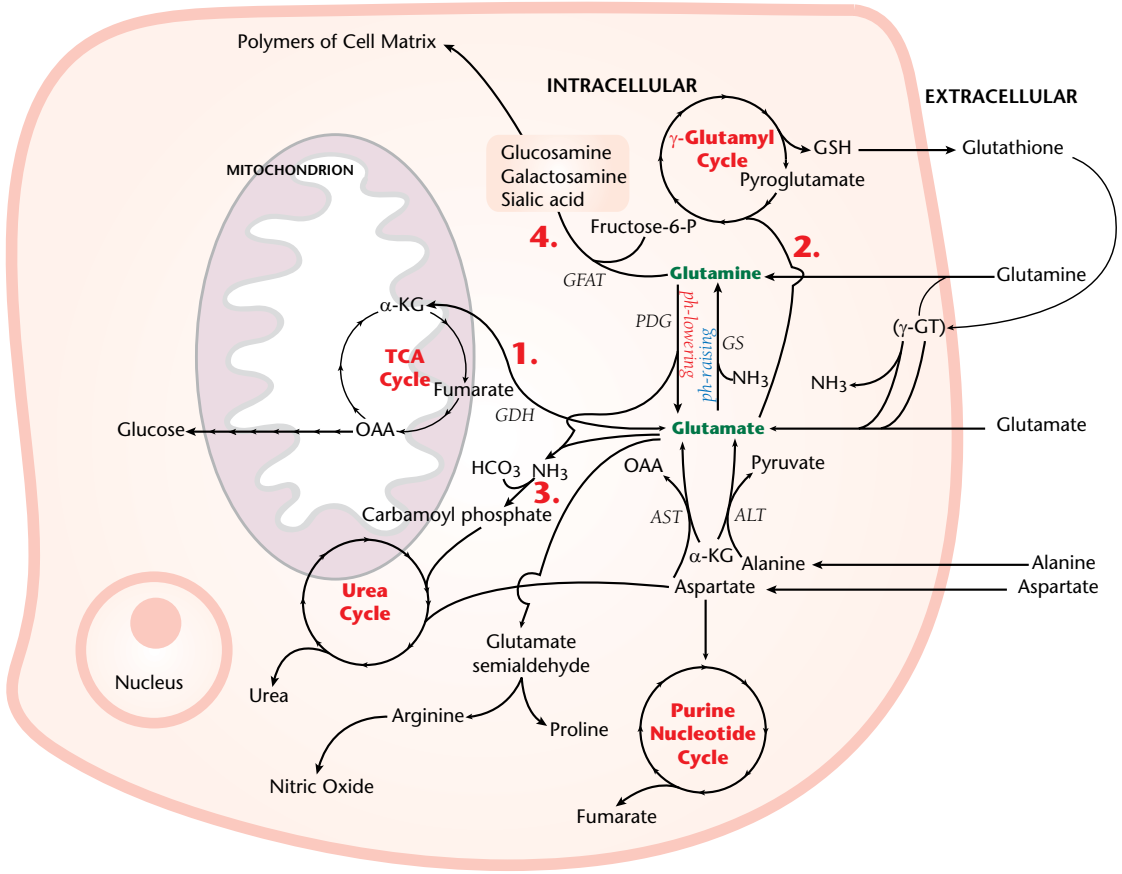


FIGURE 4.4 — Multiple Pathway Interactions of Glutamate and Glutamine

The enzymes glutamine synthetase (GS) and phosphate-dependent glutaminase (PDG) allow bi-directional control of glutamate/glutamine conversions. Glutamate and glutamine interact simultaneously with the tricarboxylic acid cycle (TCA), urea cycle, purine nucleotide cycle, and γ-glutamyl cycle (glutathione formation), exerting metabolic regulation and rate adjustments at multiple points. Total-body glutamine metabolic flux shifts between four principal processes: (1) gluconeogenesis (renal); (2) glutathione synthesis; (3) ureagenesis and proline synthesis (hepatic); and (4) glutamine-fructose-6-phosphate aminotransferase (GFAT) pathway to cell matrix polymers. Glutamate is formed by transfer of amino groups from Asp, Ala and other amino acids via the enzymes aspartate transaminase (AST) and alanine transaminase (ALT). α-Ketoglutarate (α-KG) is reformed by deamination under the action of glutamate dehydrogenase (GDH).

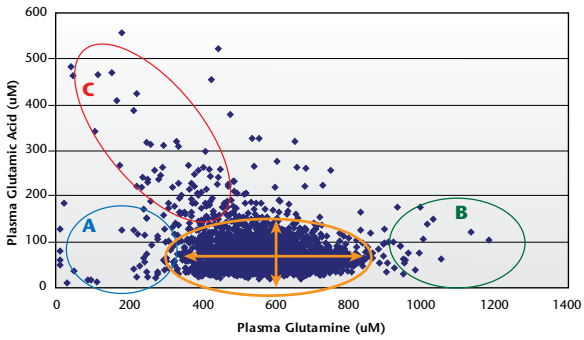


FIGURE 4.5 — **Scatter Plot of Glutamate vs. Glutamine**

Each point represents the measured glutamine and glutamic acid concentrations for an individual submitted for routine amino acid profiling from an outpatient clinic. The central ellipse with thick arrows emphasizing normal distributions encompasses more than 90% of the 2,972 points. In this normal range, there is no tendency of glutamic acid to rise as glutamine values increase. Ellipse A: individuals where glutamine has fallen to abnormally low levels, where the average glutamate still remains constant. Ellipse B: individuals with very high glutamine, none of which have elevated glutamate. Ellipse C: the region where higher glutamate levels are found in proportion to declining glutamine probably represents improperly stored and transported specimens, where conversion of glutamine to glutamate has occurred.

glutamic acid is produced and nitrogen (or ammonia) is then transformed into urea via the urea cycle. The other product, alpha-ketoglutaric acid (α-KG), can be used to produce energy by the TCA. Glutamate carries nitrogen away from exercising muscle via α-KG. Experiments done on animals show that hepatic ammonia clearance can easily be exceeded resulting in increased circulating ammonia.[104]

Glutamine is intimately involved in the detoxification of ammonia and ammonia disposal is a major contributor to systemic pH regulation. The chemical structure of glutamine suggests its role as an amine regulator. It has both α-amine and side-chain amide groups. The interchange of the side-chain nitrogen between glutamine and glutamate is a reaction common to all tissues, facilitating movement of amine groups. In acidic conditions, hydrolysis of the glutamine side chain releases ammonia (NH_3). Ammonia binds excess protons, thereby making the pH more basic. Acute acidosis stimulates renal cell uptake and metabolism of glutamine. The shift toward higher glutamine transport during ammonia stress has been demonstrated by direct measurement of brain glutamine using high-magnetic field MR.[105]

Glu and Gln regulate systemic pH in the kidney, liver and brain especially during food intake, pulmonary changes and cardiac exertion. Greatly elevated plasma glutamine is favored by chronic alkalotic conditions (insufficient bicarbonate clearance) or elevated ammonia, in which patients are in a state of metabolic fragility, lacking normal ability to respond to the pH stresses of everyday life. Conversely, chronic acidemia is associated with low glutamine. Systemic pH influences enzymes controlling Glu and Gln. The net effect of acidosis is to increase the flow of glutamine from liver to kidney, where the ammonium ion is removed, thereby lowering systemic pH. Acidosis stimulates glutamine uptake in the kidney, tending to produce low plasma glutamine, especially in individuals with impairment of the enzyme phosphate-dependent glutaminase.[108] Thus, patterns of simultaneously low or high fasting plasma glutamate and glutamine are found in patients who have chronic difficulty overcoming headaches, fibromyalgic pain, or infectious diseases. They may benefit from the decreased digestive burden and accompanying pH buffering demands achieved by lowering animal protein intake. The more rapid digestion of animal proteins places stronger demands on pH buffering systems during postprandial intervals.[109]

REFER TO CASE ILLUSTRATION 4.1

Brain Regulation

Neurons and astrocytes have transport systems and metabolic capacities that protect the brain from ammonia while stabilizing glutamate levels for use by glutamatergic neurons. Glutamate is the principal excitatory neurotransmitter in the brain. Animal studies suggest that the excitatory function of glutamate plays a key role in controlling gastric function, with high glutamate causing a depression of gastric motility.[110] Stimulation of endogenous synthesis of glutamate may contribute to chronic neurodegeneration in such disorders as amyotrophic lateral sclerosis (ALS) and Huntington's chorea.[111] The effect on Huntington's chorea is unrelated to dietary intake of glutamate. Low plasma glutamate levels, on the other hand, can impact a large class of neurons in the central nervous system, which utilize glutamic acid as an excitatory neurotransmitter. Glutamic

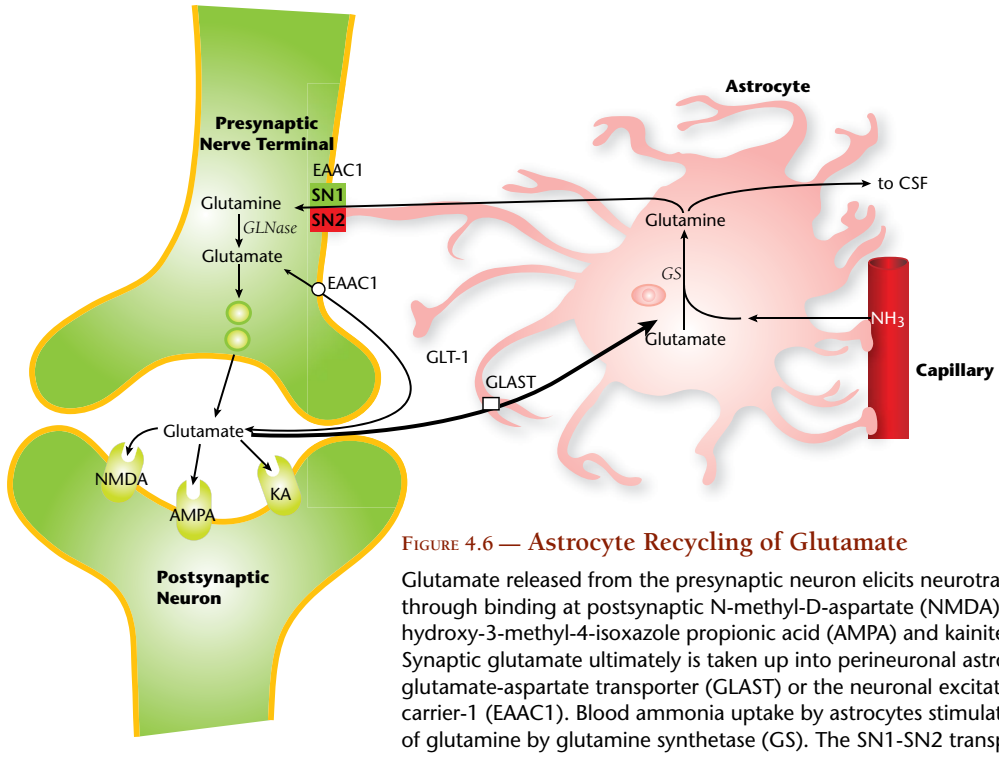


Figure 4.6 — Astrocyte Recycling of Glutamate

Glutamate released from the presynaptic neuron elicits neurotransmission through binding at postsynaptic N-methyl-D-aspartate (NMDA), α-amino-5-hydroxy-3-methyl-4-isoxazole propionic acid (AMPA) and kainite (KA) receptors. Synaptic glutamate ultimately is taken up into perineuronal astrocytes by glutamate-aspartate transporter (GLAST) or the neuronal excitatory amino acid carrier-1 (EAAC1). Blood ammonia uptake by astrocytes stimulates formation of glutamine by glutamine synthetase (GS). The SN1-SN2 transporter is used to transfer glutamine into the presynaptic neuron where it is reconverted to glutamate by glutaminase (GLNase) for vesicle storage. Reuptake of glutamate is, thus, quite different from the direct neuronal reuptake mechanism of serotonergic and most other types of neurons. The complex mechanism generates multiple sites of potential polymorphic effects that can impact the primary excitatory glutamatergic system of the brain.

acid is also important in ammonia detoxification in the brain where it combines with ammonia to form glutamine (Figure 4.6).

The immune system is influenced by moderate variations of plasma glutamate and high glutamate seems to be a causative factor in immunopathology of diseases, including AIDS. Because of such associations high fasting plasma glutamate has been proposed as a factor causing low lymphocyte activity and low T4 counts.[112] Low fasting plasma glutamate, on the other hand, can be caused by 5 weeks of trazodone antidepressant therapy, where amino acid status was found to predict response to therapy.[84] Patients with major depression who did not show lowered serum aspartate, asparagine, serine, threonine and taurine were found to not respond to such therapy. The unidirectional outward transport of glutamate from brain to blood is thought to be part of the mechanism of glutamate perturbations relevant to the pathophysiology of major depression.[21]

Notes:

REFER TO CASE ILLUSTRATION 4.2

This patient described in Case Illustration 4.2 is in need of immediate metabolic support to prevent inflammatory sequelae, especially in light of recent evidence for neuroinflammation in the brain of patients with autism.[113] Her plasma amino acid profile revealed an extremely abnormal Gln/Glu ratio. This data indicates several things about the metabolic state of the patient. She is so far away from normal Glu and Gln balance that she will have difficulty responding to pH or ammonia challenges. Neuronal maturation and cortical organization may be difficult due to altered astrocyte-neuron glutamate metabolism. The neurotoxic effects of elevated quinolinic acid are especially alarming when such an abnormal Gln/Glu ratio is found because of the known mechanism by which both agents activate receptors of glutamatergic neurons (see "Quinolinate" in Chapter 6, "Organic Acids"). Finally, the delivery of nitrogen to all organs is impaired by such a low glutamine level, possibly contributing to general developmental delay.

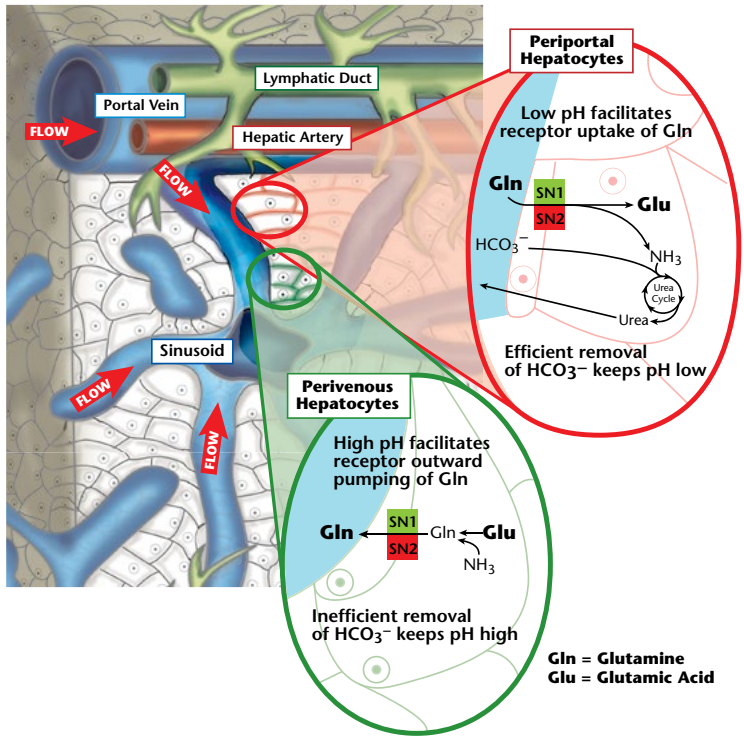


FIGURE 4.7 — The SN1-SN2 Transporter System

A section of a hepatic lobule is illustrated, showing a typical course of blood from portal to venous vessels. This is the system that filters postprandial blood before it enters extrahepatic circulation. Hepatocytes that surround the vessel near the portal end have distinctly different metabolic activities than those near the venous end. Relatively acidic pH on the portal side causes membrane transporters called SN1 and SN2 to pump glutamine inward where conversion to glutamate releases ammonia. Simultaneous utilization of bicarbonate to form urea serves to maintain relatively acidic cellular pH in the periportal hepatocytes. As the glutamine-depleted blood flows on past the perivenous hepatocytes, glutamine, synthesized from glutamate by glutamine synthetase is pumped outward by the same SN1-SN2 transporter because of the higher pH generated by the absence of urea-cycle enzymes. Only the perivenous hepatocytes contain glutamine synthetase. The enzyme is not expressed by the upstream cells near the portal end of the capillary. The net effect is to allow adjustment of the relative rates of inward and outward flow to assure blood glutamine supply in sinusoidal blood that flows toward the heart. Polymorphism of the SN1-SN2 transporter proteins can be the origin of abnormalities in plasma glutamine.

Gluconeogenesis

Recent evidence has shown how glutamine plays yet another pivotal role as a regulator of total body energy metabolism as a gluconeogenic substrate. Thus, fasting plasma or whole-blood glutamine tends to rise or fall with changes in demand for glucose production. Glutamine is involved in the hexosamine biosynthetic pathway (HBP), a nutrient-sensing system involved in the development of insulin resistance. When postprandial glucose elevation subsides, glutamine is found to surpass all other amino acids, including alanine, as a supplier of carbon for glucose synthesis.[114] Though plasma glucose may be formed from alanine or lactate released from skeletal muscle, only glutamine contributes a net gain of glucose.[114]

Glucagon, acting through hepatic receptors, can reduce plasma glutamine by 30% under conditions of constant insulin and glucose.[115] Insulin supresses the renal glutamine-glucose conversion, whereas epinephrine is a powerful stimulator of this organ-specific glutamine uptake and glucose release.[116]

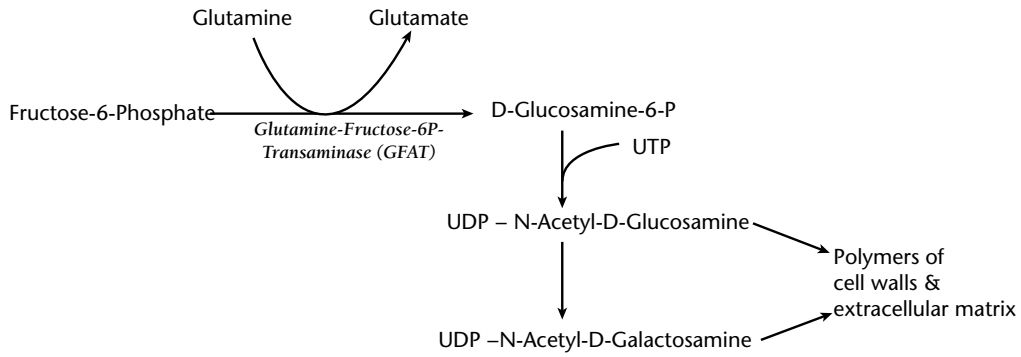


Figure 4.8 — From Glutamine to Glucosamine

Glutamine supplies the amine group for synthesis of hexosamine polymers that characterize the extracellular matrix and give identity to cells via the glycoproteins of their membranes.

These cycles are shifted toward greater glucose synthesis (gluconeogenesis) in patients with non–insulin-dependent diabetes mellitus (NIDDM),[117] and the conversion of glutamine to glucose in renal tissues is dominant.[118] Thus, glutamine is intimately involved in energy control through its major contribution to gluconeogenesis.

The enzyme glutamine-fructose-6-phosphate aminotransferase (GFAT) activates the hexosamine pathway, which is involved in energy metabolism and development of insulin resistance.[119] GFAT is the rate-limiting step to pathways for polymers of glucosamine, galactosamine and sialic acid that are used to construct the cell matrix (Figure 4.8).

In response to hyperglycemia, GFAT is overexpressed in the kidney of diabetics with concurrent stimulation of the plasminogen system and vascular injury.[120] The GFAT promoter gene is activated by angiotensin II.[121] Restriction of structural and cell-recognition polysaccharides is another potential result of low plasma glutamine.

Of Further Interest...

The SN1-SN2 Transporter System (Figure 4.7). The mammalian liver is actually two separate organs with respect to Glu and Gln metabolism. In periportal hepatocytes, glutamine uptake is active and the amide side-chain nitrogen is delivered for ureagenesis. On the perivenous side, lack of urea cycle activity to remove bicarbonate causes higher intracellular pH, driving synthesis and export of glutamine. These systems work in tandem so that any ammonia left in periportal blood is removed as it enters perivenous regions. Periportal ammonia increases only when these two systems approach saturation.

There is a strong metabolic zonation produced by the total lack of glutamine synthesis in periportal hepatocytes and the total lack of glutamine uptake for urea synthesis in perivenous hepatocytes. The perivenous cell population is much smaller than the periportal. Only 7% of all acinar hepatic cells surrounding the terminal hepatic venules account for all hepatic glutamate uptake.

In periportal hepatocytes, bicarbonate-rich portal blood enhances uptake of glutamine and deamination is driven by the bicarbonate substrate role in the urea synthesis. On the perivenous side, lower pH drives synthesis and export of glutamine. These cells contain high activities of glutamine synthetase, an enzyme totally absent in the periportal cells. The perivenous hepatocytes are highly sensitive to oxidative damage from alcohol consumption, being the first to show signs of liver disease.[106] Their loss will lead to declining plasma glutamine. Genetic defects in the SN1-SN2 transporter system produce simultaneous elevation of plasma glutamine, asparagine and histidine. Individuals with such patterns have difficulty maintaining normal tissue pH.[107]

Dietary Protein Influences

One factor controlling circulating glutamine is the total dietary supply of essential amino acids. In healthy individuals, plasma glutamate and glutamine vary inversely with dietary protein intake and the changes are attributable to changes in de novo production.[122] When humans shifted from normal to high protein intakes, glutamine dropped more than 30%.[123] In routine clinical assessments of fasting plasma amino acid profiles, it is common to find low levels of essential amino acids with high-normal or even slightly elevated glutamine.

Clinical Relevance and Treatment

Some of the more common clinical conditions with relevance to glutamine and glutamate are summarized in Tables 4.9 and 4.10, respectively. Plasma glutamine is lowered in individuals placed on high-protein diets, apparently due to decreased release from muscle and lower net synthesis of glutamine.[123] The average level of 720 μM for healthy men on normal protein intake (1 g/kg/d) fell to 444 μM when protein intake was increased by 2.5-fold during high protein intake. Plasma glutamate levels decrease rather than increase with increased dietary protein intake. This phenomenon apparently is due to the actions controlling fluxes through pathways of glutamate synthesis and utilization and the fact that little or no dietary glutamate or glutamine reaches the portal circulation.[122] When humans are fed low-protein diets, rising levels of plasma glutamine is an early-stage response to help assure adequate supply for synthesis of glutamic acid in nerves, glutathione in liver and other essential derivatives.[122]

Depressed patients have lower plasma glutamate and glutamine levels than controls,[124] consistent with other studies where excitatiory amino acids, especially glutamate, glycine and taurine were able to discriminate depressed patients from controls.[20] These perturbations of amino acid circulating concentrations may reflect altered brain metabolism or blood-brain barrier transport differences.

A glutamine synthetase mutation has been reported in which glutamine was largely absent from serum, urine and cerebrospinal fluid.[126] This form of congenital glutamine deficiency produces severe brain malformations, multiple organ failure and neonatal death. Milder variants of this genetic modification will produce less profound glutamine insufficiency with milder symptoms in older children and adults. Skeletal muscle activity is the primary contributor to plasma glutamine in the postabsorptive state.

The normal inflammatory response involves interferon-γ stimulation of the class of lymphocytes called T helper cells (TH1). In severe trauma patients, plasma concentration of glutamine is routinely lowered by approximately 50% of normal mean values.[127] Severe trauma leads to an impaired TH1 immune response related partially to the low plasma glutamine concentrations, which can lead to a poor cell-mediated immune response despite normal antibody levels.[127] Glutamine levels fall when it supplies amine groups (nitrogen) for increased protein synthesis. To spare the fall of glutamine, hepatic catabolism of other amino acids falls, restricting other biosynthetic activities dependent on the carbon skeletons.

Starvation, injury, sepsis and other protein catabolic states result in reduced glutamine in muscle and plasma.[74,128] Glutamine is considered a conditionally essential amino acid for critically ill patients because of improvements in immune function of these patients with glutamine supplementation.[129] Among patients with cancer or human immunodeficiency

TABLE 4.9 — PATIENT GROUPS LIKELY TO BENEFIT FROM GLUTAMINE[125]

Category	Examples
Severe catabolic illness	Burns
	Multiple trauma
	Bone marrow supplementation
	Acute/chronic infection
	Other critical illness
Intestinal dysfunction	Inflammatory bowel disease
	Infectious enteritis
	Necrotizing enterocolitis or intestinal immaturity
	Short-bowel syndrome
	Mucosal damage following chemotherapy
	Radiation or critical disease
	Surgical gastrointestinal patients
Immunodeficiency syndromes	Immune system dysfunction associated with critical illness or bone marrow transplantation
	Acquired immunodeficiency syndrome
Advanced malignant disease	Cancer cachexia
Low-birth-weight babies	Premature infants

Table 4.10 — Clinical Disorders Associated with Altered Plasma Glutamate

Disease	Plasma glutamate	Metabolic dysfunction
Amyotrophic lateral sclerosis	High	Low activity of glutamate dehydrogenase[154]
Acquired immunodeficiency syndrome	High	Decreased immunological reactivity[155]
Headache, fatigue, confusion, poor concentration and food intolerance due to hyperammonemia	Low	Urea cycle insufficiency[156]
Epilepsy	High	Glutamate-induced gene expression[157]
Acute liver failure	High	Impaired neuron-astrocyte glutamate flux[158]
Tumor-induced cachexia	Low Gln/Glu	Negative nitrogen balance[159]

virus infection, increased skeletal muscle catabolism, low plasma glutamine and high venous glutamate levels are common.[130] These shifts in amino acid concentration are part of the mechanism for halting the loss of body cell mass in such diseases.

Because of the favorable responses to supplementation, glutamine is recognized as a conditionally essential amino acid for patients in catabolic states.[131] Clinical responses to supplementation may be explained by glutamine's influences on the inflammatory response, oxidative stress, cell protection and the gut barrier. By inducing the production of heat shock proteins, glutamine assists the protection of cells against toxic agents or pathologic insults.[132] By modulating cytokine output, glutamine decreases the inflammatory response.[133] By increasing splanchnic glutathione production, glutamine protects the intestinal mucosa from the degenerative effects of oxidative stress.[134]

Mild hyperammonemia conditions are often seen as low fasting plasma glutamic acid levels and high glutamine levels. Symptoms include headache, irritability, fatigue, mental confusion, poor concentration and food intolerance reactions, particularly to high-protein foods. A low-protein, high-complex carbohydrate diet with supplementation of α-ketoglutaric acid, vitamin B_6 and BCAAs will be useful in correcting this condition, particularly if glutamine is high. Supplementation of α-ketoglutaric acid supplies additional substrate for ammonia removal and generates additional energy as a component of the citric acid cycle. Other agents employed against acute hyperammonemia include benzoic acid and sodium phenylacetate.[135] The pattern of simultaneously low glutamic acid and glutamine calls for EAA supplementation with cofactors to enhance the flux of amino acids in liver and normalization of glutamine production in perivenous hepatocytes.

Contraindications for Supplementation of Glutamate or Glutamine: The safety of glutamine may be considered as a special topic under the subject of optimal protein intake. Considering optimal protein intake, in turn, begs the question of the type of protein, especially whether protein sources are plant or animal based.[65] In general, consumption of amino acids in excess of requirements for utilization results in conversion of the amino nitrogen to urea. When protein intake is adjusted by addition of casein, lactalbumin, or ovalbumin, the rate of urea synthesis reaches a maximum at about 80 g protein/d for a 70 kg individual. At intake of 200 g protein/d for a 70 kg individual, maximal excretion was sustained for 24 hours. Thus, within broad limits, urea excretion may be measured to assess the degree of excess protein intake.[136] Although the mechanism is not clear, studies in rats show that total blood LDL cholesterol levels are closely related to the amount of animal protein consumed.[137] This conclusion is strongly supported by epidemiological data on humans.[65] Thus, commonly

Notes:

available measures of urea and cholesterol provide markers for assessing excessive animal protein intake.

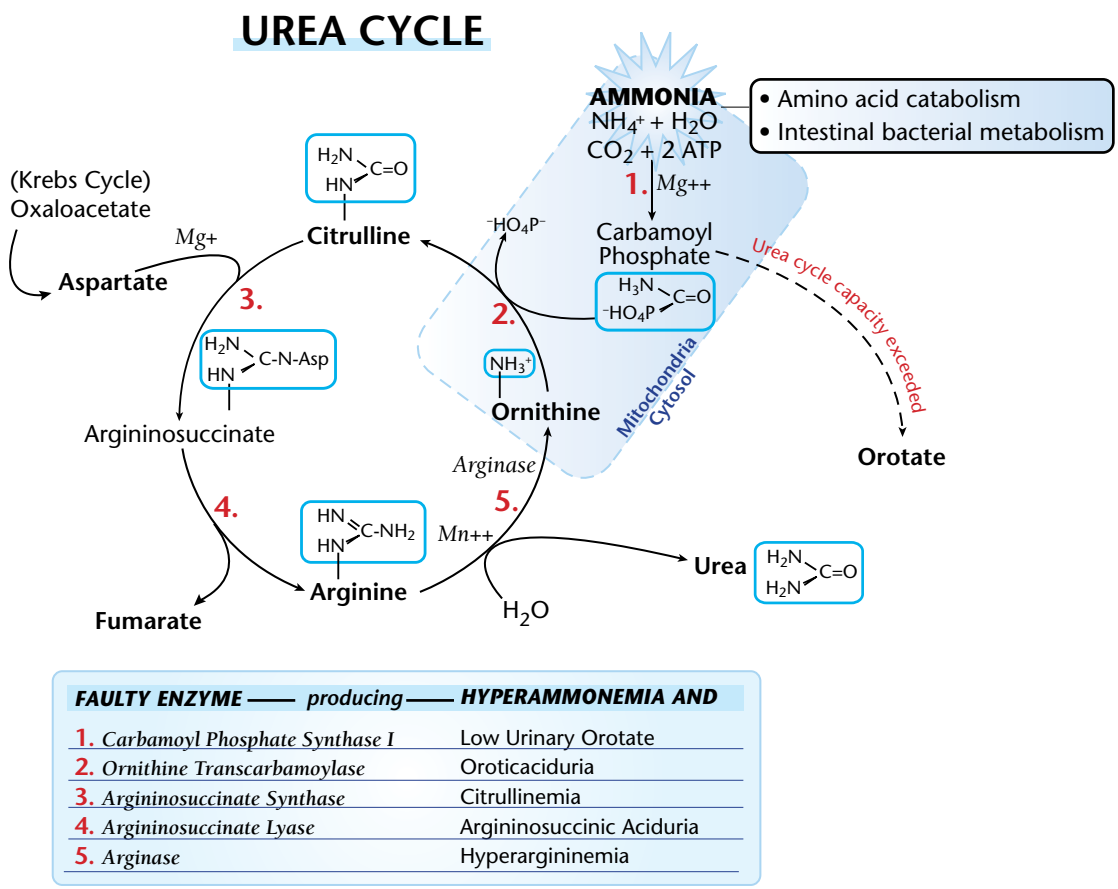
Numerous studies of high-protein effects in neonates, children and adults have been reported. Approximate doubling of cow's milk protein intake for preterm infants produced toxic effects manifested as fever, lethargy and poor feeding.[138] Follow-up at 3 and 6 years showed increased incidence of strabismus and low IQ scores in the children with lowest birth weight who had been fed the high-protein diet.[139] Other potential high dietary protein effects include loss of calcium, calcium oxalate stone formation and progression of renal disease. Individual amino acid supplementation at excessive levels also can produce a variety of effects specific to the amino acid.[140]

Glutamine is widely used in doses of 10 to 20 g per day for the reasons discussed above. As already mentioned, however, oral glutamine is not generally effective for modifying plasma glutamine. Because of the biochemical conversion of glutamine to ammonia and glutamic acid, researchers have addressed the question of toxicity from oral supplementation of glutamine. Some authors have argued that therapeutic use of glutamine is inappropriate for any condition,[141] although earlier studies had demonstrated safety in subjects receiving parenteral infusions of up to 0.570 g/kg body wt/d.[142] A review of more chronic effects of glutamine therapy concludes that no adverse effects are reported during short-term dosing as high as 50 to 60 g/d. However, patients under long-term supplementation with glutamine should be monitored for metabolic diseases such as diabetes or coronary artery disease and for psychological and behavioral changes due to glutamate neurotoxic effects.[140] Although glutamine supplementation reduces plasma nitrate in experimental animals, no such effects have been reported for humans.[143]

Glutamic acid is not generally used in clinical interventions because of toxic effects from administration of the free amino acid. Neurotoxic effects result from exceeding the capacity of mechanisms that function to regulate intracellular glutamate concentrations when plasma concentrations are sharply increased.[144–146] Postnatal administration of monosodium glutamate (MSG) to weanling rats, an animal model for the study of human obesity, produces insulin insensitivity and obesity.[147] These effects are explained by MSG-induced damage to the arcuate nucleus and subsequent interference with circadian energy expenditure.[148] MSG-induced obesity produces a general defect of adipocyte cell membrane characterized by impaired receptor proteins.[144] Reports of these effects have generated a public controversy over regulation of MSG as a food additive.

Glutamate-mediated excitotoxicity resulting from impaired astroglial uptake constitutes one of the current hypotheses explaining the progression of ALS.[149] In ALS, plasma glutamate is elevated and treatment with BCAA showed a significant benefit.[150] Schizophrenia treatments (and etiologic mechanisms) have been linked to the glutamatergic and dopaminergic excitatory amino acid systems.[151] Alterations in plasma levels of aspartate, glutamate, glycine and taurine have been suggested as neurochemical markers of epilepsy.[152] Other reviews have summarized the numerous neurodegenerative diseases involving stimulation of glutamate receptors.[153]

Notes:



FAULTY ENZYME —— producing ——	HYPERAMMONEMIA AND
1. *Carbamoyl Phosphate Synthase I*	Low Urinary Orotate
2. *Ornithine Transcarbamoylase*	Oroticaciduria
3. *Argininosuccinate Synthase*	Citrullinemia
4. *Argininosuccinate Lyase*	Argininosuccinic Aciduria
5. *Arginase*	Hyperargininemia

FIGURE 4.9 — **The Urea Cycle**

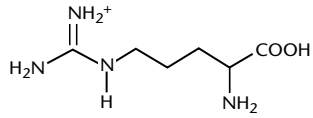
This figure illustrates the transfer of nitrogen from ammonia through the various intermediates to urea. The partial structures are included to assist in following the flow of nitrogen atoms. The first nitrogen is transferred to ornithine from carbamoyl phosphate and the second is carried to citrulline by aspartic acid. The reloading of aspartic acid requires several steps that ultimately serve to gather the aspartate nitrogen from another molecule of ammonia. Enzymes for the formation of carbamoyl phosphate and for driving the four reactions of the cycle are shown in the inset. Collectively, these four enzymes represent a high incidence of genetic defects that manifest as hyperammonemia and the other amino or organic acidurias shown.

The Urea Cycle and Nitrogen Management

The detoxification of ammonia is a critical metabolic process that requires the functioning of the urea cycle (Figure 4.9). Hepatic urea cycle activity changes as needed to manage the varying ammonia clearance demands due to dietary nitrogen content fluctuations. Thus amino groups are supplied to drive transamination reactions or are removed to prevent toxic ammonia buildup. Subtle impairments of the liver's urea cycle function can lead to sub-clinical or mild hyperammonemic conditions. Symptoms include chronic fatigue, headache, irritability, occasional diarrhea or nausea, lack of concentration, mental confusion and intolerance of foods, particularly high-protein ones. Urea cycle impairments tend to produce low blood urea nitrogen (BUN) levels.

Amino acid profiles include several markers of urea cycle function, including arginine, ornithine, citrulline, glutamine and aspartic acid. Ammonia toxicity problems can deplete α-ketoglutaric acid and vitamin B_6. Supplementation with these nutrients combined with a low-protein, high-complex carbohydrate diet and branched-chain amino acids can reduce ammonia loads and help to normalize urea cycle function.

Arginine (Arg)



Arginine provides a starting material from which products are formed that serve diverse functions (Figure 4.10). The hepatic urea cycle responds to protein intake and ammonia clearance demands. It is catalyzed by four enzymes, one of which, ornithine transcarbamoylase (OTC), is located in the mitochondrial matrix, whereas the other three are cytosolic (Figure 4.9). One enters as carbamoyl phosphate and the other as aspartic acid. Collectively, the four enzymes comprise the greatest incidence of genetic polymorphisms of any area of metabolism.[160] The urea cycle enzyme arginosuccinate synthetase (ASS) is ubiquitously distributed in human tissues for net synthesis of arginine or nitric oxide (NO). In the kidney, ASS and the enzyme arginosuccinate lyase (ASL) cause the net conversion of citrulline into arginine to maintain total-body arginine levels (Figure 4.10). Arginine supply can be increased in kidney proximal tubular cells when there is a nitrogen source available such as aspartic acid. The synthesized arginine is passed back into circulating blood. Arginine also is the direct precursor to the cell regulator nitric oxide. ASS can also produce nitric oxide from arginine in the presence of nitric oxide synthase.

Pathways of protein synthesis in all tissues and creatine synthesis in skeletal muscle account for the greatest quantity of total-body arginine flux. The total physiological impact of arginine, in addition to its role in the urea cycle, includes its role in cell regulation via production of nitric oxide and polyamines such as spermine.[161] Arginine insufficiency quickly leads to hyperammonemia due to failure of ammonia removal. Citric acid, cis-aconitic acid, isocitric acid and orotic acid appearance in urine are other biochemical markers of this condition (see Chapter 6, "Organic Acids").

Arginine has been found to be an essential amino acid in many metabolic balance studies.[162] Arginine is the first amino acid to be depleted when mammalian cells are grown in cell culture, indicating high cellular turnover of arginine compared with other amino acids such as leucine.[163] Thus, arginine is one of the few compounds that needs to be supplied from dietary sources, even though human tissues contain the enzymes necessary for its synthesis. Low levels of arginine in plasma or urine could be reflective of low dietary intake of arginine-containing foods such as legumes, whole grains and nuts.[164] On the other hand, chronic high blood levels of arginine may be conducive to tumor growth. Agents that induce arginine restriction are being investigated as anticancer therapy.[165] The precise role of NO in cancer is poorly understood, but it has the potential to stimulate tumor induction, promotion and progression,

TABLE 4.11 — CLINICAL SYMPTOMS OF HYPERAMMONEMIA AND UREA CYCLE DISORDER

Symptoms of Hyperammonemia	Symptoms of Urea Cycle Disorder	
Anorexia	General	Hyperammonemia
Irritability		Poor growth
Heavy or rapid breathing	Head, ears, eyes, nose and throat	Papilledema
Lethargy	Pulmonary	Tachypnea or hyperpnea
Vomiting		Apnea and respiratory failure (in latter stages)
Disorientation	Abdominal	Hepatomegaly
Somnolence	Neurologic	Poor coordination
Asterixis (rare)		Dysdiadochokinesia
Combativeness		Hypotonia or hypertonia
Obtundation		Ataxia
Coma		Tremor
Cerebral edema		Seizures and hypothermia
		Lethargy progressing to combativeness to obtundation to coma
		Decorticate or decerebrate posturing

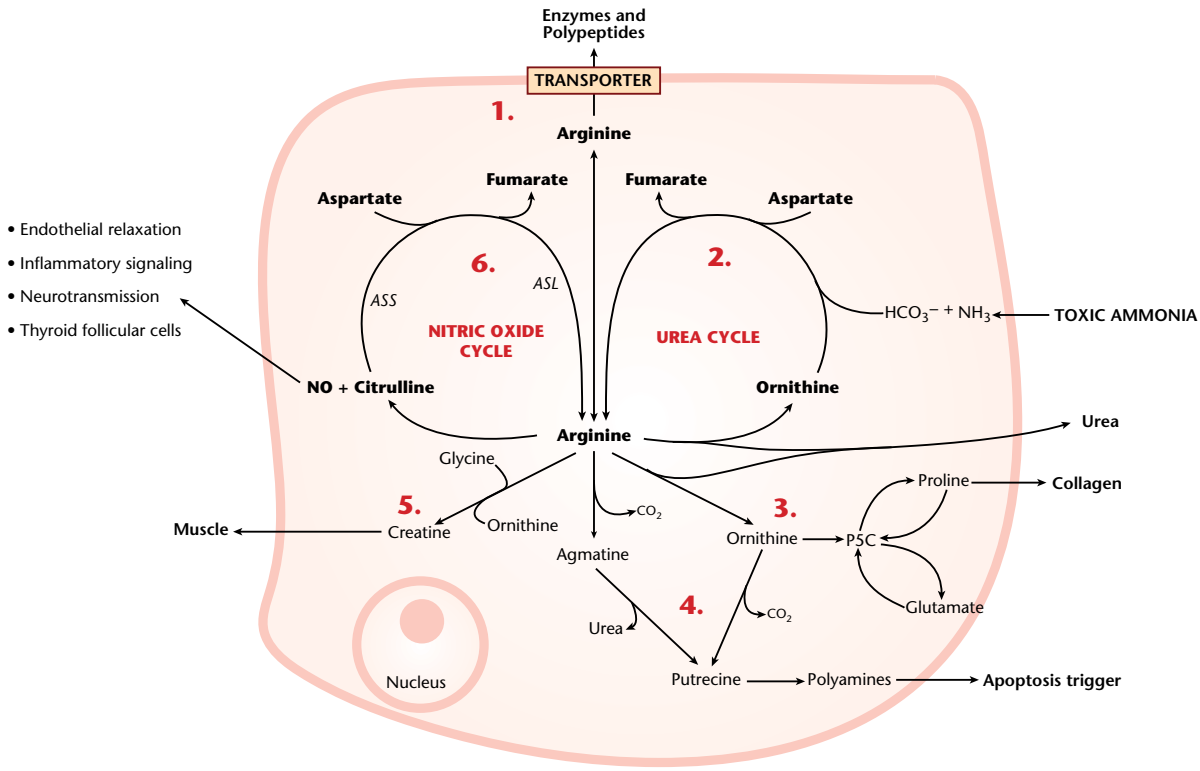


FIGURE 4.10 — Overview of Arginine Metabolism

In clockwise order from the top: (1) Arginine transporters perform regulated supply of the amino acid to protein synthesis needs of all tissues. (2) Urea formation clears toxic ammonia. (3) Catabolism to proline can help maintain collagen synthesis. (4) Up-regulation of polyamine formation is a signaling mechanism for apoptosis. (5) Net synthesis of creatine supplies the substrate for energy storage in muscle as creatine phosphate. (6) Reversal of a urea cycle step by nitric oxide synthase is the route to nitric oxide for multiple cell signaling functions. Renal tubule cells can convert circulating citrulline back to arginine that is transported to blood for return to the arginine pool. P5C = pyrroline-5-carboxylate.

as well as angiogenesis, cell adhesion and other pro-carcinogenic factors.[166] Regarding patient candidacy for arginine supplementation, the central question is whether the endogenous biosynthetic potential can meet the total-body demand. Depending on glutamine status, as much as 60% of absorbed arginine is metabolized by enterocytes and never enters portal circulation. Arginine status depends on activities of enzymes in small intestine, kidney, liver and nitric oxide-producing cells. Pathway 6 of Figure 4.10 illustrates how renal tubular cells can increase blood arginine from a supply of citrulline and aspartic acid, whereas the citrulline is mainly produced in the small intestine.[167]

Proline biosynthesis for collagen production can arise from either glutamic acid or arginine. If the pathway from glutamic acid is inhibited, then arginine deficiency can result from the increased conversion of arginine to proline. Individuals with this problem display low plasma arginine, ornithine, citrulline and proline.[168] The importance of intestinal activities of this enzyme is further illustrated by the hyperammonemia associated with intestinal resection surgery in rats.[169] In stable short-bowel patients, an arginine-free diet produces reduced levels of plasma arginine, ornithine and hydroxyproline within 5 days.[170]

Intestinal bacteria can decarboxylate arginine to form agmatine, which may increase the virulence of *Helicobacter pylori*.[171] Agmatine is also produced in human tissues[172] and may have an important neurotransmitter role.[173]

Clinical Relevance: Arginine and nitric oxide are involved in vascular tension. Nitric oxide is produced in endothelial cells and diffuses into the underlying layer of

smooth muscle cells, where it elicits release of the final modulator of muscular relaxation, cyclic guanosine monophosphate (cGMP). Asymmetrical dimethylarginine (ADMA) regulates nitric oxide formation as discussed in the next section.

Many of the reported effects of arginine in human health are due to nitric oxide-related cell responses. There is evidence of a relative deficiency of arginine in diseased coronary arteries. Arginine administration reversed the effect of nitric oxide inhibitors and improved coronary vasomotor tone in angina patients with angiographically narrowed coronary arteries.[174] The treatment resulted in greater dilation of diseased arteries, including stenosed arteries. Arginine administered at the end of surgery reduces intraperitoneal adhesion formation, a major cause of infertility and pain following surgery.[175]

Arginine has a protective effect against the hypercholesterolemia induced in animals by simultaneous methionine- and lysine-enriched diets.[176] The mechanism for the protective effect is unknown. Lysine infusion (0.5 mmol/kg) in 10- to 14-year-old humans also produced increases in plasma levels of arginine and ornithine, apparently through an inhibitory effect on arginase. The lysine infusion also resulted in increases of blood ammonia and urinary orotic acid.[177]

Other cell-regulatory effects of NO listed in Figure 4.10 are discussed further in Chapter 10, "Hormones". Nitric oxide participates in the cascade of events leading to immune cell activation. A special form of nitric oxide synthase in neurons serves to regulate their function and NO formation in the thyroid follicular cells regulates blood flow locally as a mechanism to regulate thyroid activity.

Arginine may play yet another role as a supplier of polyamines to stimulate healing in the wound response. Mechanical wounding causes an increase in the expression of the arginine decarboxylase 2 gene and concurrent decreases of spermine with increases of putrescine concentrations, indicating these metabolic changes are associated with the healing response.[178] Administration of arginine after trauma-induced hemorrhage significantly improves macrophage function and interleukin-6 levels.[179]

Arginine is one agent found to increase sperm count and motility, although the response is quite variable. Of 15 patients with low sperm counts who received arginine hydrochloride over a 3-month spermatogenesis cycle, 3 pregnancies (20%) were obtained.[180] Enhancement of penile erection is another effect of supplemental arginine, especially when given along with ornithine to spare the utilization for urea cycle function. The effect on penile erection is mediated by nitric oxide relaxation effects on vascular sphincter muscles.[181]

Interpretation and Treatment: Arginine released from dietary protein is absorbed in the jejunum and ileum of the small intestine by a specific transport system also used for lysine and histidine. This intestinal transport competition is one reason that heavy supplementation with any one of these amino acids should be done with caution.

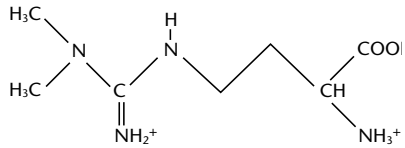
High levels of arginine in plasma or urine may represent a functional block in the urea cycle. The arginase enzyme requires manganese and the failure of this metabolic role may give rise to the early signs of manganese deficiency appearing as ammonemic symptoms.[182] If ammonia toxicity symptoms (confusion and disorientation) and signs (elevated urinary citrate) were present, a low-protein diet with the avoidance of lysine-containing foods would be advisable to reduce the ammonia load. Additional vitamin B_6 and α-KG may also be useful to remove excess intracellular ammonia.

In light of the favorable clinical responses to arginine supplementation of up to 9 grams per day in hypertensive patients, one must consider adverse reactions. Mammary tissue has a high capacity for arginine catabolism due to high activities of arginase, ornithine aminotransferase and pyrroline-5-carboxylate reductase.[183] The principal products are proline and ornithine. High doses of L-arginine were found to have inhibitory effects on in vivo nitric oxide formation in rat substantia nigral cells.[184] Additional potential adverse effects of excessive arginine intake due to pro-oxidant formation are discussed in Chapter 9, "Oxidative Stress and Aging."

Patients with some types of cancer may benefit from an arginine-restricted diet. Some tumor cell lines are dependent on arginine for growth due to an inability to express ASS. Selective elimination of arginine from the circulation of animals causes tumor regression. Among human cancers studied, sarcomas, invasive breast carcinoma and renal cell carcinoma, were sometimes found to be ASS deficient,[185] but the efficacy of arginine restriction in human cancer patients has not been investigated.

Notes:

Asymmetric dimethylarginine (ADMA)



ADMA is an endogenous inhibitor of angiogenesis.[186] Many angiogenic factors require endothelium-derived NO to exert their effects. Arginine is the compound from which NO is formed and ADMA is a naturally occurring compound that inhibits NO synthases.

Formation of ADMA from Arginine: Under the action of an enzyme called methionine-dependent protein arginine N-methyltransferase (PRMT), ADMA is formed by methylation of arginine residues of nuclear proteins (Figure 4.11).[187] On hydrolysis, the ADMA residues are released to pass into blood, available for binding to NOS to modulate the rate of NO production. A principal mechanism of regulating concentrations of ADMA is through the action of the catabolic enzyme dimethylarginine dimethylaminohydrolase (DDAH) that degrades ADMA to citrulline. ADMA formation is increased by methionine loading, producing endothelial dysfunction. This effect can be reversed by administration of ascorbic acid that enhances DDAH activity.[188] More than 4 mmol (> 1 g/d) of ADMA is extracted by the liver (700 times the amount circulating in blood).[189] The other principal mechanism for regulation of ADMA is renal clearance, allowing ADMA above renal thresholds to pass into urine.[190] In addition, endothelial DDAH expression is stimulated by all-*trans* retinol.[191] On the other hand, nerve damage causes marked increase in the expression of ADMA as part of the mechanism to protect neuronal cells from the effects of excess NO.[192]

To summarize the mechanistic studies, there are multiple dietary and nutrient-dependent steps involved in the control of ADMA synthesis and clearance. L-Arginine stimulates NO synthesis to overcome the inhibitory effects of high ADMA. Low-fat meals help to reduce the synthesis of ADMA by PRMT. Vitamin A induces expression of DDAH, causing a lowering of ADMA. Antioxidants prevent the inhibition of DDAH, thus enhancing the removal of ADMA. Normalization of elevated homocysteine with vitamins B_6, B_{12} and folic acid reduces ADMA by controlling endothelial oxidative effects. In addition, individuals with persistent ADMA elevations may be managed with hormonal interventions such as acetylcholinesterase (ACE) inhibitors and natural estrogen replacement therapies. Exercise has a strong

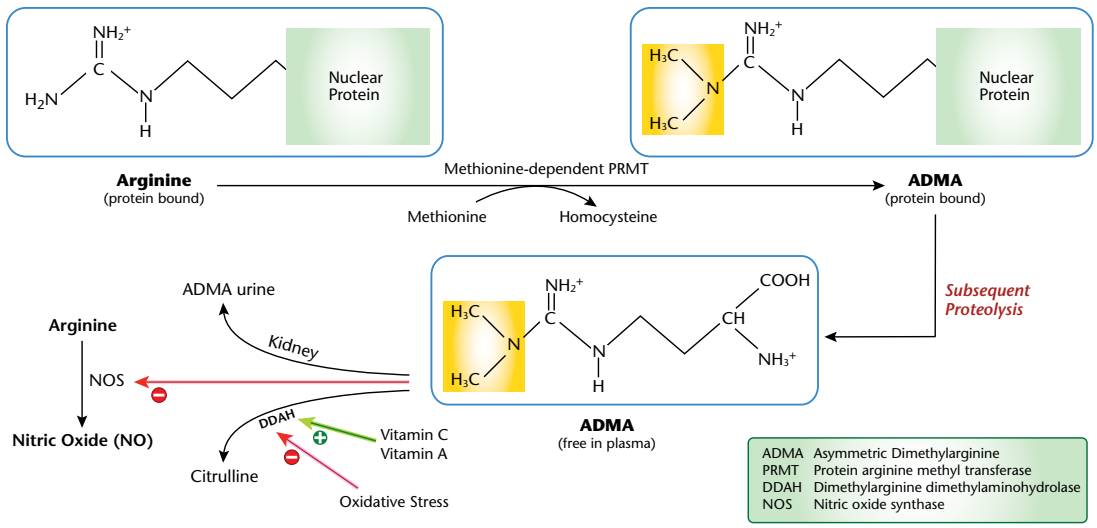


FIGURE 4.11 — ADMA Synthesis, Clearance, & Regulation of NOS

Methylation of the terminal nitrogen atom of the arginine side chain in nuclear proteins produces protein-bound ADMA that is released on proteolysis. The released ADMA binds to NOS, slowing its activity in NO formation, thus regulating the rate of NO synthesis. The half-life of ADMA is governed partly by DDAH activity that causes degradation to citrulline. Because DDAH is susceptible to damage by cellular oxidants, its protection by vitamin C and vitamin A allows normal ADMA removal and, thus, normal NO formation.

positive effect on the arginine/ADMA ratio. Pain-free walking distance is linearly correlated with lower ADMA levels in atherosclerotic patients.[193]

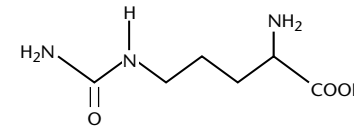
Clinical Relevance: High plasma ADMA signals lack of nitric oxide stimulation of cell activities. Levels of ADMA high enough to inhibit nitric oxide synthase have been found in renal failure,[194,195] hypertension, hypercholesterolemia, preeclampsia,[196] diabetes mellitus,[197] tobacco use and aging.[198] Atherosclerosis is marked by impaired vasodilation in response to normal physiological stimuli.[199] A number of standard heart disease risk factors (smoking, hypertension, hyperlipidemias) are related to vasodilatory impairment, but the strongest correlation may be the ratio of arginine to ADMA in plasma.[200] This ratio has been used to assess the balance of stimulatory and inhibitory effects on NO synthesis.[201] Elaboration of endothelium-derived nitric oxide affects the behavior of circulating T lymphocytes and monocytes.[202]

Patients with atherosclerosis or with risk factors for atherosclerosis have a defect in endothelium-dependent vasodilator function, which is related to decreased activity of NO. In hypercholesterolemia, elevated plasma levels of ADMA reduce NO synthesis. The lipid-induced impairment of angiogenesis can be reversed by oral administration of arginine. This mechanism may account for the benefit of arginine in nutrient-based interventions for fibrosarcoma.[203] A synthetic NOS inhibitor has been found to have potent antiangiogenic effects in multiple myeloma induced in mice.[204] These findings point up the need to assess arginine and ADMA status when arginine therapy is considered.

In patients with type-2 diabetes mellitus, plasma ADMA increases from 1.0 to 2.5 mmol/L 5 hours after ingestion of a high-fat meal, indicating that ADMA may contribute to abnormal blood flow responses and to atherogenesis in this population.[197] In essential hypertension, urinary excretion rate of nitrite plus nitrate (or NO production) is lowered from 77 to 56 µmol/mmol creatinine, whereas plasma levels of ADMA increase from 1.1 to 2.4 µM, compared with normotensive controls.[205] This shows that the two molecules have an inverse relationship. Other findings suggest that ADMA plays an important role in the pathogenesis of hypertension-associated loss of kidney function.[206]

Treatment: The inhibitory effect of ADMA on NO production may be overcome with the use of supplemental arginine.[207] Oral administration of 14 to 21 grams of arginine daily normalizes plasma arginine-ADMA ratios and attenuates monocyte and T-lymphocyte adhesiveness. For some patients, these changes may lead to lower rates of atherosclerotic plaque formation and lower risk of heart disease.[208] Such responses are modulated by other dietary and lifestyle factors. Acute coronary events for middle-aged men in Finland, where animal protein intake is among the highest, do not show a relationship to dietary arginine intake.[209]

Citrulline (Cit)



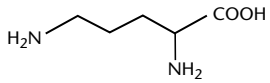
The amino acid citrulline gets its name from its high concentration in the watermelon *Citrullus vulgaris*. In human kidneys, citrulline and aspartic acid are united by argininosuccinate synthetase (ASS) to produce arginosuccinate (Pathway 6 of Figure 4.10). The degradation of arginosuccinate to fumarate and arginine is a primary mechanism for sustaining plasma levels of arginine. The same enzyme acts in liver cells to complete the urea cycle (Step 3 in Figure 4.9). Plasma citrulline has been used as a marker for reduced small intestinal mass,[210] intestinal injury and rejection of small bowel transplants.[211] Plasma citrulline rises in proportion to enterocytes mass, reflecting the unique capacity of enterocytes for net conversion of glutamine into citrulline.

Both classic and type II citrullinemia are associated with known gene alterations. Plasma citrulline elevation is also used as a marker of postoperative intestinal failure.[210] Nitric oxide (NO) synthesis from arginine results in lower production of citrulline. Low availability of NO may be due to inactivation of NO or inhibition of synthesis, which may be detected by lowered production of citrulline.[212]

High plasma or urinary citrulline can indicate a functional block in the argininosuccinate synthetase (ASS) enzyme, leading to a buildup of excess ammonia in the system.

Supplementation of magnesium and aspartic acid may help to drive this reaction forward. Arginine restriction may also be used to decrease citrulline levels. Plasma citrulline declined by 30% in adult males receiving an arginine-free diet,[181] and protein restriction in one infant resulted in lowered plasma citrulline.[213] Arginine loading results in increased citrulline values.[214]

Ornithine (Orn)



In the final step in which urea is produced in the urea cycle, ornithine is also produced (Figure 4.9). The enzyme ornithine carbamoyl transferase then initiates another turn of the cycle by catalyzing the reaction of ornithine with carbamoyl phosphate. The most common inherited condition of the urea cycle enzymes is ornithine transcarbamylase (OTC) deficiency where hyperammonemia is the dominant clinical feature. The incidence is estimated to be as high as 1:20,000. A report appearing in 2006 indicated that 341 mutations of the OTC gene had been reported. [215] Patients with these inherited traits may show elevated plasma glutamine, proline, lysine, valine and methionine, but ornithine is usually normal.[216] An alternative scenario for ornithine metabolism is genetic polymorphic impairment of ornithine transport into the mitochondria. These patients show hyperammonemia and hyperargininemia along with the hyperornithinemia, leading to the common abbreviation of HHH for this genetic scenario. HHH patients have elevated plasma ornithine even though the urea cycle fails due to inadequate ornithine in the mitochondrial matrix. Hyperammonemia, again, is the cardinal sign that is detected by plasma ammonia measurement or by urinary orotic aciduria as described in Chapter 6, "Organic Acids." Supplemental ornithine (6 g/d) helps patients with HHH to achieve lower ammonia, while raising plasma ornithine to even higher levels.[217] Many polymorphisms of the OTC gene display as less severe symptoms that may lead to diagnosis only late in life. One report describes a case that was diagnosed at 32-years-old after a history of recurrent episodes of vomiting, meat refusal, lethargy and convulsions since childhood.[10] Another adult-onset form produces postpartum coma in women with the polymorphism.[218]

When hepatic failure is induced in pigs, both ornithine and citrulline become greatly elevated.[219] High levels are found when there is a blockage of the urea cycle at the critical ammonia incorporation step, leading to a higher body burden of ammonia.

α-Ketoglutaric acid, vitamin B_6 and magnesium are useful in clearing chronic minor excesses of ammonia.[220] A low-protein diet will also decrease nitrogen as a source of ammonia. Gut bacteria are also a major source of ammonia. Ornithine is also a precursor to the polyamines, putrescine, spermidine and spermine.[221] High ornithine levels may have effects on these regulatory amines.

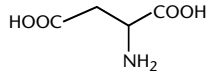
Cases of low levels of ornithine are rare, but could suggest impaired control of growth hormone (GH). Ornithine serves as a source of polyamines that regulate cellular metabolism. Ornithine can stimulate release of GH from the pituitary,[222] and thus may serve as an important anabolic substance for the body. Growth hormone responsiveness to ornithine stimulation has been used to assess abnormal growth rates in children.[223] Urea cycle effects due to low ornithine may be corrected with arginine supplementation.[162]

In patients with urea cycle deficits, sodium benzoate therapy has been used to lower nitrogen load by causing increased excretion of glycine. However, the therapy has been shown to cause insufficiency of carnitine[224] and it can cause glycine insufficiency.[225]

Refer to Case Illustration 4.3

Notes:

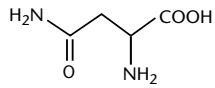
Aspartic Acid (Asp)



Aspartic acid, or aspartate, is pivotal in amino acid metabolism. Aspartic acid aids in the detoxification of ammonia via the urea cycle (Figure 4.9). It is also a precursor of nucleic acids used in DNA and RNA. Low levels of aspartic acid may reflect lowered cellular energy-generating capacity, experienced as chronic fatigue. Aspartic acid can enter the citric acid cycle by conversion to oxaloacetic acid in the presence of B_6 and α-ketoglutaric acid and equimolar amounts of citric and aspartic acids drive both the citric acid and urea cycles, in the presence of those cofactors. These metabolic considerations suggest that, when aspartate is high, B_6 and α-ketoglutarate may assist the conversion to oxaloacetic acid. Magnesium and zinc are also relevant cofactors that can help normalize high aspartic acid.

Simultaneous administration of aspartic acid and arginine leads to increases in neurostimulatory amino acids in the brain[226] and to multiple organ-specific changes.[227] Increases of GABA in brain, ornithine and proline in liver and serine, glycine and alanine in all tissues were found when aspartic acid was given along with arginine. Aspartic acid administration has been shown to increase stamina and resistance to fatigue.[228]

Asparagine (Asn)



Asparagine is needed in all tissues for protein synthesis and it serves a special function for attachment of carbohydrate residues to membrane proteins by which cells are identified. The enzyme, aspartate ammonia ligase, converts aspartate to asparagine by addition of ammonia to form the amide group of the side chain. This link to aspartic acid has two potential consequences in amino acid profiles. Individuals with normal activity of the ligase tend to have aspartic acid and asparagine in similar relative positions, depending largely on the availability of aspartic acid. On the other hand, reports showing high aspartic acid with low asparagine suggest polymorphism of the ligase that causes reduced activity.

Although, the plasma and urinary levels of asparagine can vary over relatively wide ranges with little clinical consequence, evidence of physiological roles of asparagine comes from treatment of lymphoblastic leukemia with L-asparaginase. This enzyme cleaves asparagine to aspartic acid and ammonia, resulting in depletion of asparagine levels and cleavage of the asparagine attachment site for polysaccharide chains used in cell identity. Serum asparagine concentrations decline approximately ten-fold during therapy.[229] Administration of asparaginase produces decreases of serum albumin, cholesterol, insulin, fibrinogen and clotting factors, along with dramatic reduction in serum thyroxin-binding globulin.[230] Asparagine-rich proteins such as antithrombin III are reduced as a result of the lowered plasma asparagine concentrations.[231] Monitoring of plasma asparagine levels is a useful means of determining dosage schedule of L- asparaginase.[232] Plasma asparagine does not fall in individuals who are resistant to the enzyme.

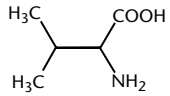
The evidence from such chemotherapeutic applications suggests that other patients with chronic low asparagine levels may experience some degree of the metabolic fates described. However, there are no reports that allow firm conclusions of such effects.

Notes:

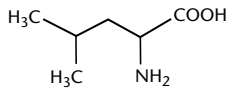
Essential Amino Acids for Proteins and Energy

The Branched-Chain Amino Acids (BCAA):

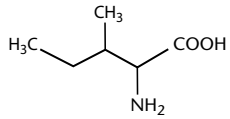
Valine (Val)



Leucine (Leu)



Isoleucine (Ile)



The branched-chain amino acids valine, leucine and isoleucine are used for peptide and protein synthesis. They do not serve as precursors to other non-peptide products as do most other essential amino acids. The BCAA are ubiquitous among the great variety of proteins that serve as enzymes, transports and structural components of cells, where their hydrocarbon side chains provide the majority of the hydrophobic inner protein domains. The BCAA make up 35% of muscle proteins and comprise almost 50% of the amino acids in dietary protein. Collectively they make up the largest supply of circulating EAA. The factors that govern their plasma concentrations act so that the flow into metabolic pathways meets whole-body demands during times of metabolic crisis and renewal.

During strenuous muscle activity, BCAA serve as a source of energy, because they participate in transamination reactions that result in flow of alanine and glutamine from skeletal muscle to the liver and kidney, where they may form glucose. The carbon skeletons of isoleucine and valine are catabolized to succinate, a key intermediate in the citric acid cycle. In the brain, the BCAA play a role in the control of amino acid-derived neurotransmitter metabolism by competing with other large neutral amino acids like tryptophan for the pumping systems controlling the blood-brain barrier. That means that brain synthesis of serotonin and dopamine from dietary tryptophan and tyrosine is dampened due to the presence of BCAA in food because the BCAA reduce the rate of transfer across the blood-brain barrier. Because of their nitrogen-sparing effects, the BCAA have been used clinically for recovery from surgery, trauma and sepsis and in diabetic regulation. The stress-induced decrease of muscle protein synthesis can be prevented by supplementation with a balanced BCAA formula.[233]

The Branched-Chain Ketoacid Dehydrogenase Complex: In skeletal muscle, the BCAA are converted to their respective ketoacids via a branched-chain amino acid transaminase, requiring the cofactor pyridoxal 5-phosphate, which is the biochemically active form of vitamin B_6 (pyridoxine). This is the primary reason that vitamin B_6 is critical for amino acid metabolism.[234] High levels of BCAA in plasma may result from inadequate vitamin B_6. BCAA utilization is impaired and BCAA excretion in urine is elevated when the cofactor is lacking.

The ketoacids are catabolized by the branched-chain α-ketoacid dehydrogenase complex (BCKDC). This enzyme is very active in skeletal muscle, which is the primary site for catabolism of BCAA.[235] The full BCKDC system is a multienzyme complex with subunits dependent on the nutrient-derived cofactors thiamin as thiamin pyrophosphate (ThPP), riboflavin (FAD), niacin (NAD) and pantothenic acid (coenzyme A, vitamin B_5). In addition, lipoate must be attached to one of the subunits as a prosthetic group. The net effect of these BCAA catabolic reactions is to pass carbons into the citric acid cycle for energy production. Normally, high urinary or plasma levels of BCAAs indicate deficiencies of vitamin B_6 or the vitamins required by the BCKDC. Adequacy of the nutrient cofactors for BCKDC can be measured in urine (Chapter 6, "Organic Acids"). A genetic mutation of BCKDC produces extreme elevations of plasma and urinary BCAA seen in maple syrup urine disease.[236]

Pantothenic acid supplementation has also proven useful in these situations since it conserves the sulfur-containing amino acids by improving cellular energy-yielding pathways.[237] Chronic depletion of BCAAs will eventually show up as low urine and plasma levels, with loss of muscle mass and protein synthesis capabilities sometimes experienced as alopecia and sarcopenia.

Catabolic and Anabolic Hormone Effects: Metabolic acidosis associated with the catabolic state inhibits BCKDC, so the BCAA tend to rise. On the other hand, the BCAA tend to fall when the BCKDC responds in

a way that transmits catabolic and anabolic hormone signals to exert metabolic control. Under a variety of stressful conditions associated with changes in circulating glucocorticoids the BCKDC complex is activated (Figure 4.12). In renal failure, two catabolic signals, glucocorticoids and acidification, stimulate activation of BCKDC, resulting in BCAA degradation, thereby lowering plasma concentrations of BCAA.[238]

Anabolic hormones act in an opposing manner by inactivating BCKDC, thus decreasing the breakdown of BCKA and raising plasma levels of the BCAA.[239] In females, the sudden need for protein synthesis for uterine reconstruction after menses is availed by a supply of amino acids. Amino acid mixtures and testosterone have similar anabolic effects in older men.[240] Testosterone injection increases net protein synthesis without changing amino acid transport,[241] and plasma BCAA rise along with testosterone and dehydroepiandrosterone sulfate (DHEA-S) during intense athletic training.[242] The corresponding removal of BCAA from energy-yielding pathways during such anabolic phases causes increased oxidation of glucose and fatty acids, often accompanied by increased appetite.

A reported case of high plasma BCAA was unremarkable until the patient became comatose during an infection and died at the age of 4 1/2 years.[243] Propionic acidemia resulted from mutations in genes encoding propionyl-CoA-carboxylase. Plasma BCAAs were elevated in spite of the associated acidosis that should have activated BCKDC and consequently degraded the BCAA.The two missense mutations found in this case did not result in clinical presentation until an infection caused failure of metabolic compensation. This case demonstrates that late onset of symptoms may be found in older populations with less disruptive mutations.

Late-onset propionic acidemia patients are found to suffer from abnormal psychomotor development and show mental and psychological disabilities.[244] Accumulation of toxic metabolites can impair mitochondrial energy production, especially in regions of the brain with a high rate of aerobic (mitochondrial) metabolism, such as in basal ganglia.[245,246] Toxic substances have been shown to cause hyperammonemia in propionic academia by inhibiting urea cycle enzymes.[247]

An active insulin response is required for clearing of amino acids from plasma. BCAA levels are increased in non–insulin-dependent diabetes mellitus (NIDDM) and the sustained elevated levels may be a causative factor in diabetic microangiopathy.[248] Skeletal muscle is a major destination for plasma BCAA. Their incorporation into muscle protein is dependent on insulin-like growth factor-I (IGF-I), but for different reasons. IGF-I stimulates protein synthesis, whereas insulin inhibits protein breakdown by stimulating amino acid uptake. Muscle protein synthesis is increased when plasma amino acid levels are raised, as demonstrated in studies using phenylalanine infusion.[249] The net effect is that decreasing IGF-I levels with insulin insensitivity leads to higher plasma BCAA levels. Conversely, raising BCAA levels in plasma in the presence of a normal insulin response increases lean body mass in response to exercise.

Elevations in Plasma: Excessive dietary intake can affect plasma levels of these amino acids and will generally cause elevation in urinary amino acid levels. Such excessive intake is common among athletes, especially body builders who may consume large amounts of protein powders in addition to maintaining a high-protein diet. An enriched infusion of BCAA increases nocturnal respiration in normal adults[250] and normalizes food intake in patients on total parenteral nutrition. The flow of BCAA into blood suppresses gastric emptying after a meal containing protein to allow proper digestion of protein in the acid milieu of the stomach.[251]

Leucine-Specific Considerations: Leucine is among the most abundant amino acids in animal tissues. It has been proposed as a regulator of protein metabolism because it surpasses all other amino acids in inhibiting muscle protein degradation. Because of reduced rates of release from skeletal muscle, plasma essential amino acids tend to fall when leucine is abundant.[252] The finding of such a regulatory role for a single amino acid reinforces the benefit of balanced intake of all essential amino acids. It is not recommended to supplement leucine alone because it can have undesired effects. Instead, a balanced amino acid mixture is appropriate.

Notes:

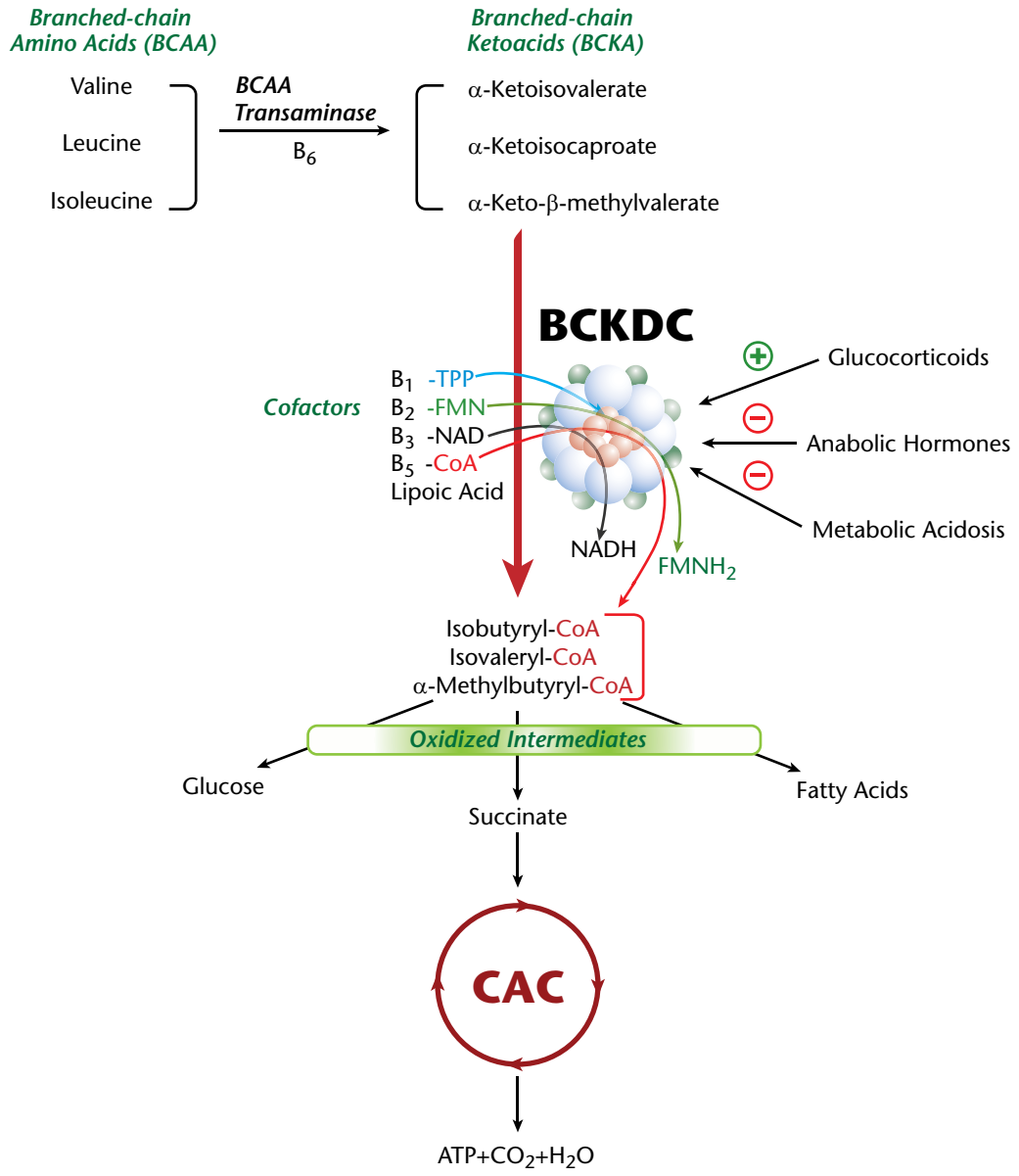
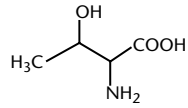


Figure 4.12 — Multiple Levels of Plasma BCAA Regulation

The non-reversible committed step of the catabolic pathway is controlled by the branched-chain ketoacid dehydrogenase complex (BCKDC). The BCKDC contains multiples of three separate enzymes shown as colored spheres. Cofactors derived from five B-complex vitamins are required for the full enzyme activity to accomplish this single step. Hormones and metabolic factors regulate the BCKDC via two other enzymes that activate (phosphatase) or inactivate (kinase) in the BCKDC enzyme complex. The phosphatase and kinase, in turn, respond to various hormonal controls, transmitting their signals to the metabolic level. The net effect is to regulate tissue building and breakdown via plasma amino acids. Normal responses to hormones depend on activity of the BCKCD that, in turn, depends on supply of the essential nutrient cofactor precursors.

Threonine (Thr)



Threonine (Thr) is typically the second or third limiting amino acid in vegetarian diets, following methionine or lysine. This means that dietary supply of proteins with balanced content of threonine is critical for protein synthesis and tissue maintenance. Since threonine is metabolized differently in humans than in most animals (Figure 4.13), data from animal studies should be interpreted with caution.[253] Results from animal experiments can still shed light on some aspects of threonine assessment. An early study of dietary influence in rats showed plasma concentrations of threonine and threonine flux into brain shifting in proportion to long-term dietary protein content.[254] Plasma threonine then increased from 3- to 5-fold when the animals were fed a very high-protein meal (50% casein), indicating slow hepatic clearance of threonine. α-Amino-N-butyric acid (α-ANB) is a competitor for the threonine transporter. Animals maintained on low-protein diets with added α-ANB have low threonine with decreased growth and food intake, which could be corrected by adding threonine to the diet. These results were interpreted as evidence that individual amino acid supplements can induce imbalanced amino acid status.[255]

The matter of disposition of the α-ketobutyrate from threonine catabolism is of importance and is discussed in Chapter 6, "Organic Acids." The urinary organic acid α-hydroxybutyrate (AHB) is a marker of hepatic glutathione synthesis via the conversion of homocysteine to cysteine. The contribution of threonine catabolism to urinary AHB levels should be relatively constant for a given individual. Additionally, threonine catabolism is not likely to significantly contribute to urinary AHB because mitochondrial threonine dehydratase favors oxidation of the precursor, α-ketobutyrate, to propionate rather than reduction to AHB. Evidence for this in humans is found in an analysis of plasma threonine versus urinary AHB in retrospective data from analyses of a general outpatient population in a clinical laboratory[550] (Figure 4.14). There is no trend to higher AHB levels as plasma threonine varies over a range of 20 to 200 μmol/L, confirming that increasing levels of plasma threonine produce no proportional increase in urinary AHB.

Exercise induces greater uptake of threonine than any other amino acid in cardiac tissue of thoroughbred horses.[256] Humans on high-protein diets, however, may have elevated plasma threonine due to the limited number of pathways for clearing this amino acid. In the early stages of dietary protein deficiency, plasma threonine is uniquely stable in contrast to most other essential amino acids.[51] This effect is likely due to the lack of metabolic requirements for threonine other than protein synthesis. After 12 months of human growth hormone (GH) treatment, children with GH deficiency had higher fasting serum concentrations of most amino acids, whereas

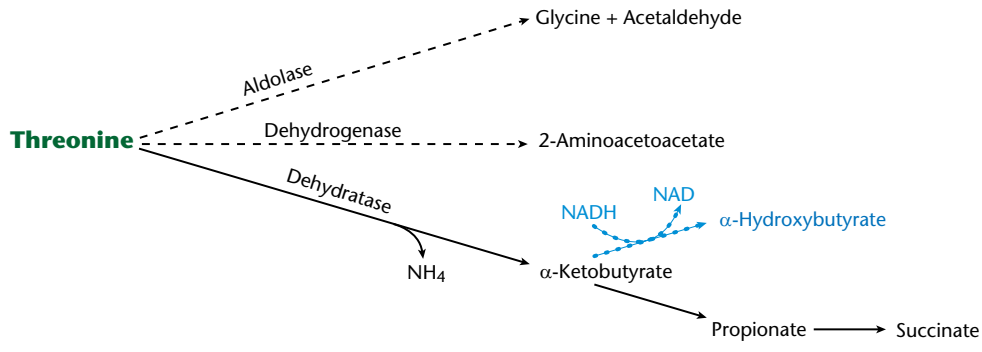


FIGURE 4.13 — Differentiation of Human Threonine Metabolism

Biochemical pathway charts may indicate the three catabolic pathways for threonine shown in this diagram. Man and chimpanzees have weak expression of genes for the aldolase and dehydrogenase enzymes, leaving only the deydratase pathway for threonine clearance. The mitochondrial location of this enzyme results in efficient oxidation of α-ketobutyrate to propionate. Reduction to α-hydroxybutyrate is favored when cytosolic conversion of homocysteine to cysteine produces α-ketobutyrate (Figure 4.20). Dashed arrows indicate pathways active in bacteria and most vertebrates and solid lines indicate human pathways. The light blue arrows indicate the improbable conversion in mitochondria.

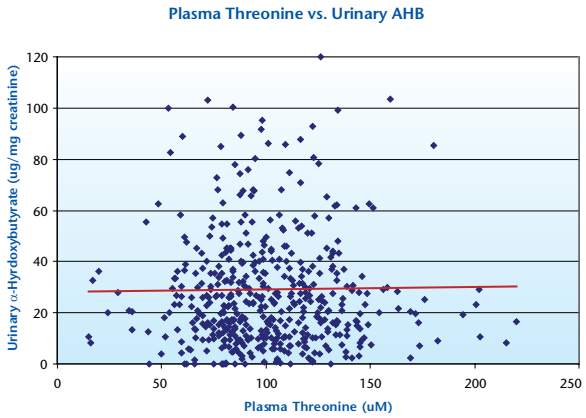


FIGURE 4.14 — **Plasma Threonine vs. Urinary α-Hydroxybutyrate**

No increase of urinary α-hydroxybutyrate is found over the entire range of plasma threonine in 486 general outpatient profiles where plasma amino acids and urinary organic acids were performed. The evidence argues against significant activity of ketobutyrate dehydrogenase in clearing of excess threonine and it suggests the conclusion that urinary α-hydroxybutyrate is largely derived from the glutathione synthetic pathway illustrated in Figure 4.20.

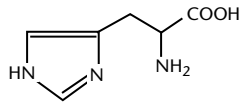
threonine decreased significantly.[257] The GH-induced retention of nitrogen and increased amino acids was unable to prevent threonine losses.

Interpretation and Treatment: A pattern of low EAA levels with threonine above the 4th quintile indicates inadequate assimilation of dietary protein. This may be due to low protein intake or poor digestive factors. The opposite pattern of low threonine while other EAA are normal may be due to factors such as hormonal imbalance causing a catabolic state.

Oral threonine doses of 7.5 g/d can raise plasma threonine levels to the upper range of normal.[258] In multiple sclerosis (MS), supplementation of threonine is a potential non-sedating, non-toxic approach to the management of spasticity. The effect is due to threonine-assisted glycine generation and postsynaptic inhibition of the motor reflex arc in the spinal cord.[258] The plasma phenylalanine-lowering effects of threonine are described under the section, "Phenylalanine (Phe) and Tyrosine (Tyr)" later in this chapter.

Generation of glycine from threonine can provide for special glycine needs in conditions demanding glycine, such as glutathione loss due to oxidative stress or toxicant challenge requiring glycine conjugation.

Histidine (His)



Although histidine cannot be synthesized in human tissues, nitrogen balance in healthy adults is sustained even on a histidine-free diet. A histidine-free diet caused a 26% linear decline in whole-body protein turnover, whereas urinary excretion of nitrogen, urea, creatinine and 3-methylhistidine were not affected.[259] Plasma histidine declined steadily over 48 days to 38% of initial values, whereas urinary histidine dropped by 51% over the first three days, then continued to decline to 18% of initial levels. The rate of release from hemoglobin and carnosine, both of which are in large supply in well-nourished individuals, suggests that these two sources can act as a reservoir of His. Carnosine (β-alanyl-histidine) is abundant in skeletal muscle (see the section, "Carnosine and Anserine" later in this chapter).

Histidine, Folate Deficiency and Anemia: As discussed more fully in the Chapter 2, "Vitamins" and Chapter 6, "Organic Acids", the appearance of formiminoglutamate (FIGLU), a degradation product of histidine catabolism, in urine is a functional marker for folate status (Figure 4.15). The histidine catabolic pathway provides a sensitive source for such a biochemical marker due to the relatively high flux of excess histidine through this pathway.[260] The FIGLU test sensitivity may be increased by administering three 5 g doses of L-histidine hydrochloride monohydrate in 4-hour intervals as a metabolic challenge on the day before the overnight urine collection.[14] The histidine challenge dose causes a rapid rise of histidine that has been found to alleviate the anemia associated with folic acid deficiency. Since chronic folic acid deficiency reduces the flow of histidine through the catabolic pathway, one might think that histidine losses would be lowered and there would be little effect of added histidine. The explanation apparently lies in the inefficiency of renal histidine recovery (causing histidine to be the most abundant amino acid in healthy urine). Histidine that is not catabolized in the liver due to folate deficiency simply spills into urine because the renal threshold is easily exceeded. Thus, folic acid deficiency results in increased urinary loss of histidine, leading to histidine depletion and lack of availability for protein synthesis. Synthesis of hemoglobin falls as

histidine levels become a limiting factor (histidine comprises 10% of the amino acids in hemoglobin). Thus, the histidine challenge procedure for assessing folic acid status provides an instance where the test for a deficiency alleviates one of its effects (anemia).

Other studies confirm that histidine supplementation can overcome anemia in folic acid-deficient patients, apparently by stimulating hemoglobin synthesis. In eight folic acid-deficient patients, doses of 5 g L-histidine hydrochloride monohydrate dissolved in apple juice, taken 3 times daily, produced an average increase in reticulocytes from 0.5 to 8.9 %, whereas mean hemoglobin rose from 8.1 to 12.1 g/dL.[14] In folic acid deficiency, urinary excretion of histidine and the histidine metabolite FIGLU is increased (see Chapter 6, "Organic Acids"). Folic acid deficiency seems to exacerbate the relatively poor renal resorption of histidine, causing greater losses. With histidine administration, urinary histidine increased by 4-fold, whereas FIGLU excretion increased by 10-fold compared with normal controls.[261]

Histidine is converted into histamine by a single-step reaction catalyzed by histidine decarboxylase (Figure 4.15). In extrahepatic tissues, histamine is cleared by methylation via transfer from S-adenosylmethionine. Hepatic tissue clears circulating histamine via a copper-dependent amino oxidase, whereas postprandial excess histidine is removed by conversion to glutamic acid via FIGLU. Histamine elicits physiological responses by binding to receptors in target tissues. Histamine receptor types H1, H2 and H3 are found in a variety of tissues and cell types.[262]

Histamine is synthesized and stored in mast cells, basophils and enterochromaffin cells. In the allergic response, mast cells degranulate, releasing histamine that binds to H1 receptors in the smooth muscles of airway, gastrointestinal tract and cardiovascular tissues. The H1 receptor is antagonized by antihistamines. In the stomach, histamine released by enterochromaffin-like cells binds to H2 receptors initiating a cascade of events that lead to secretion of hydrochloric acid.

Hypothalamic neurons that project into most cerebral areas of the brain synthesize histamine as a neurotransmitter, exerting control over sleep, wakefulness, hormonal secretion, cardiovascular control, thermoregulation, food intake and memory formation. Because of the enhancement of histamine neurotransmission, histidine has been found to serve as a beneficial adjuvant for selected antiepileptic drugs. Thus, histidine (500 mg/kg) augmented the protective effects of phenytoin without augmentation of unwanted effects on memory and motor performance.[263] Similar effects were found with carbamazepine in rats.

Histamine is critical for brain arousal[264] and plays a suppressive role in seizure development and sleep disorders.[15,16] The relationship of histamine to changing histidine concentrations depends on the relative rates of histamine formation and degradation. In the mouse model, peripheral loading with a high dose of histidine produces the same antinociceptive effect as activation of brain histamine.[265] Animals given oral L-histidine at 1,000 mg/kg twice daily showed increased histamine levels in the brain, although plasma histidine did not change.[266] Thus, the brain can utilize excess histidine to increase histamine and modulate neuronal activity. (See the SN1 and SN2 transporters discussed in the "Of Further Interest" box on page 193.)

REFER TO CASE ILLUSTRATION 4.4

Interpretation of Laboratory Results: Chronic high rates of histamine production tend to produce lowered levels of plasma and urinary histidine because of increased utilization. The origin of the histaminic stimulation should be investigated and supplementation of histidine may need to be done carefully to avoid further promoting histamine output. Moderate dosing with formulas containing all essential amino acids in balanced proportions are generally safe and effective. Low plasma histidine levels have been associated with rheumatoid arthritis.[267] Histidine supplementation (around 4,000 mg per day) raises levels in blood and reduces arthritic symptoms in rheumatoid arthritic patients.[267] In addition to the low folate contributions described above, low dietary protein intake or malabsorption syndromes should be considered as explanations of low histidine levels, particularly if low levels of other essential amino acids exist.

Elevated plasma or urinary histidine can result from muscle protein breakdown because skeletal muscle is relatively rich in histidine. High 3-methylhistidine (discussed below) may confirm this situation. Finding a pattern of elevated histidine, glutamine and asparagine is indicative of either genetic polymorphism or toxic interactions at the SN1-SN2 transporter system because this is the only mechanism known to uniquely affect these

three amino acids. Outpatient data collected at Metametrix, Inc., show a strong positive relationship between histidine and glutamine in plasma amino acid profiles. A similar relationship is found for histidine and asparagine and such associations are lacking for the other EAA. These population-based correlations of Gln, Asn and His provide evidence that SN1-SN2 transporter system effects may be dominant factors in regulating His levels.

Refer to Case Illustration 4.5

Salicylates and steroids lower histidine levels and folic acid deficiency leads to increased catabolism in addition to increased renal losses of histidine.[268,269] Histidine supplementation of diabetic mice raises plasma and tissue concentrations of histidine in a dose-responsive manner with consequently improved hyperglycemia, hyperlipidemia, oxidation and inflammation.[270]

Mammary histidine decarboxylase activity and expression of H1 and H2 receptors undergo significant increase during pregnancy and lactation.[271] This response increases the risk of histidine deficiency for women during pregnancy and lactation.

Contraindications: In rats, excessive histidine intake has been associated with hypercholesterolemia[272] and increased hepatic glycogen.[273] Oral L-histidine should be used with caution in zinc- or copper-deficient patients because rapid rises of plasma histidine cause stripping of the elements from albumin binding sites and tissue zinc depletion.[274] This effect is easily offset with extra zinc intake. However, patient responses to oral histidine may be due to changes in zinc status. Food intake suppression caused by L-histidine supplementation may be due to loss of taste due to zinc depletion rather than to neuronal effects of the added histidine.[275] Gene expression for metallothionein 1, the most abundant form in the liver, was markedly lowered in rats fed a histamine-excessive diet for five days, apparently due to complexation of copper and zinc.[276] Reports of such effects should lead to caution regarding aggressive histidine, except where laboratory data indicate a histidine-depleted or folic acid deficient status.

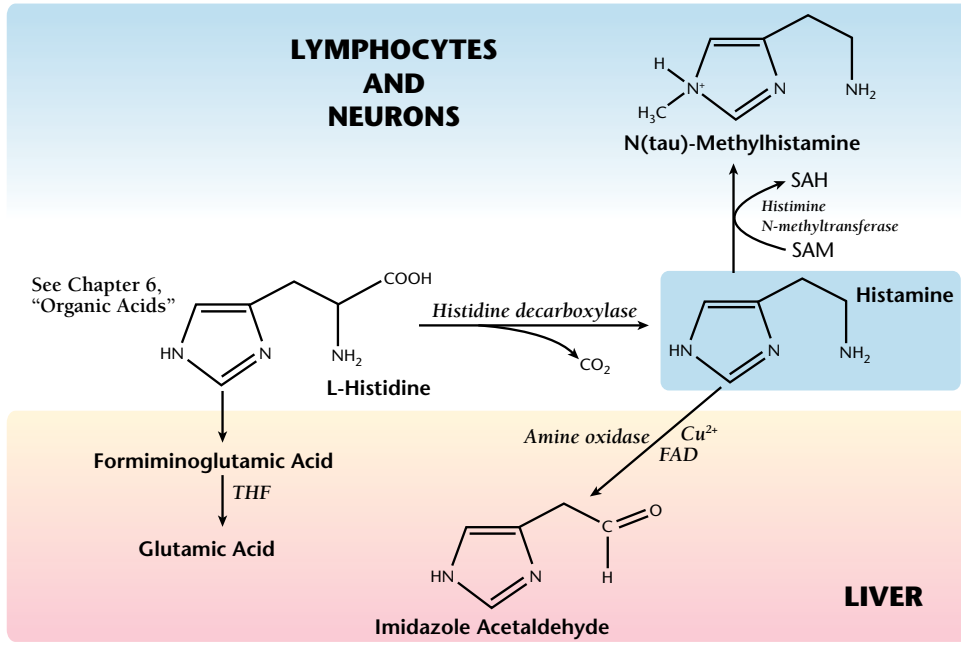
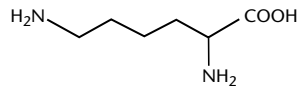


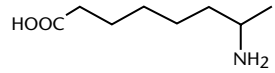
Figure 4.15 — **Histidine Metabolism**

Histidine decarboxylase converts L-histidine into histamine in lymphocytes and histaminergic neurons. In those tissues, methylation of the ring nitrogen causes the removal of histamine from the cell and ultimate excretion of N(tau)-methylhistamine. Hepatic clearance of circulating histamine is facilitated by the alternative pathway of copper-catalyzed oxidation to imidazole acetaldehyde.

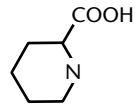
Lysine (Lys)



alpha-Aminoadipic Acid (α-AAA)



Pipecolic Acid



Lysine metabolism is unique in that its degradation does not involve an initial transamination, so lysine plasma levels are not elevated like other amino acids by deficiency of vitamin B_6. Instead, lysine undergoes a unique reaction with α-ketoglutarate in the mitochondria to form a stable intermediate called saccharopine (Figure 4.16) that is further degraded to alpha-aminoadipic acid (α-aminoadipic acid, α-AAA). Elevated α-aminoadipic acid is a clue that a patient is consuming large amounts of lysine.[277] Elevated lysine can result from deficiency of any of the three enzymes required for the catabolic pathway. Some evidence also points to low mitochondrial α-KG as a cause of high plasma lysine.[278] Since further conversion of α-aminoadipic acid to α-ketoadipic acid involves a transamination requiring vitamin B_6, elevation of α-aminoadipic acid could result from vitamin B_6 deficiency.

The normal hepatic mitochondrial pathway through saccharopine to α-aminoadipic acid and glutamate is strongly influenced by availability of α-KG. The hepatic conversion of lysine to pipecolic acid is a peroxisomal pathway used for removal of excess lysine under conditions of abnormal loading.[279] In the brain, however, lysine catabolism leads primarily to pipecolic acid formation. This tissue-specific catabolic end product is of significance because pipecolic acid modulates neuronal activity as discussed under "GABA Receptor Actions of Pipecolic Acid," on the following page.

Lysine is high in meats and dairy, whereas it is lower in corn and many plant proteins. This accounts for the higher risk of lysine deficiency among people whose diet is low in animal products and restricted in a variety of vegetables. Plasma concentrations of lysine and its metabolite, carnitine, decrease in parallel fashion with dietary intake of lysine when vegans, lacto-ovo-vegetarians and meat eaters are compared.[280]

Growth and Fatty Acid Metabolism: Lysine deficiency retards the growth rate of pigs, causing plasma IGF-I levels to fall 52% lower than controls.[281] Lysine is the precursor for the synthesis of L-carnitine,[282] which shuttles fatty acids into the mitochondria for β-oxidation. Serum triglycerides are high in individuals on a lysine-restricted diet because carnitine is essential in the transport of fatty acids.[283]

The Gut and Anxiety: Enhancement of dietary lysine has been proposed as part of the treatment plan for irritable bowel syndrome.[284] In rats, dietary lysine deficiency increases stress-induced anxiety with enhanced serotonin release and greater fecal volume.[284] In pigs, transportation stress caused lowering of plasma lysine and arginine. With lysine and arginine supplementation, the effect was reversed, along with lowering of plasma cortisol.[285] Lysine fortification of wheat reduces anxiety and stress in humans.[286] The mechanism may be inhibition of stress-induced diarrhea via specific inhibition of 5-HT4 serotonin receptors. Lysine has not been shown to affect other classes of serotonin receptors.[287] These findings can have relevance for 5-hydroxytryptophan supplementation, which induces tachycardia via 5-HT4 receptors.[288] In addition to their effect on diarrhea-predominant irritable bowel syndrome, 5-HT4 antagonists (1) block corticosteroid secretion, (2) inhibit 5-HT-induced tachycardia and (3) reduce anxiety.[289]

Oral dosing with L-lysine (1 g/kg) reduced the effect of stress on ureagenesis and reduced plasma arginine.[290] These responses suggest that requirements for arginine

Notes:

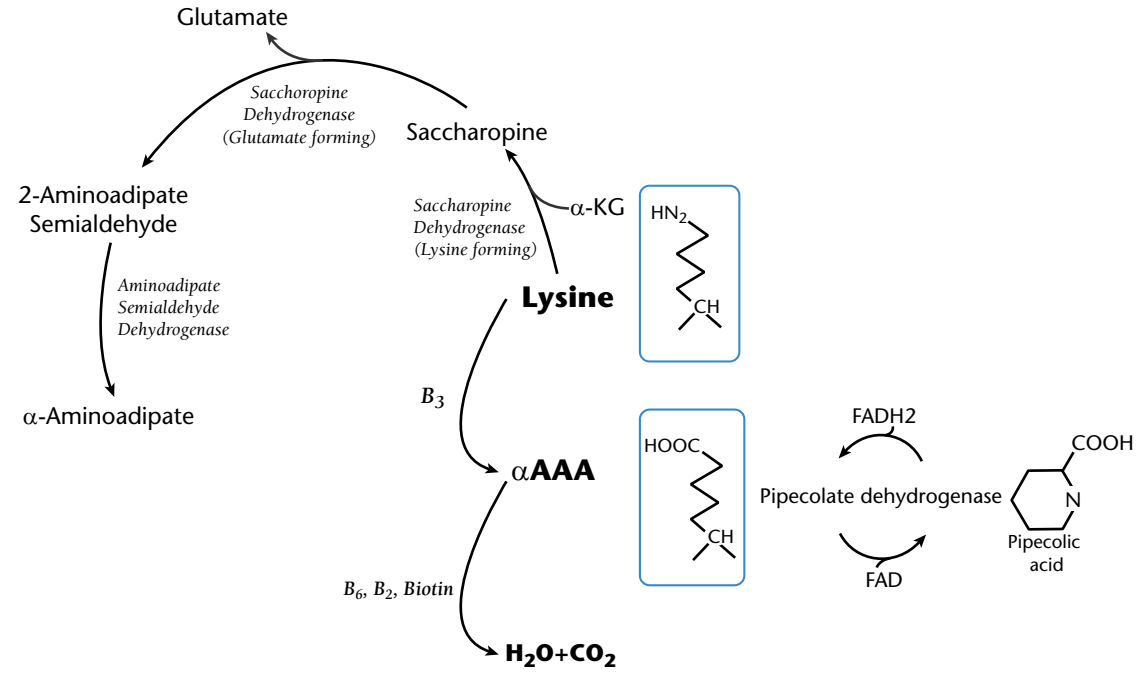


Figure 4.16 — **Lysine Catabolism**

The first step in lysine catabolism is dependent on availability of mitochondrial α-KG to form the stable intermediate, saccharopine. Genetic deficiency of the enzyme saccharopine dehydrogenase produces hyperlysinemia that may respond to the B-complex vitamins required for the alternate pathway. The bifunctional enzyme, saccharopine dehydrogenase, is also used to cleave the peptide, releasing glutamate and α-aminoadipate semialdehyde. Ultimately, α-aminoadipate is formed. The alternative, reversible pathway involving pipecolate formation occurs in peroxisomes, leading also to α-AAA formation.

are increased during lysine-modulated stress reduction. The efficacy and safety of using customized mixtures rather than individual high-potency essential amino acids can be understood in light of these effects from excessive or insufficient lysine.

Older therapeutic uses of lysine include herpes simplex infections, cardiovascular disease and osteoporosis.[291] The effect on herpes simplex, the most extensively studied application, is apparently ascribed to competitive inhibition with lysine residues on the surface of the viral particles.[292] The effect makes lysine a useful prophylactic at serum concentrations of 165 μM for cases of recurrent herpes simplex labialis.[293] A similar mechanism may be ascribed to the competition of free plasma lysine with the lysine side chains of proteins susceptible to glycation. Oral lysine supplementation also reduces nonenzymatic glycation of glomerular basement membrane collagen and albuminuria in diabetic rats.[294] Amino acid mixtures with high levels of L-lysine showed anticataractous and antidiabetic effects in rats.[295] In diabetic human subjects lysine treatment increased insulin sensitivity and decreased blood sugar an average of 27%.[296]

GABA Receptor Actions of Pipecolic Acid: Pipecolic acid (PA) binds to GABA receptors in the brain. Although pipecolic acid alone has little effect on brain activity,[297] it can potentiate the suppressing effects of barbiturates on electrically and chemically induced convulsions.[298] Pipecolate binding sites are most abundant in cerebral cortex, thalamus and olfactory bulb,[299] and there is evidence that they are identical with the GABA receptors.[300]

Refer to Case Illustration 4.6

Bicuculline and picrotoxin are agents that induce convulsions similarly to strychnine. Coadministration of pipecolic acid protects against bicuculline- and picrotoxin-induced convulsions induced via inhibition

of GABAergic neurons.[301] In rat brain, pipecolic acid enhances GABA response without affecting glycine responses.[302] In cerebral cortex slices, pipecolic acid inhibits neuronal GABA uptake and/or enhances its release, especially in the presence of β-alanine.[303] Patients with liver cirrhosis have elevated levels of GABA and pipecolic acid in plasma. And plasma pipecolic acid is closely correlated with plasma ammonia concentration.[304] This study also revealed that the degree of portal hypertension in cirrhotic patients may be predicted by plasma PA. Intracerebroventricular injection of pipecolic acid in chicks altered feeding and increased sleep-like behavior,[305] and the hyperphagic effect was attenuated by both GABA-A and GABA-B receptor antagonists.[305]

Elevated plasma lysine can enhance pipecolic acid formation in the brain. Central nervous system effects of lysine loading like those found in Zellweger syndrome can be due to modulation of GABA receptor binding by L-pipecolic acid.[306] Pipecolic aciduria and elevated α-aminoadipic acid are found in the cerebrohepatorenal syndrome of Zellweger.[307]

Genetics: Hyperlysinemia is found in many inborn errors of metabolism, including urea cycle abnormalities, pyruvate carboxylase deficiency and L-2-hydroxyglutaric aciduria.[9] Patients with one of the forms of ornithine carbamoyl transferase (OCT) deficiency that causes elevated lysine may benefit from extra α-ketoglutarate. They may have concurrent high plasma levels of glutamate, glutamine, citrulline and argininosuccinic aciduria (see Table 4.12). The evidence indicates that lysine degradation is regulated by bioavailability of mitochondrial α–KG. Children with Down's syndrome have higher plasma lysine compared to controls.[308]

In contrast to hyperlysinemia, low lysine concentrations are found in the autosomal recessive disorder, lysinuric protein intolerance (LPI), caused by mutations in the gene encoding the transporter of the dibasic amino acids lysine, arginine and ornithine.[309] Deficiencies of lysine, arginine and ornithine are exacerbated in this condition because post-prandial hyperammonemia leads to avoidance of dietary protein. As a result, attempts to resolve the lysine deficiency with protein intake are unsuccessful. Supplementation with L-lysine, however, has been shown to normalize low plasma lysine that is characteristic of LPI.[310] To avoid profuse diarrhea it was necessary to limit lysine doses to 7.3 mg/kg per dose, given 3 times daily for 3 days.

Inability to absorb lysine may lead to bacterial overgrowth from lysine that passes into the transitional gut where bacterial growth rates are maximal. From general principles milder polymorphic forms of LPI are expected to be encountered producing low plasma and urinary lysine, possibly in the presence of normal levels of other amino acids.

Interpretation of Laboratory Results: A low lysine level is an indication of need for both L-carnitine and lysine supplementation, particularly if there are signs of muscle weakness, fatigue, or high serum triglycerides.[311] Although lysine is not known to have direct influence on brain function via neurotransmitters, the influence of energy deficit due to lack of carnitine may be suspected as a contributing factor in cases such as that illustrated in the accompanying case illustration.

REFER TO CASE ILLUSTRATION 4.7

High levels of lysine in fasting plasma or urine are rare and if the cause is not identified as lysine supplementation, high levels may indicate an impaired ability to metabolize lysine. Proper metabolism of lysine utilizes vitamins B_3 and B_6. Vitamin C and iron are also needed to normalize lysine.[312] Elevated lysine in urine may also be a result of urea cycle abnormalities that produce elevated plasma arginine. The high arginine seems to cause excessive lysine spilling into urine by competition for a shared transport protein active in amino acid resorption in renal tubules. Also, carnitine supplementation tends to raise plasma lysine through a sparing effect on lysine conversion to carnitine.[282]

TABLE 4-12 — GENETIC VARIANTS AFFECTING PLASMA LYSINE

Lysine (pl)	Glu + Gln (pl)	α-KG (u)	Genetic Variants	Nutrient Therapy
High	High	Low	OCT def, Citrullinemia, Arginosuccinic aciduria	α-KG
Low	Low	High	α-KG dehydrogenase deficiency	Thiamin, lipoate

Plasma (pl), urine (u), alpha-ketoglutaric acid (α-KG) and ornithine carbamoyl transferase (OCT)

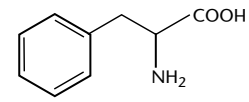
The high positive correlation between homocysteine and α-amino adipic acid suggests that they share metabolic marker status in the etiology of atherosclerosis and myocardial infarction.[313] Elevations suggest reduced tissue capability of amine group transfer and inhibited lysine catabolism or excessive lysine intake. Elevated α-aminoadipic acid is a risk factor for heart disease. Unlike homocysteine, α-aminoadipic acid is not vitamin B_{12} dependent and thus its elevation may indicate a specific need for vitamin B_6.[314] In a mentally retarded girl with a number of dysmorphic features, Raynaud's phenomenon, hypotonia and petit mal seizures, treatment for three months with vitamin B_6 increased lysine conversion to α-AAA measured in blood and urine.[277]

Contraindications: Monitoring plasma concentrations of lysine is recommended in long-term oral dosing with lysine, since excessive use of lysine may lead to kidney pathology.[315] A plasma level above 165 µmol/L is required to obtain the herpes-inhibiting effect. This level is in the upper-normal range. Prolonged supplementation at levels over 3 g per day can result in elevated plasma and urinary lysine concentrations. Such levels generally signal excessive intake that will only add to the total-body nitrogen load, challenging ammonia clearance capacity.

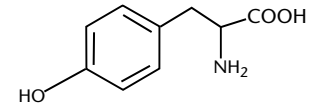
Notes:

Neurotransmitter Precursors

Phenylalanine (Phe)



Tyrosine (Tyr)



In addition to the direct neurotransmitter roles of glycine, aspartic acid, glutamic acid and γ-amino butyric acid (GABA), the aromatic amino acids (phenylalanine, tyrosine and tryptophan) are converted to catecholamines and serotonin by enzymes in adrenal, intestinal, brain and peripheral nervous tissue. Plasma levels of these amino acids influence neuronal concentrations of the respective neurotransmitters. The blood-brain barrier, formed by astrocyte end plates that surround blood sinuses in the brain, filters the entry of specific amino acids, preventing rapid changes in concentrations within the neuron. The transport system operates on all amino acids that have large, neutral side chains (the LNAA). In addition to the aromatic amino acids, the BCAA are also LNAA. This transporter sharing is a reason that supplying an equal amount of any single amino acid in the presence of other LNAA reduces the rate of transfer into the brain compared with dosing with the pure amino acid. Thus, tryptophan alone causes more serotonin increase in the brain than a mixture of tryptophan with other LNAA. The neurons store their respective neurotransmitters in granules and synaptic release initiates neural responses.

Phenylalanine and tyrosine are in the chemical family of aromatic amino acids because they have benzene or para-hydroxy benzene rings of their side chains. Phenylalanine is an essential amino acid that is ubiquitous in the structure of major proteins except for collagen. Since human tissues normally possess an enzyme that will convert phenylalanine to tyrosine, both of these amino acids are precursors to the catecholamines (DOPA, dopamine, norepinephrine and epinephrine), melanin and thyroid hormone (Figure 4.17). The catecholamines are involved in basic nervous system activities such as movement, memory, attention, problem solving, desire,

motivation, "fight or flight" response, heart rate and stroke volume, anaphylaxis and energy production via glycolysis or lipolysis. Although the conversion of phenylalanine to tyrosine is a facile reaction in most individuals, a significant number of people have difficulty achieving a rate of this reaction needed to meet demands for clearing phenylalanine and generating tyrosine. Dietary tyrosine intake is approximately 18% lower than that for phenylalanine,[316] so if the conversion from phenylalanine to tyrosine is blocked, the adequacy of tyrosine becomes a clinical problem.

The enzyme that catalyzes the phenylalanine to tyrosine reaction, phenylalanine hydroxylase, requires molecular oxygen as a reactant and it requires iron, tetrahydrobiopterin (BH_4) and the reduced form of niacin as cofactors. BH_4 can be produced in normal human metabolism from GTP, but there are a number of known genetic polymorphisms for phenylalanine hydroxylase and there are metabolic circumstances that prevent adequate rates of recycling for BH_4, making it a conditionally essential nutrient. Because of this situation, the biosynthesis and regeneration for BH_4 is fully described in Chapter 2, "Vitamins."

Other potentially significant aspects of the tyrosine metabolite reactions are the requirement of the dopamine to norepinephrine step for copper (Cu^{2+}) and the need for S-adenysyl methionine (SAMe) for the conversion of norepinephrine (or noradrenaline) to epinephrine (adrenaline).

Genetics (PKU and HPA): The inherited metabolic disorder of phenylketonuria (PKU), which has a prevalence of 1 in every 15,000 births worldwide, results in greatly elevated phenylalanine in plasma and urine due to insufficient activity of phenylalanine

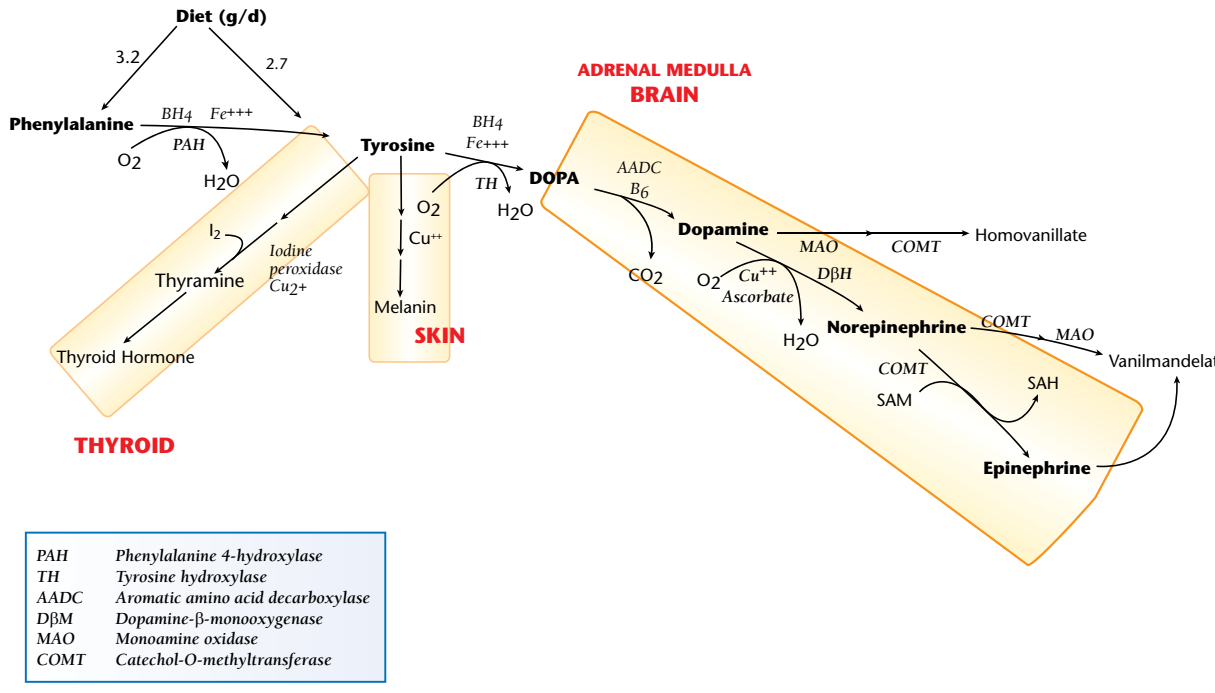


FIGURE 4.17 — **Neurotransmitters and Hormones from Phenylalanine and Tyrosine**

The values for dietary intake from the 1988–1994 NHANES III survey show only slightly lower intake of tyrosine than phenylalanine. Three distinctly different classes of non-protein, cell regulatory products, thyroid hormone, melanin and catecholamines are formed in thyroid, skin and adrenal medullary tissues, respectively, from tyrosine. The phenylalanine hydroxylase enzyme used for the conversion of phenylalanine to tyrosine is also required for the further hydroxylation of tyrosine to form dihydroxyphenylalanine (DOPA). Subsequent steps form the catecholamines that are cleared by MAO and COMT reactions. Note the multiple requirements for tetrahydrobiopterin (BH_4), iron (Fe^{+++}) and copper (Cu^{++}).

hydroxylase (PAH), the enzyme necessary for conversion of phenylalanine to tyrosine. To date, there have been more than 400 mutations occurring in all segments of the gene encoding PAH.[317] Some mutations cause phenylketonuria; others cause non-PKU hyperphenylalaninemia (HPA). The majority of individuals with HPA have mild clinical phenotypes.[38]

In humans with PKU, plasma phenylalanine is 30-fold higher than normal and early symptoms include vomiting, irritability, an eczema-like rash and a mousy odor to the urine. Some may also have subtle signs of nervous system function problems, such as increased muscle tone and hyperreflexia. Later, severe brain symptoms occur, such as mental retardation and seizures. Most symptoms of untreated PKU are avoided by early identification and management.

Management of PKU requires frequent monitoring of phenylalanine. Urine phenylalanine correlates with plasma concentrations,[318] whereas blood spot phenylalanine is also suitable for screening.[319] The ratio of phenylalanine to tyrosine calculated from plasma concentration data has been proposed as an even more sensitive way of detecting not only PKU presence, but also the heterozygous carriers of the disorder.[320]

Some mutations cause PKU; others cause non-PKU hyperphenylalaninemia, whereas still others are silent polymorphisms (without phenotypic expression) present on both normal and mutant chromosomes. As the residual enzyme activity falls in patients with each type of mutation, the relative proportions of phenylalanine converted to tyrosine decrease.

The ratio of plasma phenylalanine to tyrosine is shown for a large outpatient population (n = 2,969) including males and females over 13 years of age are shown in Figure 4.18A. The linear trend line shows an average relationship near 1:1. Very low phenylalanine concentrations are usually associated with correspondingly low tyrosine. The cluster of several measurements where phenylalanine is 40 to 50 µM and tyrosine is below 10 µM indicates abnormally low PAH activity. Figure 4.18B shows a histogram of the same population with biochemical markers of phenylalanine-tyrosine ratios associated with HPA and PKU of 1.14 and 1.39 µM, respectively.[321] For distinguishing mild phenotypes of PAH deficiency, plasma tyrosine may be more sensitive than plasma phenylalanine because the phenylalanine elevation may be moderate, whereas tyrosine may be very low. These kinds of abnormalities are undetectable by standard neonatal inborn error screening, but easily determined by quantitative amino acid analysis.

The histogram shows the distribution of the Phe/Tyr ratios from Figure 4.18A. The horizontal axis is the value of the ratio and the vertical axis is the number of individuals in each segment. The data are quite normally distributed. The cutoff values of 1.14 and 1.39 are those reported by Mallolas as biochemical phenotype markers of carriers of mutations leading to hyperphenylalaninemia or PKU.[321]

Cofactors and the Phe/Tyr Ratio: Even for individuals with normal phenylalanine hydroxylase activity, deficiency of any of the related nutrients—iron, BH_4, or niacin (vitamin B_3)—creates a functional metabolic block at this step, resulting in low tyrosine or a high ratio of phenylalanine to tyrosine in plasma.[322] Regarding distinguishing heterozygous PAH deficiency patients, suppression of plasma tyrosine is more sensitive than plasma phenylalanine for revealing the metabolic severity among the many mutation possibilities. In other words, the metabolic outcome of single nucleotide polymorphic (SNP) mutations is revealed by testing of plasma tyrosine concentrations. In these patients the tyrosine-lowering effects were very apparent when measurements were made 4 hours after a standard meal containing 140 kcal of protein.[323] Apparently the insulinemic effect of the meal drives available tyrosine into tissues, lowering blood levels even below fasting levels. Therefore, the relatively high percentage of people who are carriers of

Notes:

one or more mutation in the PAH gene may have difficulty maintaining normal plasma tyrosine levels and the potential for tyrosine insufficiency is even greater in PKU patients treated with low-phenylalanine diets.

Although PKU is usually caused by a congenital deficiency of PAH, it also can result from defects in the metabolism of biopterin, which is a cofactor for the hydroxylase enzyme. GTP cyclohydrolase is the rate-limiting enzyme in the conversion of GTP to BH_4. No nutrient-derived cofactors are required by this enzyme. Deficiency of GTP cyclohydrolase can limit the rate of conversion of phenylalanine to tyrosine causing progressive encephalopathy and dihydroxyphenylalanine (DOPA)-nonresponsive dystonia.[324] The impact of insufficient BH_4 from impaired cyclohydrolase activity affects not only the formation of tyrosine, but also the next required step in catecholamine biosynthesis in which DOPA is formed. Human tissues can produce BH_4 from GTP, though the rate of production may be inadequate to meet optimal demands in some individuals and supplementation of BH_4 has been employed for affected individuals.

Interpretation of Laboratory Results: Excessive protein intake or a metabolic block in the conversion of phenylalanine to tyrosine can elevate phenylalanine. Iron is a cofactor required for the enzyme and supplementation improves the conversion of phenylalanine to tyrosine. If phenylalanine is high, vitamin C, niacin, iron, or BH_4 and a low-phenylalanine diet may be indicated. In a murine model of PKU, hepatic iron accumulates, whereas hepatic copper and zinc decrease compared with control and PKU phenylalanine-restricted mice, demonstrating that hyperphenylalaninemia alters the metabolism of iron, copper and zinc.[325] Other studies show that high plasma phenylalanine can adversely affect bone status.[326] High plasma phenylalanine seems to interfere with cholesterogenesis, producing lower plasma cholesterol and coenzyme Q_{10}, although the

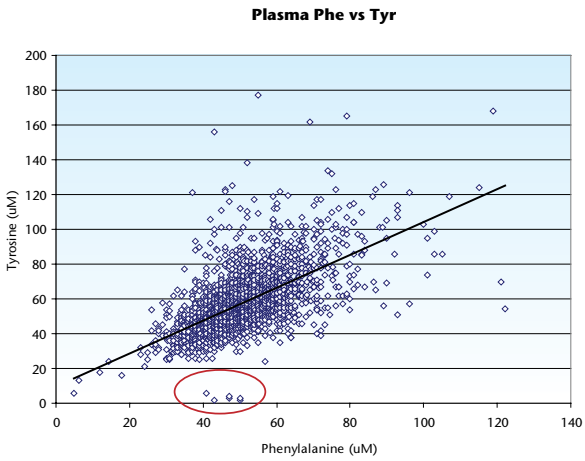


FIGURE 4.18A — Relationship of Plasma Phenylalanine and Tyrosine in a Large Outpatient Population

The data shown was collected from 2,969 patients in a general outpatient population. All ages for both male and female are included. The linear trend line shows an average relationship near 1:1. The average Phe/Tyr ratio is 1.1. Very low phenylalanine concentrations are associated with correspondingly low tyrosine. The cluster of several measurements where phenylalanine is 40 to 50 and tyrosine is below 10 uM (circled in red) are indicative of abnormally low PAH activity. Similarly, at higher phenylalanine values those points that fall far below the trend line identify individuals with possible inhibition of the Phe ➔ Tyr conversion. This relationship is more easily seen when the data are plotted as the histogram of the Phe/Tyr ratio as in Figure 4.18B. No restriction for dietary supplementation of amino acids was made, so the individuals with tyrosine concentrations above 120 are likely to be using dietary tyrosine or phenylalanine supplements.

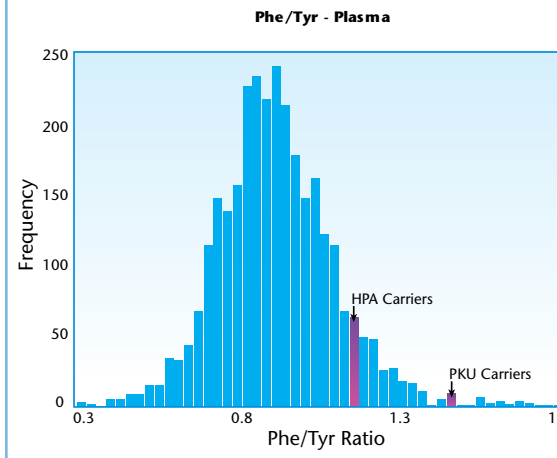


FIGURE 4.18B — Histogram of the Phe/Tyr Ratio

The histogram shows the distribution of the Phe/Tyr ratios from Figure 4.18A. The horizontal axis is the value of the ratio and the vertical axis is the number of individuals in each segment. The data are quite normally distributed. The cutoff values of 1.14 and 1.39 are those reported by Mallolas as biochemical phenotype markers of carriers of mutations leading to HPA or PKU.

special diets used to control phenylalanine intake may contribute to the effect.[327,328]

Published data on the causes and effects of low plasma phenylalanine are rare. The general phenylalanine requirement for protein synthesis means that phenylalanine-depleted individuals will have difficulty maintaining organ reserve.

Treatment for Phenylalanine and Tyrosine Abnormalities: Regarding the elevated phenylalanine detected as neonatal PKU, the reader is referred to one of the excellent reviews of PKU, including the low-phenylalanine diet.[329] Recent reports indicate the need for long-term maintenance of the phenylalanine restriction.[330] Recent studies have shown that the effectiveness of supplemental BH_4 is low because it is rapidly degraded. A better response was found with use of sepiapterin, a compound in the normal biochemical recovery pathway for BH_4.[331] Other work on the possible effects of BH_4 in hypercholesterolemia alerts us to adverse effects of oxidation products of BH_4 on endothelial nitric oxide formation.[332] When there is a deficiency of iron, vitamin C can help restore tyrosine formation because it enhances intestinal absorption of iron. Relatively high doses of niacin are usually included in low-phenylalanine diet formulas (22 mg/100 g powder) for use in PKU treatment. The extra niacin assures adequate NADH for reduction of dihydrobioptyerin back to BH_4.

Refer to Case Illustration 4.8

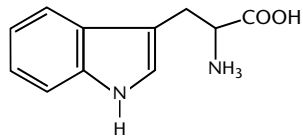
Supplementation of threonine at 50 mg/kg per day resulted in a significant decrease of plasma phenylalanine levels.[333] The mechanism for this effect is unknown, so this treatment is not a standard practice. Although tyrosine supplementation is an obvious consideration for remediation for the restricted conversion from phenylalanine in PKU, more effective therapy may be achieved with mixtures of large neutral amino acids to restore balance in the transport of amino acids across the blood-brain barrier.[334] These considerations apply to the various milder forms of PKU that have been discussed.

Assuming the patient has not been supplementing with tyrosine, high tyrosine levels may indicate a failure of one or more of the pathways of utilization. Vitamin B_6 may help normalize high tyrosine levels.[335] Tyrosine has been used as a treatment for depression and blood pressure modulation.[336]

Low levels of tyrosine may reflect a chronic deficiency state leading to deficits in the biogenic amines. High levels of stress lead to depletion of phenylalanine.[337] In addition to supplemental tyrosine, iron and vitamin C are potential nutritional interventions for low tyrosine.[322,338] Caution is needed regarding iron therapy because of the potential for inducing oxidative stress. Assessment of iron status is generally recommended before recommending iron. If tyrosine is low, then formation of thyroid hormone may be compromised. Possible symptoms include hypothyroidism, chronic fatigue, autonomic dysfunction, depression, impaired learning or memory, or behavioral disorders.[26] Plasma tyrosine has been proposed as a useful assessment of thyroid function. Low plasma levels of tyrosine have been associated with hypothyroidism.[339,340] Since the low-phenylalanine diets used to treat PKU are also low in tyrosine, they may produce a tyrosine deficit. Since the enzyme defect is prior to the formation of tyrosine, supplementing dietary tyrosine to enhance tyrosine-dependent functions should have no adverse effect on PKU patients. End-stage renal disease patients become tyrosine deficient because of their inability to sustain adequate rates of conversion of phenylalanine to tyrosine. Their routine dialysis therapy frequently results in iron depletion. Tyrosine dietary supplementation has been proposed as a therapy in these patients.[341]

Notes:

Tryptophan (Trp)



In addition to supplying about 3% of total-body protein composition, tryptophan serves as precursor to serotonin and niacin. As shown in Figure 4.19, tryptophan is metabolized via the serotonin pathway and excreted as 5-hydroxyindoleacetate or via the kynurenine pathway from which xanthurenic acid, quinolinic acid and kynurenic acid intermediates may be found in urine. In addition, unabsorbed dietary tryptophan may be metabolized by intestinal bacteria, producing indican as a final excretory product. The significance of abnormalities in concentrations of these compounds is discussed more fully in Chapter 6, "Organic Acids." Tryptophan monooxygenase starts the two-step conversion to serotonin, whereas tryptophan dioxygenase initiates conversion to kynurenin. To summarize, nutrients that are involved at various steps in tryptophan metabolism are BH_4, iron and vitamins B_6 and B_3. In the pathway from tryptophan to serotonin, there is only one intermediate, 5-hydroxytryptophan.

Approximately 10% of dietary tryptophan is normally used for serotonin synthesis. Serotonin is well known for its roles in mood, sleep, emesis, sexuality and appetite, whereas it has been implicated in neuropsychiatric disorders such as depression, migraine, bipolar disorder and anxiety.[342]

Neuropsychiatric Disorders: Numerous studies have demonstrated that plasma tryptophan is an indirect marker of changes in brain serotonin synthesis.[343] Serotonin is produced in the brain and in the numerous chromaffin cells in the small intestine where it plays a large role in regulating motility. Rapid tryptophan depletion and reduced serotonergic activity is associated with impulsive behavior.[344,32] Plasma tryptophan and the tryptophan-neutral amino acids ratio have been proposed as useful indicators of brain serotonergic activity and neuropsychiatric disorders in diabetic school children.[345] A pathophysiologic role of decreased brain serotonin in primary fibromyalgia has been suggested

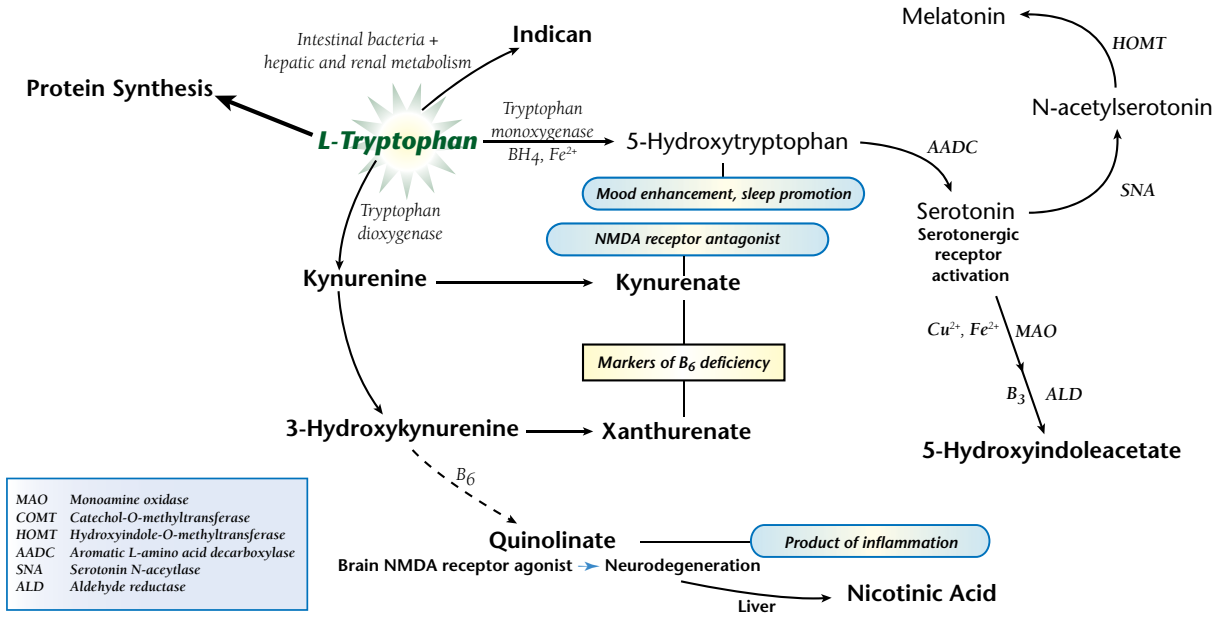


FIGURE 4.19 — **Formation and Catabolism of Serotonin, Nicotinate and Indican**

Almost every protein in human tissues requires a few residues of tryptophan for completion of its synthesis. Hepatocytes and, under interferon-gamma stimulation, macrophages and astrocytes divert tryptophan into the kynurenine pathway. In the liver, this pathway can lead to production of nicotinic acid. Other special tissues use tryptophan for production of serotonin, whereas intestinal bacteria may degrade tryptophan, as indicated by the appearance of indican in urine. Kynurenate and quinolinate can become end products in neural tissue where they modulate glutamatergic neurons via actions at the NMDA receptors.

from observations that these patients have lower plasma tryptophan.[346]

Tryptophan has been shown to help induce sleep in insomniacs due to increased serotonin production in the brain stem. Plasma tryptophan levels are increased with sleep deprivation because of decreased utilization.[347–349] Tryptophan has been used extensively to treat depression and it is thought to potentiate the therapeutic action of tricyclic antidepressants. Low plasma levels of tryptophan have been reported in depressed patients[350] and are correlated with the degree of depression.[351]

Interpretation and Treatment: It is important to remember that, for the chronically ill patient, the special roles of tryptophan just discussed may be of minor significance compared with tryptophan requirements for protein synthesis. Full recovery for the patient with long-term gastrointestinal, skeletal muscle, or hepatic organ reserve loss, for example, requires high activity of the entire protein synthetic system where tryptophan tends to be the limiting factor. The practical application of this knowledge is to prefer L-tryptophan* rather than 5-HTP for repletion. In cases where serotonin synthesis must be sustained at high levels, concurrent use of both forms may be considered. Although 5-HTP can serve only as a precursor to serotonin, its use can spare approximately 10% of tryptophan intake for protein synthesis.

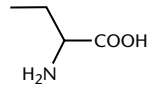
High plasma levels may indicate either excessive intake of tryptophan or poor metabolic utilization. Niacin supplementation may be needed if the conversion of tryptophan to niacin via the kynurenin pathway is inhibited due to vitamin B_6 insufficiency. For patients with low plasma levels, oral supplementation of tryptophan or the metabolic equivalent, 5-hydroxytryptophan (5-HTP) is effective in maintaining adequate tissue supply of tryptophan. Used alone or with amitriptyline, tryptophan is effective against depression in general practice.[352] It is useful because it provides tryptophan for numerous other functions in the body. In contrast, 5-HTP uptake is correlated with serotonin tissue concentration and synthesis rates in healthy humans.[353] Levels in plasma may not rise with supplementation in depressed patients, presumably due to high utilization rates.[354,355]

The principal catabolic product of serotonin that appears in urine is 5-hydroxyindole acetate (5-HIA). Low urine levels can indicate lowered production of the neurotransmitter (see Chapter 6, "Organic Acids").

Contraindications: Tryptophan and 5-HTP should be used with caution in patients on selective serotonin reuptake inhibitors (SSRI), as adverse effects have been reported.[356] Tryptophan is utilized in a variety of reactions whereas 5-HTP is the direct precursor to serotonin. For practitioners intending to remove the patient from SSRI use, care must be taken when titrating the doses of SSRI and 5-HTP. Tryptophan in a free-form balanced amino acid mixture does not appear to cause adverse reactions for patients on SSRI medications.

** The FDA ban on OTC tryptophan remains in effect. Only with a doctor's prescription can people obtain L-tryptophan.*

alpha(α)-Amino-N-Butyric Acid (AANB)

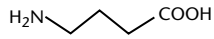


Although glutamine appears to be the origin of the neurotransmitter, α-amino-N-butyric acid in the brain,[357] details of the biosynthetic pathway have not been elucidated. Exercise reduces rat brain levels of α-amino-N-butyric acid levels,[358] and it is also decreased in cerebrospinal fluid of patients with Parkinson's disease.[95] Chronic alcohol intake causes elevated levels of plasma and tissue concentrations of leucine, alanine and α-amino-N-butyric acid.[359] This effect was prevented by the addition of pyruvate, dihydroxyacetone and riboflavin to their diet.[359,360] Because blood α-amino-N-butyric acid is specifically elevated by high blood alcohol, the ratio of α-amino-N-butyric acid to leucine has been proposed as a marker for alcoholism.[279] Studies have shown that the effect is related to hepatic functional loss rather than a specific alcohol intake effect.[361–363]

Elevated plasma α-amino-N-butyric acid indicates vitamin B_6 insufficiency since this amino acid is metabolized via the usual hepatic transamination reaction requiring pyridoxal phosphate.[364]

Notes:

Gamma(γ)-Aminobutyric Acid (GABA)



Gamma-aminobutyric acid is an inhibitory neurotransmitter in the central nervous system. GABAnergic neuronal defects are thought to be involved in the etiology of epilepsy.[365] Granule cells of the dentate gyrus have the unique capacity to release both glutamic acid and γ-aminobutyric acid. Granule cell firing causes GABA receptor activation that mediates antiepileptic and neuroprotective actions.[366]

In the brain, GABA is produced from glutamate in a single decarboxylation reaction. Isoforms of glutamic acid decarboxylase, the enzyme used for conversion, are expressed in tissues other than the brain, where they play a role in diseases of autoimmune character, including neurological disorders and insulin-dependent diabetes.[367] Although the subject of such autoimmune mechanisms is beyond the scope of this book, the presence of the enzyme for its formation means that GABA can arise in various tissues, even though its function is not known. GABA may be synthesized from glutamic acid and ornithine in muscle, but concentrations in plasma reflect CNS levels derived from glutamic acid.

Low levels in plasma are characteristic of one subset of patients with depression.[368] The neurodegenerative condition, Huntington's disease, also manifests as lowered levels of GABA as neuron loss proceeds.[369] Vitamin B_6 deficiency impairs GABA formation, offering one option to help assist patients with inadequate GABA production.[370] In animal models of seizure, lysine has dose-dependent anticonvulsant effects that appear to be due to GABA receptor modulation.[371]

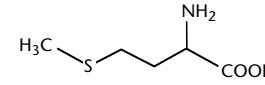
Elevated plasma GABA may reflect low conversion to succinic acid (a vitamin B_6-dependent reaction) for utilization by the citric acid cycle. Gamma-hydroxybutyric aciduria is characteristic of genetic variants with succinic semialdehyde dehydrogenase deficiency. The inevitable retardation of language development in children with this polymorphism is sometimes accompanied by other autistic features.[372] This disorder constitutes one of the potential genetic origins of autism.

Notes:

Sulfur-Containing Amino Acids for Methylation and Glutathione Synthesis

Humans meet their sulfur amino acid needs largely by intake of protein containing the essential amino acids cysteine and methionine. Methionine is a major methyl ($-CH_3$) donor in the body and is required for the synthesis of acetylcholine, choline, creatine and epinephrine (Figure 4.20). Cysteine, a product of methionine, is a component of glutathione, coenzyme A and taurine. All connective tissue proteins, sulfo-mucopolysaccharides and antibodies require adequate metabolism of the sulfur-containing amino acids.

Methionine (Met)



Methionine is the essential precursor in sulfur amino acid metabolism. Low methionine levels may adversely affect these crucial pathways and may reflect a poor-quality protein diet. Methionine is one of the amino acids most frequently found to be deficient, because the methionine content of poor-quality protein sources is very low. Plasma methionine concentrations reflect total-body utilization of cysteine for synthetic reactions in tissue maintenance. Any chronic detoxification challenge requiring glutathione conjugation or oxidative challenge requiring glutathione redox activity tends to deplete methionine and lead to low plasma methionine levels. For example, even dietary intake of tannins found in vegetables and tea increases the rate of methionine utilization to support hepatic tannin clearance.[373] Urinary as well as plasma levels of methionine fall when humans are placed on a methionine-free diet.[374]

Methionine is the principal sulfur-containing dietary component. The metabolism of methionine to homocysteine with transfer of the methyl group to form the active methyl compound, S-adenosylmethionine, is shown in Figure 4.20. Alternatively, hepatocytes express betaine homocysteine methyltransferase that uses betaine as a substrate for methyl group transfer to homocysteine. The figure emphasizes the reciprocal regulation of the two enzymes, methionine synthase and cystathionine-β-synthase. These enzymes respond to oxidative stress in such a way that homocysteine is diverted from transmethylation with formation of methionine to transulfuration

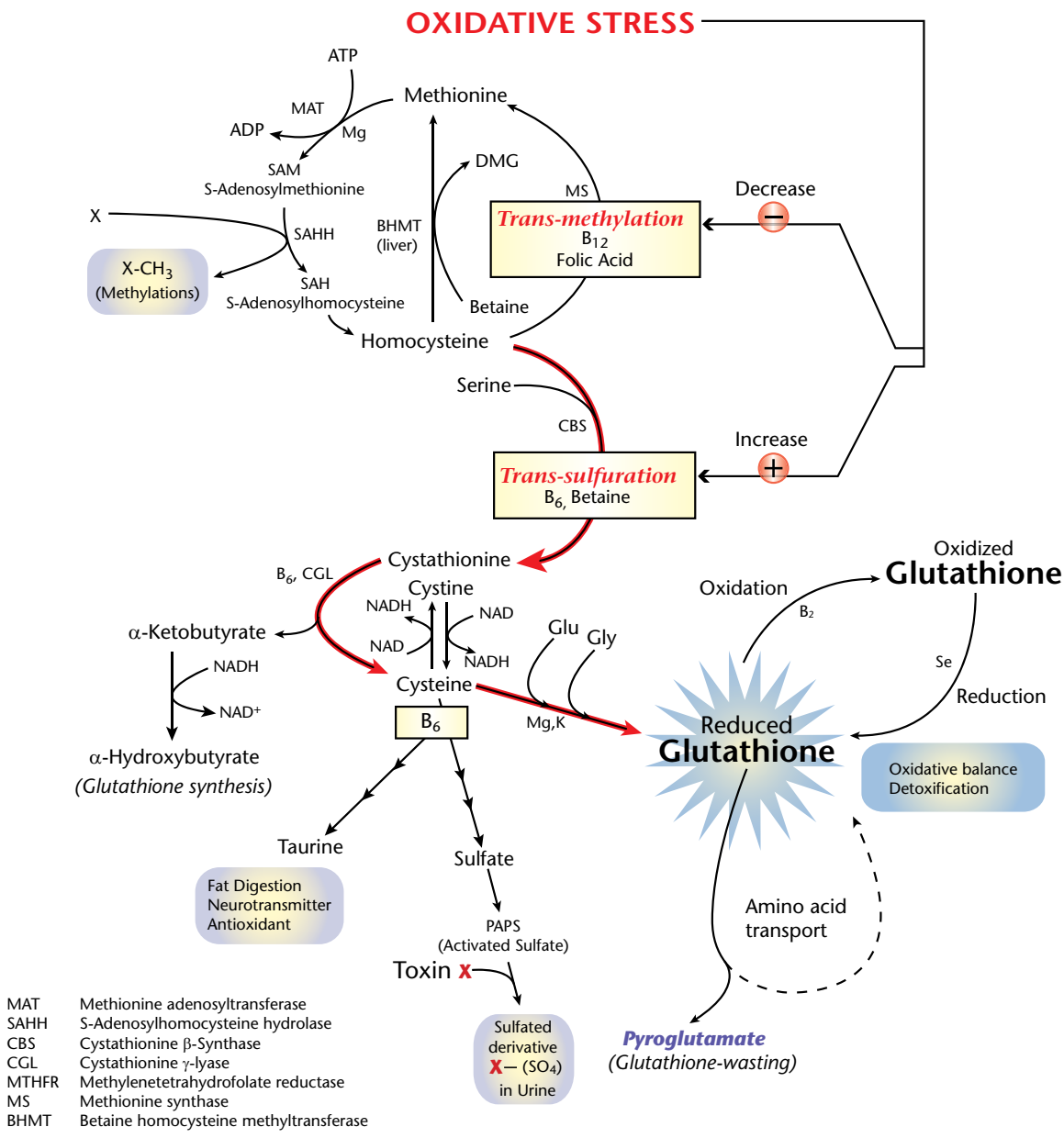


FIGURE 4.20 — **Relevant Sulfur-Containing Amino Acid Pathways**

The essential amino acid, methionine, is used in a cyclic manner to supply methyl groups for the multiple pathways of biosynthesis, cell regulation and detoxification. The regeneration is initiated by the trans-methylation reaction catalyzed by MS. Under conditions of increased oxidative stress, however, homocysteine is diverted into the trans-sulfuration pathway to form cysteine for sustaining glutathione levels. This diversion is carried out by reciprocal regulation, in which MS is inhibited, whereas CBS is stimulated by oxidants formed as a result of the oxidative stress. Other demands for cysteine are shown as products formed from taurine or sulfate. Under conditions where homocysteine conversion to methionine is the dominant flow, folate and vitamin B_{12} status are the critical micronutrient factors. During chronic oxidative stress, vitamin B_6 becomes the critical micronutrient. Functional vitamin B_6 deficiency causes accumulation of homocysteine and failure to maintain glutathione, taurine and sulfate status. Even in normal vitamin B_6 status, chronic oxidative challenge can cause depletion of methionine and homocysteine that ultimately restricts the formation of glutathione, taurine and sulfate by substrate depletion. For furthur details see Figures 2.11 and 6.21.

to allow greater rates of glutathione synthesis. Increased cellular glutathione concentrations serve to modulate the oxidative stress. Other aspects of cysteine metabolism are discussed in the section "Cysteine."

The importance of evaluating methionine status before using aggressive methionine supplementation is emphasized by studies showing that high-methionine diets induce hypercholesterolemia in several species.[176] One possible mechanism is excess methyl group stimulation leading to hepatic accumulation of S-adenosylmethionine with toxic consequences of hepatic methionine adenosyltransferase stimulation and spermine synthase repression associated with elevated glutathione.[94,375] The cholesterol accumulation effects were reversed by adding cysteine, further indicating that methylation pathway stimulation is involved to mediate the effect. It is interesting to speculate that such methionine-mediated methylation stimulation may be a factor in the multitude of adverse health effects associated with high-animal protein diets.[65]

Methionine chelates metals and may be useful as a supportive therapy in chelation of lead.[376] Methionine has a similar chelation effect in cobalt intoxication.[377] The chelating property extends to nutrient elements such as copper, which can be depleted by excess methionine. In addition, methionine toxicity causes growth inhibition and hematologic changes leading to hemolytic anemia.[378] See "Cystathionine" below for a discussion of methionine toxicity biomarkers more sensitive than plasma methionine levels.

S-Adenosylmethionine (SAMe, SAM, or AdoMet): Methionine must be activated through formation of the adduct S-adenosylmethionine (SAM) by the action of the enzyme SAM synthetase. In diseases where the activation process is impaired, treatments may be accelerated by supplying the active form as oral SAM. SAM is a normal constituent of human cells, however it can become a conditionally essential nutrient, making it an effective therapeutic intervention.[379,380] Impaired activity of SAM synthetase results from liver disease induced by alcoholism and the toxic pathological consequences of the disease are reversed by SAM therapy.[381,382]

Clinical Relevance, Interpretation and Treatment: Methionine metabolism disorders have a diverse symptomatology, including allergic chemical sensitivities, headaches, eye strain, muscle weakness, brittle hair, hair loss, myopia, mild myopathy, osteoporosis and cardiovascular symptoms. The pathways are heavily dependent on vitamins B_6, B_{12} and folate (Figure 4.20).[383] When methionine is high, vitamin B_6, α-ketoglutarate and magnesium may be needed to support utilization. This scenario can be distinguished by concurrent elevated homocysteine. High methionine with low homocysteine indicates that the elevation is due to inhibition of methyl transfer to adenosine (possibly due to polymorphism in methionine synthase) and SAM supplementation may be needed. Other biomarker scenarios have been summarized in Chapter 2, "Vitamins." Low plasma methionine indicates inability to sustain methionine levels during overnight fasting. A balanced free-form amino acid mixture is generally safe and effective for raising low methionine status.

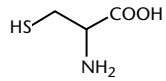
Cirrhotic patients have significantly subnormal values for cysteine, glutathione and albumin. In addition, they have elevated methionine during a high-protein liquid diet and low taurine during either mixed-foods diets or total parenteral nutrition. There appear to be multiple abnormalities in sulfur metabolism in cirrhosis.[384] Reduced urinary excretion of methionine was observed in patients with gastric resection, ileocecal resection and abnormal gut flora, whereas patients with jejuno-ileal bypass showed increased excretion of methionine, probably a consequence of the impaired liver function.[385]

Supplemental methionine given to a patient who has normal plasma methionine, indicating that they are already replete in the amino acid (with normal plasma levels), increases the risk of production of the heart risk factor, homocysteine, as well as the risk of methionine toxicity discussed above. Folate, vitamins B_{12} and B_6 and betaine may be helpful to clear homocysteine.[383] Methionine loading (50 mg per kg body weight) prior to measuring plasma homocysteine has been used to test sufficiency of these nutrients for this metabolic pathway.

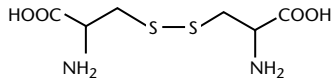
The pattern of lower plasma methionine, S-adenosylhomocysteine, cystathionine, cysteine and total glutathione and higher concentrations of SAH and oxidized glutathione in autistic children, has been described as an indication of increased vulnerability to oxidative stress. Intervention with folinic acid, betaine and injectable methylcobalamin normalized the metabolic imbalance and produced improvements in autistic symptoms.[386]

Notes:

Cysteine (Cys)



Cystine (Cyss)



Most of the sulfur in food is in the form of protein-bound cysteine/cystine. High-sulfur foods such as eggs and legumes, therefore, are high-cysteine foods. Cysteine residues are found at the catalytic site of many enzymes because of the unique substrate-binding properties offered by the electron-rich sulfur atom. The same electron-dense character makes sulfur compounds such as reduced glutathione and DMSA some of the best heavy metal-complexing agents (see Chapter 3, "Nutrient and Toxic Elements"). The cysteine-rich protein, keratin, which is so abundant in hair, confers extremely high metal-binding capacity to hair. Cystine (Cyss) is the oxidized dimer of cysteine (Cys) in which the sulfhydryl group is converted into a disulfide with extraction of two hydrogens similar to that shown for glutathione in Figure 4.21. Some assays oxidize the specimen so that all cysteine is converted to cystine; therefore, these laboratory reports may show only cystine. The term tCys indicates the combined presence of the two forms in the original specimen, whereas cyst(e)ine is generally used to indicate a combination of reduced (cysteine) and oxidized (cystine) forms.

It is important to distinguish between the concentrations of protein-bound and free cysteine. Although virtually all proteins of blood plasma contain cysteine residues, normally there is very little cysteine present in the unbound or free form that is measured in plasma or urine amino acid testing. Both the reduced (sulfhydryl) and oxidized (disulfide) forms occur in proteins. When two cysteine residues in a single polypeptide chain join to form cystine, the structure is greatly stabilized. These points are relevant to laboratory assessments because most laboratories do not report the reduced (–SH) forms and the oxidized (–S-S-) forms that are reported are in dynamic equilibrium with the reduced forms. Therefore, abnormal levels have a direct relationship to the total levels of reduced plus oxidized forms.

Glutathione: Glutathione is a tripeptide, γ-glutamyl-cysteinyl-glycine. As much as half of the total-body flux of cysteine may be accounted for by turnover of glutathione.[387] Under oxidizing conditions, two tripeptides join via disulfide formation to create a double molecule (Figure 4.21). The enzyme, glutathione reductase requires copper and FAD (from vitamin B_2). This ability

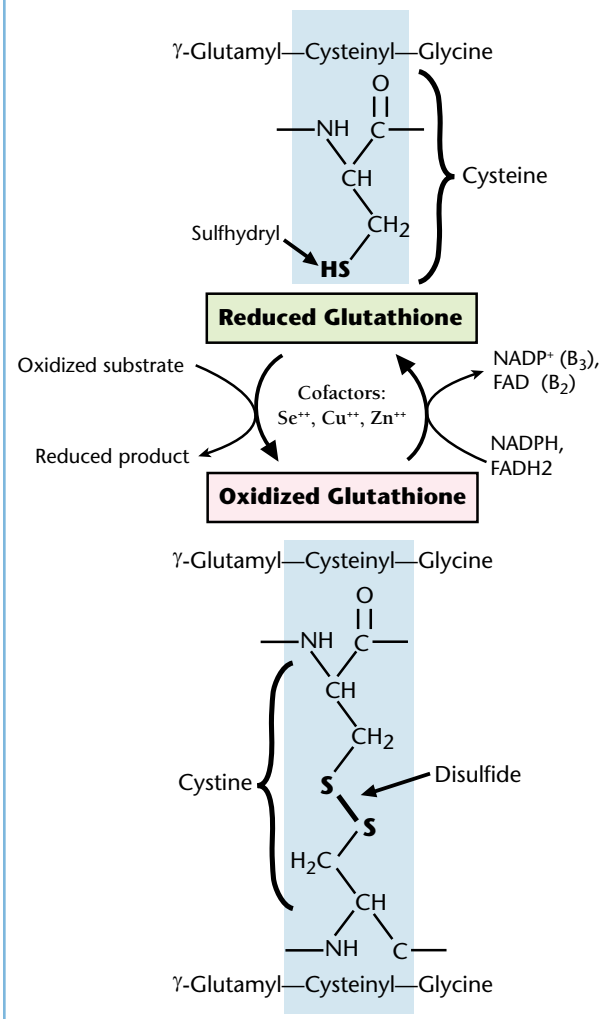


FIGURE 4.21 — Reduced and Oxidized Glutathione

The central cysteinyl residue of the glutathione tripeptide undergoes oxidation as electrons are accepted from substrates for the enzyme glutathione peroxidase. Glutathione reductase then uses reduced NADH and FADH2 to regenerate reduced glutathione. Various isoforms of the enzymes require the cofactors shown. The –SH group of reduced glutathione (GSH) becomes the –S-S- group of oxidized glutathione (GSSG).

to buffer oxidizing and reducing conditions, which shift with the flow of NAD^+ and NADH, allows glutathione to help regulate oxidative cell processes. Hepatic phase II detoxification also consumes glutathione as it participates in many reactions to conjugate and excrete toxicants (see Chapter 8, "Toxicants and Detoxification"). In high-oxygen environments, cysteine is lowered due to the high glutathione demand.[388]

Hepatic cholesterol biosynthesis is modulated by dietary cyst(e)ine. Cysteine has been found to be significantly elevated in patients with primary biliary cirrhosis and other forms of liver disease.[389] Hypercholesterolemia can be induced in laboratory animals by feeding excessive cystine.[390] The effect may have limited impact on heart disease risk, because the increase in cholesterogenesis is accompanied by 65% lower levels of the heart disease risk factor lipoprotein(a). In fact, other studies in rats have demonstrated that if, instead of imposing a gross excess of cysteine, the amino acid is added at moderate levels, plasma cholesterol is lowered significantly and HDL cholesterol is increased.[391] These effects are thought to result from enhanced conversion of cholesterol to bile acids. Dietary supplementation with N-acetylcysteine (NAC) does not increase the circulating concentrations of tCys.[392] The variation in responses to increased oral cyst(e)ine underscore the benefit of testing for individual amino acid profiles to determine patient status before implementing amino acid therapies.

Cysteine plays a regulatory role in the physiological control of body cell mass.[130] Plasma tCys is highly correlated with serum creatinine.[393] Plasma tCys rises with age, probably due to declining glomerular filtration rates accompanied with increasing urinary tCys.[394] It may explain the elevated tCys found in diabetic patients.[395]

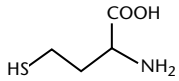
Correct specimen collection and storage are very important because plasma cystine undergoes slow degradation even when stored at –20°C. If a specimen takes more than 48 hours to reach the laboratory, the cystine level may be significantly lowered from the level in the freshly drawn blood. Therefore, it is helpful to record the specimen collection time on the test request form when ordering laboratory assessments.

Interpretation and Treatment: Patterns of plasma cystine, serine, cystathionine and taurine can indicate the location of a metabolic conversion block. Concurrent low cystine and serine, for example, indicate a restriction of the serine-glycine supply (see "Precursors of Heme, Nucleotides and Cell Membranes" section on the following pages). Low cystine levels may impair cellular synthesis of taurine (see Taurine section on the following pages), in which case plasma and urinary taurine will also be low.

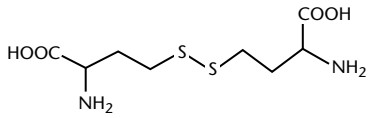
Low plasma cyst(e)ine may reflect a dietary deficiency of methionine and/or cysteine in which case N-acetylcysteine (NAC) would be an appropriate intervention. High dietary intake of condensed tannins as in heavy users of tea can lower plasma cystine.[373] Elevated plasma or urinary cystine can reflect either excessive dietary intake of cystine rich foods or impaired metabolism of cystine. High levels can indicate a need for vitamin B_6 and folic acid.[396]

Notes:

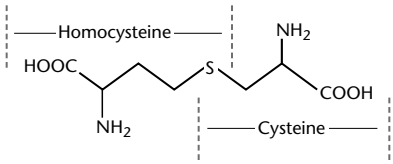
Homocysteine



Homocystine (HCys)



Cystathionine



The relation between homocysteine and homocystine is parallel to that described above for cysteine and cystine. Values reported from clinical laboratories are also similarly related so that total homocystine may reflect the sum of reduced and oxidized forms in the original specimen. Elevated homocysteine tends to coincide with high homocystine.

Age, gender, folate, serum vitamin B_{12}, serum creatinine and multivitamin usage are determinants of plasma homocysteine concentration (discussed in Chapter 2, "Vitamins"). In population studies, total plasma homocysteine concentrations are higher in men than in women, increase markedly with age and are inversely correlated with blood folate, serum vitamin B_{12} and serum creatine. Users of multivitamins have lower homocysteine levels than nonusers.[394]

Elevated homocysteine commonly affects the central nervous system (CNS), primarily as mental retardation, seizures and stroke.[397] Elevations indicated by increased plasma or urinary levels are etiologic factors in osteoporosis due to impaired cross-linking of collagen.[398] Smoking has been shown to increase the incidence of homocysteinuria, probably due to lowered levels of the controlling vitamins.

Moderately elevated levels of homocystine are associated with a significantly increased risk of atherosclerosis. High levels in body fluids of this amino acid have been associated with increased risk of cardiovascular disease as well as ocular, neurological, musculoskeletal and joint abnormalities. A common cause of this metabolic disorder is impaired function of the enzyme cystathionine β-synthetase for conversion to cystathionine (see Figure 4.20). This enzyme is vitamin B_6-dependent and supplementation of the vitamin has been used to treat elevated homocysteine. A second route in metabolism of homocysteine is remethylation to methionine using vitamin B_{12}, folic acid and betaine (trimethylglycine). Supplementation of betaine has proven useful for people with high homocysteine who do not respond to vitamin B_6.[399] Adequate intake of vitamins B_6 and B_{12}, folic acid and magnesium is necessary for insuring proper methionine metabolism to prevent the accumulation of homocysteine.

For lowering of elevated homocysteine, in addition to the supplementation of folate, B_{12} and B_6, some have advocated the use of betaine to support the betaine-methyl transfer reaction in reformation of methionine. Recent evidence demonstrates that plasma betaine concentration is, indeed, inversely related to homocysteine levels. Because betaine levels are raised by folic acid supplementation, additional betaine supplementation is less likely to be effective when the B vitamins are used at appropriate doses.[400] In patients with homocysteinuria due to cystathionine β-synthase deficiency, endothelial function is improved by 1 to 2 g/d vitamin C.[401] Although the molecular origin of this effect is unclear, the protective role of vitamin C for tetrahydrobiopterin, the cofactor for nitric oxide synthase, is a good candidate mechanism.

Although high plasma homocysteine is widely recognized as a cardiovascular disease risk factor, individuals with low homocysteine are also at risk of other conditions related to sulfur amino acid metabolism. The risk of hypohomocysteinemia derives from the fact that homocysteine is the normal precursor for formation of cysteine and thus for production of glutathione, taurine and sulfate from methionine.

Individuals with low homocysteine have limited capacity for up-regulation of glutathione synthesis in response to oxidative stress and certain kinds of toxin exposure. The most common treatment for low homocysteine is administration of sulfur-containing amino acids such as N-acetylcysteine and taurine. Preformed glutathione and inorganic sulfate salts (potassium sulfate) may also be employed to support hepatic and renal demands for toxin removal through sulfation and mercaptan formation. Plasma methionine and urinary sulfate, pyroglutamate or α-hydroxybutyrate may be

preformed for confirmation of significant cysteine deficit (see Chapter 6, "Organic Acids").

Hypohomocysteinemia causes reduced availability of cysteine. Cysteine restriction causes limitation in production of sulfate, taurine and glutathione.[414] The limited production ability is exacerbated in conditions that cause increased demand for any of the sulfur compounds produced from homocysteine. Alcohol intake greatly increases the production of taurine,[415] and many drugs and xenobiotics increase sulfate requirement for conjugation and elimination.[416] One of the body's main uses of sulfate and taurine is in the phase II liver detoxification.

Taurine is involved in the formation of bile acids, whereas the sulfation pathway is required for removal of steroid hormones, phenolic compounds and numerous drugs. Glutathione metabolic activities include phase II conjugation reactions, prostaglandin synthesis and reduction/oxidation reactions. Indeed, a survey of the literature shows that a reduction in glutathione is associated with diseases impacting virtually every major organ system (see Table 4.13). Any condition that increases oxidative stress tends to increase the demand for hepatic glutathione production. Thus oxidative stress draws homocysteine into glutathione synthesis, potentially causing a drop of plasma homocysteine to levels where total-body glutathione status is critical.[414] The studies cited here are only a small fraction of those suggesting that detection of low plasma homocysteine is of clinical utility in any scenario requiring increased use of glutathione, taurine, or sulfate.

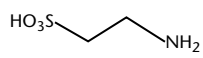
Autistic patients have lower plasma concentrations of homocysteine, methionine, SAMe, cystathionine, cysteine and total glutathione and higher concentrations of SAH, adenosine and oxidized glutathione. The biochemical abnormalities were normalized by a targeted nutritional intervention trial with folinic acid, betaine and methylcobalamin. These findings led to a conclusion that chronic oxidative inactivation of methionine synthesis through adenosine inhibition of SAH hydrolase is a propagating factor in autism.[386]

Cystathionine is the product of the vitamin B_6-dependent degradation of homocysteine. It is produced by conjugation of serine with homocysteine via the action of cystathionine-β-synthase, the pyridoxal-5-phosphate-dependent enzyme that is deficient in hyperhomocysteinemia. Increase in cystathionine-β-synthase activity accounts for the principal effects of vitamin B_6 supplementation in lowering homocysteine. Cystathionine lyase cleaves cystathionine to cysteine and α-ketobutyrate, the immediate precursor of α-hydroxybutyrate (see Chapter 6, "Organic Acids"). High cystathionine levels may indicate a functional vitamin B_6 or serine deficiency. Metabolites of cystathionine stimulate superoxide generation in leukocytes, placing increased demands on antioxidant capacity.[417] A decreased ratio of plasma cystathionine to homocysteine is a toxicity biomarker of methionine excess. In rats, as methionine intake is increased to levels that produce toxic effects, ratio values suddenly fall from 1.5 to less than 0.3.[378] This finding indicates that the conversion of homocysteine to cystathionine is rate limiting in disposal of excess methionine.

TABLE 4-13 — PATHOLOGIES AND DISEASES ASSOCIATED WITH LIMITED GLUTATHIONE STATUS

Organ Pathology Associated With Decreased Glutathione Status[402]	Specific Conditions Associated With Reduced Glutathione Status
Hepatic	Schizophrenia[403]
Cardiovascular	Autism[404]
Lungs	Cataracts[405]
Kidney	Accelerated aging[406]
Genitourinary	Hyperlipidemia[407]
Endocrine	Hepatitis C[408,409]
Gastrointestinal	AIDS[410]
Gallbladder	Adult respiratory distress syndrome[411]
Musculoskeletal	Diabetes[407,412]
Neurological	Cystic fibrosis[411]
	Symptoms associated with environmental toxicity[413]

Taurine (Tau)



Taurine is the two-carbon, sulfur-containing, β-amino acid, β-aminoethylsulfonic acid. Taurine serves antioxidant roles in various tissues, but it does not form a part of general protein structure like most of the other amino acids that have been discussed. Because taurine is a normal component of skeletal muscle, dietary fish and meats can supply significant taurine. Many cooked meats supply taurine in the range of 100 to 200 mg/100 g wet weight.[418] Consumption of a meat-restricted diet results in very low taurine intake. Taurine requirements for optimal human function are quite significant, especially in certain disease states. Thus, taurine is a conditionally essential nutrient that can be synthesized from cysteine when vitamin B_6 is adequate.

The cysteine relationship has been demonstrated by showing that cysteine supplementation results in normalization of plasma taurine concentrations in children receiving home parenteral nutrition.[419] Taurine is important during mammalian development, especially for cells of the cerebellum and retina.[420–422]

Care must be taken when comparing plasma and whole-blood (blood spot) taurine concentrations because the level in whole blood is more than five times higher, indicating an active pumping mechanism to sustain erythrocyte levels. In cats where dietary taurine is usually much higher, whole-blood levels respond to taurine supplementation slower than plasma levels.[423] Taurine status has been extensively studied in patients on long-term total parenteral nutrition where 10 mg taurine/kg/d intravenously normalizes plasma and blood cell taurine concentrations.[424] During supplementation, urine taurine-creatinine ratios rose to approximately five times normal. Another tissue distribution effect is found in horses, where cardiac tissue-plasma ratio is highest for taurine.[256] Plasma levels of taurine rise when glutamine is supplemented in stressed rats and patients with severe trauma.[425]

The antioxidant potential of taurine accounts for some of its clinical effects. When taurine is added to supplementation of vitamin E and selenium, diabetic retinal abnormalities are reduced in a dose-dependent fashion.[426] Patients with diabetic nephropathy also respond positively to taurine and vitamin E.[427]

Zinc Deficiency: Urinary taurine becomes elevated in zinc deficiency, apparently due to the decreased rate of protein synthesis. The cysteine that is not drawn into formation of Cys-tRNA is degraded to taurine and sulfate. If dietary intake is normalized, the degree of taurine elevation may be used to infer the severity of zinc depletion.[428]

Inflammation: High plasma taurine is found associated with various stress reactions,[429] apparently mediated by release of interleukins.[430] A high plasma level can indicate excessive production of taurine due to an inflammatory process mediated by white blood cells. Taurine is found in high concentrations in heart muscle and white blood cells. Taurine is involved in control of chemical oxidation by white blood cell phagocytes in response to respiratory burst activity. Inadequate taurine supplies allow the oxidative activity to go unchecked, leading to excess oxidative damage and formation of aldehydes. Individuals with this condition are allergy prone and often extremely sensitive to environmental chemicals.

Cardiovascular and Neurological Effects: Taurine deficiencies have been implicated in both neurological (epilepsy) and cardiovascular dysfunction. Heart and brain cells require taurine for intracellular retention of calcium, magnesium and potassium. Taurine has been used successfully in the treatment of congestive heart failure and is implicated in night blindness, arrhythmia, angina, hypercholesterolemia and atherosclerosis.[431] Beta-agonist drugs cause a reduction in the body pool of taurine.[432,433]

Notes:

Taurine also stabilizes platelets against aggregation. Platelets from taurine-depleted animals are twice as sensitive to aggregation as platelets from those receiving taurine. In addition, human subjects with normal taurine status show increased resistance to platelet aggregation by 30 to 70% when supplemented with taurine at 400 or 1,600 mg/d, respectively.[434] Cats have a high requirement for dietary taurine and they easily develop dilated cardiomyopathy on low taurine diets. The same effect is seen in dogs on low-protein diets. Taurine supplementation helps to reverse the cardiomyopathy.[435]

Increased plasma taurine is found in patients suffering from episodic acute psychosis, characterized by sensory perceptual distortions. In such patients, oral loading with either serine or glycine can increase synthesis of taurine from homocysteine and serine, inducing psychedelic symptoms.[436]

REFER TO CASE ILLUSTRATION 4.9

Interpretation and Treatment: In the rat model, bile duct ligation causes elevated plasma taurine associated with reduced hepatic taurine conjugation to form bile acids. The absence of bile acids produced a gut-derived endotoxemia with lowered hepatic glutathione and increased lipid peroxidation that were offset by cholesteramine treatment.[437] Higher plasma taurine values are part of a pattern of amino acids associated with major depression.[20] Depression is accompanied by decreased excitatory amino acids such as glutamate and increased inhibitory amino acids such as taurine.

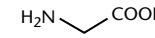
Taurine in the form of taurocholic acid is a key component of bile. Low taurine may accompany fat digestion problems,[438] fat-soluble vitamin deficiencies and high serum cholesterol levels.[439] Concurrent low cysteine is also relevant in taurine depletion. Low or low-normal cystine may indicate rapid conversion of this amino acid to taurine.

When plasma levels are low, the clinician can consider supplementation with taurine and vitamin B_6. Plasma taurine is easily raised by taurine supplementation of the diet. Cysteine addition to formulas used for home parenteral nutrition normalizes plasma taurine concentrations in children with short-gut syndrome.[419] Choline supplementation may stimulate taurine synthesis due to its sparing effect on methyl group supply by methionine. Methionine is more available to supply homocysteine for transulfuration, a requisite step for taurine biosynthesis.[440]

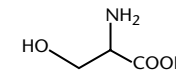
β-Alanine is a competitive inhibitor of the taurine transport system required for moving taurine across cell membranes. Addition of 3% β-alanine to the drinking water of mice causes a 35 to 50% reduction of taurine content of spleen and thymus with concurrent decline of functional immune response.[441] An individual with elevated plasma β-alanine who also shows high taurine (unsupplemented) may have impaired immune function that is responsive to taurine supplementation or other measures that lower β-alanine.

PRECURSORS OF HEME, NUCLEOTIDES AND CELL MEMBRANES

Glycine (Gly)



Serine (Ser)



The amino acid with the simplest structure, glycine, is also one of the most versatile. Glycine is classified as conditionally essential because clinical deficiency effects have long been known. Patients can become glycine-depleted due to the many metabolic demands for glycine, including heme biosynthesis for blood formation, collagen formation for growth and repair, glycocholic acid formation for digestion, glycine conjugation in detoxification and direct neurotransmitter action in brain function (Figure 4.22).

Collagen, the most abundant protein in the body, is one-third glycine and all enzymes, transport proteins and membrane receptors contain roughly 10% glycine. The glycine cleavage system, discussed below, transforms the single carbon atom of glycine into a key starting material for the multitudes of metabolic requirements for single carbon chemistry. Glycine is required for porphyrin synthesis to initiate the pathway leading to heme formation and for every cell function, including formation of RNA or DNA, synthesis of glutathione, bile acids and phase II conjugation reactions. To a lesser extent, glycine can also be used directly as an energy source or for synthesis of glucose. Glycine is

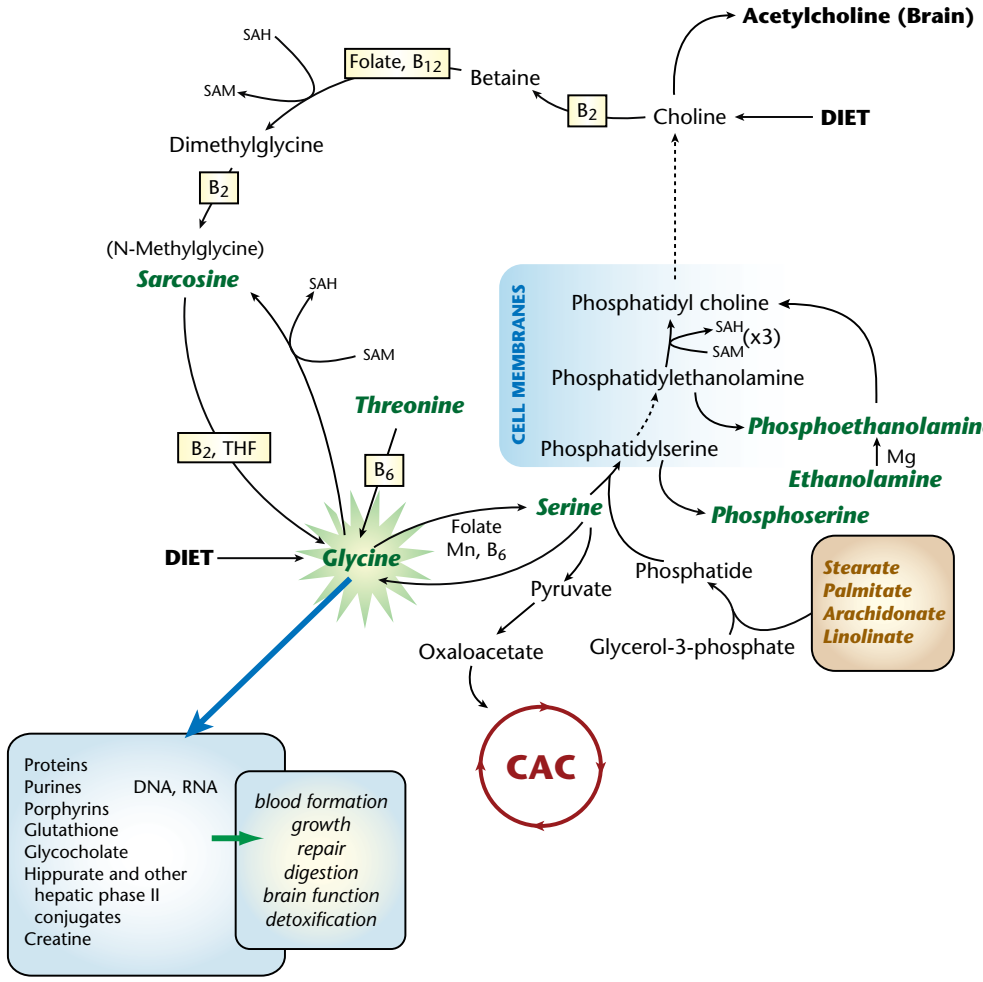


FIGURE 4.22 — **Metabolic Roles of Glycine and Serine**

One main point of this figure is to illustrate the reasons for glycine being the most heavily utilized of all amino acids. Its roles span from forming one-third of the most abundant protein in the human body (collagen) and participating in the initial step of heme synthesis, to highly variable roles in detoxification and synthesis of glutathione. The close biochemical association of serine means that the two amino acids tend to rise and fall in tandem as metabolic demands shift. Serine is the precursor for biosynthetic pathways that produce the phospholipids of cell membranes. See Figure 4.26 for detail about the limitation of human tissues for phosphatidyl serine conversion, indicated by the dashed arrow to phosphatidylethanolamine. Variations in status of cofactors, especially folate and vitamin B_6 and variations in activity of the glycine cleavage system illustrated in the following figure can produce glycine-serine ratio abnormalities. Dietary intake of glycine-containing proteins or choline from fats and oils can supply exogenous glycine. Little glycine is formed by synthesis from non-protein metabolites, so restriction of dietary protein supply can restrict the myriad processes dependent on glycine and serine.

the precursor of serine for the membrane components, phosphoethanolamine and phosphoserine.

Detoxification Roles of Glycine: The role of glycine in detoxification is especially critical during episodes of multiple exposures to several classes of chemicals. Glycine is used for direct conjugation to many compounds, including benzoic acid. Benzoic acid is a food component, food additive and a product of intestinal bacterial action on unassimilated phenylalanine (Chapter 6, "Organic Acids"). Because glycine is also one of the precursor amino acids for glutathione, individuals experimentally stressed with benzoate show inhibition of glutathione synthesis.[442] Plasma glycine is lower in patients with aspirin overdose than in healthy individuals,

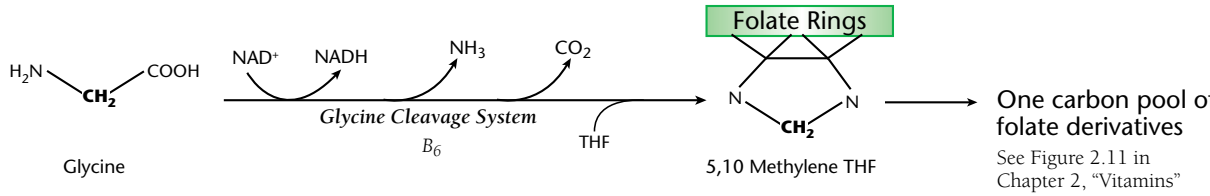


FIGURE 4.23 — The Glycine Cleavage System

The reaction catalyzed by the glycine cleavage system enzymes results in the total destruction of glycine with the capture of the central carbon atom as 5,10-methylene-THF. Thus, glycine availability enhances the capacity for maintaining the single carbon pool for biosynthesis and methylation reactions.

suggesting depletion of available glycine, which is used for formation of the excretory product, salicyluric acid.[22] These reactions are part of phase II hepatic detoxification. High serine and glycine found in hemodialysis patients may be normalized with erythropoietin (EPO) therapy.[443] The stimulation of erythropoiesis increases the rate of utilization of glycine for heme formation.

Genetics: A well documented reason for elevated plasma and urinary glycine is the condition of nonketotic hyperglycinemia (NKH) due to failure of the glycine cleavage system (GCS). The GCS is composed of a group of mitochondrial enzymes that mediate the reversible conversion of glycine to serine (Figure 4.23). Pyridoxal phosphate and tetrahydrofolate are cofactors in this reaction. By this pathway glycine is a major supplier of the "one-carbon pool" of folic acid intermediates that are pivotal to many biosynthetic, detoxification and gene-regulating reactions.[444] Plasma serine and glycine concentrations are under significant genetic control via a single major gene locus encoding the enzyme, serine hydroxymethyltransferase.[445] Individuals with profound nonketotic hyperglycinemia, who survive infancy, are severely mentally retarded, demonstrating the pathological potential of excessive glycine neuroinhibitory action.[225] Clinical presentation in atypical, mild NKH is heterogeneous, most often presenting in adults as mild mental retardation and behavioral problems.[446] Residual GCS function may be present, producing less severe elevations of glycine in plasma and CSF. Genetic polymorphisms of the glycine cleavage complex produce a specific glycine elevation rather than the concurrent high levels of glycine and several other ketoacids found in other abnormalities of branched-chain amino acid metabolism.

The GCS serves to maintain normal blood glycine levels by providing a clearing mechanism for high levels and a biosynthetic route from serine during episodes of falling

Notes:

levels. The efficiency of this system makes it highly unlikely that oral glycine will produce elevated plasma glycine in individuals with normal GCS function. The several known genetic variants are summarized in Table 4.14.[447]

Because of their neurotransmitter roles, glycine and serine have been subjects of investigations into psychiatric disorders. Serine is a critical component in the biosynthesis of acetylcholine, an important CNS neurotransmitter used in memory function and mediator of parasympathetic activity. Depressed subjects show lower glycine levels than controls.[20,21] Depression is accompanied by elevation of the serine/glycine ratio.[20] Patients suffering from episodic acute psychosis display a disturbance of serine-glycine metabolism,[436] and a higher serine-glycine ratio is observed in depressed individuals.[20] Recent evidence has linked the severity of depression with lowered plasma serine, alanine and glutamate.[124]

Table 4.14 — Manifestations of Non-Ketotic Hyperglycinemias

Form	Manifestations
Severe form	Arrhythmia, myoclonic jerks, profound hypotonia and death within the first few weeks
Mild form 1	Psychomotor retardation and growth failure
Mild form 2	Initially normal development followed by a progressive loss of developmental milestones, spinocerebellar degeneration and other symptoms of motor dysfunction

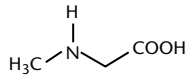
The evidence that has been summarized points to a direct relationship between plasma glycine levels and brain neurotransmitter effects. Neuronal activity is overstimulated by elevated plasma glycine levels and underactivated by low plasma glycine, leading to neuromotor or psychotic manifestations, respectively. Treating glycine insufficiency with oral glycine is quite effective. Elevated glycine is more difficult to manage, requiring steps to increase glycine utililzation and clearance. In the severe form of NKH, blood glycine is extremely elevated, often reaching values above 1,000 µM. Severe forms have been treated with moderate success with benzoate (to lower glycine by conjugation and excretion) and carnitine.[448] Treatment with 250 to 750 mg/kg/d of sodium benzoate have achieved sufficient reduction of cerebrospinal fluid glycine to reduce seizure activity.[225] Glycine potentiation of glutamatergic (NMDA) receptors may be the cause of myoclonic encephalopathy and seizures. Thus, some affected infants have been treated with benzoate along with NMDA antagonists such as dextromethorphan.[449] The NMDA agonistic role of glycine also places patients with hyperglycinemia at higher risk of glutamate toxicity during febrile episodes that cause elevation of the even stronger coagonist, quinolinic acid. In other words, the neuronal degenerative risk of elevated glycine is exacerbated by concurrent high quinolinate as further explained in Chapter 6, "Organic Acids."

Interpretation and Treatment: Low serine is associated with disordered methionine metabolism and methionine intolerance. Individuals with elevated levels of homocysteine, a heart disease risk factor, also have low levels of plasma serine and both factors show normalization with vitamin B_6, B_{12} and folate treatments.[450] Serine combines with homocysteine via a B_6-dependent step to produce cystathionine, which is then metabolized to cysteine and taurine. During periods of elevated total plasma homocysteine in humans, increased amounts of serine may be diverted to lower plasma homocysteine. Increased demand for glutathione synthesis increases serine conversion to cysteine, which is one of the three amino acids required for the creation of glutathione.

Plasma serine levels are lower than normal in homocysteinuria patients on folate therapy, compared with healthy adults.[450] Renal transplant recipients with mild elevations of serum creatinine also have lower than normal serine levels. Treatment of renal transplant patients with pyridoxine, folic acid and vitamin B_{12}, cofactors required for homocysteine metabolism, caused declines in plasma homocysteine levels, with a concurrent rise in plasma serine levels. Treatment of both pyridoxine responsive and non-responsive homocysteinemic patients with betaine lowered plasma homocysteine and normalized plasma serine levels.

In summary, low fasting plasma glycine and serine levels indicate chronically increased demand for glycine, including possible high rates of degradation for loading of folate through the glycine cleavage system. Conversely, elevated glycine can result from enzyme polymorphisms that decrease activity of the GCS, in which case there is potential for increasing the various clearing enzyme activities by raising concentrations of substrates such as benzoic acid or cofactors derived from folic acid, vitamin B_6 and niacin. Elevated serine may respond to betaine and, in hemodialysis patients, high serine and glycine may be normalized with erythropoietin (EPO) therapy.[443]

Sarcosine (N-Methylglycine)



A rarely discussed aspect of its biochemistry expands the scope of sarcosine's potential clinical significance. Simply put, sarcosine can serve to buffer the methyl group or single carbon supply system. In times of excess supply, methyl groups may be transferred from SAM to glycine, forming sarcosine. Those carbons may then be recovered as 5,10-THF to sustain the single carbon pool during overnight fasting. Genetic polymorphisms in a key enzyme, sarcosine dehydrogenase, can prevent the transfer of the sarcosine N-methyl group, resulting in sarcosinemia that can be related to low methylation capacity. Moderately elevated plasma sarcosine can be an indicator of polymorphisms for this enzyme. Adequate methylation is important for gene regulation, detoxification and neurotransmitter formation. Therefore, identification of individuals with such difficulties can have clinical significance.

On most metabolic pathway charts the only route to sarcosine originates with choline and proceeds through betaine (trimethylglycine) and dimethylglycine to yield sarcosine. The conversion of sarcosine to glycine is the final methyl group removal step (right to left in Figure 4.24). This pathway is a principal source of single carbon units as the dehydrogenase enzymes convert the methyl groups to 5,10-methylene-THF. However, when SAM and glycine are in abundance, the reverse reaction, N-methylation of glycine, also produces sarcosine (left to right in Figure 4.24).[451] The enzyme that catalyzes this reaction, glycine-N-methyltransferase, plays an important role in the overall economy of total-body methyl group supply. See Figure 2.12 in Chapter 2, "Vitamins," to review the single carbon forms of folate. During postprandial periods of high glycine clearance, the rate of 5,10-methylene-THF formation can easily exceed the rate of single carbon utilization, resulting in folate trapping. Postprandial methionine flow encourages hepatic formation of SAM that also might accumulate because its rate of utilization is strongly regulated. Clearing of SAM by glycine methylation stores excess methyl groups as sarcosine.[452] The advantage to the cell is in providing a temporary methyl group storage site in sarcosine. Up to 14% of dietary methionine may be converted to sarcosine by this pathway.[453]

Clinical Relevance and Treatment: Since the involvement of sarcosine in methyl-group conservation is a relatively new discovery, clinical associations are tentative. Elevated plasma or urinary sarcosine may become a part of the metabolic pattern analysis that allows methylation status assessment. The glycine N-methylation pathway can become important as a means of maintaining methylation status in conditions like autism, where a block has been demonstrated in cycling of homocysteine to methionine.[386] This block tends to produce relative excesses of SAMe, which is relieved by normal glycine methylation. In autistic patients, elevated sarcosine is a sign of exacerbation of methylation difficulties due to blockage in release of homocysteine. Similarly, individuals with SNPs affecting tetrahydrofolate reductase may have exacerbation of symptoms if there is concurrent difficulty with the sarcosine methyl buffering system. Figure 4.25 shows the distribution of plasma sarcosine in a set of 599 sequentially accessioned outpatient specimens.[550] Sarcosinemia is clearly present in a significant

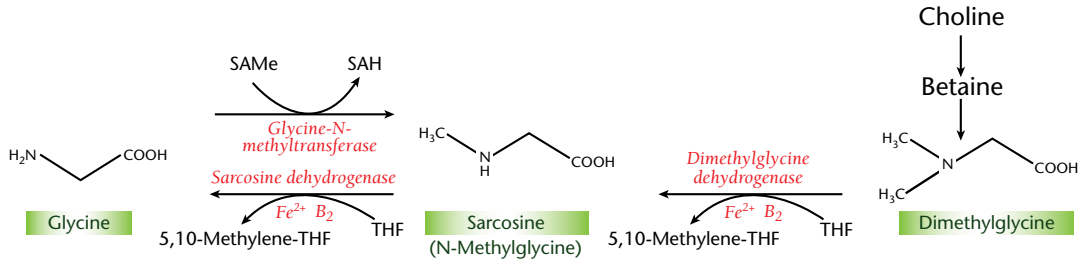


FIGURE 4.24 — Methylglycine Conversions

Sarcosine (N-methylglycine) is formed largely by single carbon transfer from dimethylglycine via dimethylglycine dehydrogenase. A similar reaction converts sarcosine into glycine. A small amount of sarcosine may be formed by transfer of a methyl group from SAMe to glycine.

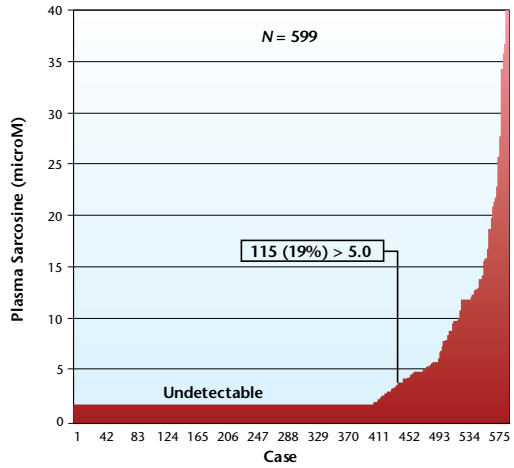
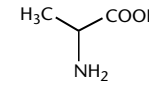


FIGURE 4.25 — **Plasma Sarcosine Distribution of a Large Outpatient Population**

Sarcosine was measured by the standard ion exchange chromatographic method. The detection limit for this method was 2.0 μM, so much of this typical sampling of an outpatient population is shown below this level. One hundred and fifteen individuals, or 19% of the population is above the 5th quintile cutoff of 5.0, indicating slow clearance of sarcosine. Many patients had levels above 15 μM consistent with more severe clearance issues.

portion of this patient population. Sarcosinemia due to defects in the enzyme, sarcosine dehydrogenase, which catalyzes the conversion of sarcosine to glycine, has been extensively reviewed.[453] Sarcosinemia can also result from folate deficiency because of the folate requirement for the sarcosine-to-glycine reaction. Sarcosine is one of the amino acids found elevated in plasma for patients with Parkinson's disease.[95] Since several amino acids show CSF-plasma ratios lower than controls, Parkinson's disease appears to involve a disruption of amino acid transport. Sarcosine has also been found helpful as adjunct therapy for schizophrenia because it delivers glycine to the brain, where it activates NMDA receptor glutamatergic response, modulating dopamine release.[454–456]

Alanine (Ala)



During strenuous exercise, muscle protein is degraded to amino acids that must be recovered to prevent loss of energy. Alanine transaminase (AT) enzymes are highly active in muscle. They provide a mechanism for the removal of ammonia by transferring amine groups from a variety of amino acids to pyruvate, forming alanine. Thus, alanine is the major carrier of amino acid nitrogen from muscle to liver where its carbon skeleton is converted to glucose (via glucogenesis). This carrier role of alanine is significant because the metabolism of amino acids in skeletal muscle is a major contributor to overall protein metabolism.

Sustained exercise requires that glucose be constantly supplied to muscle tissue. The alanine that flows from muscle serves as substrate for hepatic alanine aminotransferase (ALT) that catalyzes the reverse of the muscle reaction. Alanine is deaminated so that pyruvate is made available for gluconeogenesis and the amine group is used in formation of glutamate or glutamine. Glutamine may then carry the amine group into the urea cycle for final disposal as urea. The vitamin B_6-dependent enzyme ALT is one of the most abundant enzymes in hepatic tissue, which has led to measurement of plasma ALT as a standard marker for liver disease.[457]

Glutamine is the dominant regulator of blood glucose via renal gluconeogenesis (see Glutamine). Hepatic alanine clearance assists glutamine production by transamination where the alanine carbon skeleton becomes pyruvate while glutamate (and thus glutamine) is formed, drawing α-ketoglutarate away from mitochondrial compartments.

Clinical Relevance and Treatment: The transaminase enzymes require pyridoxal-5-phosphate derived from vitamin B_6. In vitamin B_6 deficiency, transaminase activity falls. Because of the strong dependence on

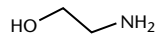
Notes:

transamination reactions for alanine clearance, high alanine levels can indicate vitamin B_6 need. Similar to the response of glutamine, plasma alanine may actually rise when dietary protein is restricted, due to increased de novo synthesis.[458]

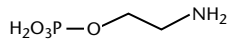
A pattern of low alanine and other amino acids in plasma or urine can be a sign of need for supplementation of alanine, especially if symptoms of easy fatigability between meals are present. In hypoglycemic adults, infusion of alanine to raise plasma alanine from 138 to 1,481 µmol/L improved some aspects of cognitive performance.[459]

Huntington's disease seems to be characterized by a defect in cellular uptake or metabolism of neutral amino acids resulting in low plasma alanine. Genetic polymorphism of the genes that control cell uptake of alanine in non-Huntington's patients may account for variability seen in plasma alanine.[460]

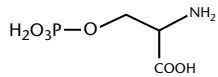
Ethanolamine (EtN)



Phosphoethanolamine (PE)



Phosphoserine (PS)



These three amino acids are closely related structurally and they share principal roles in phospholipid metabolism (Figure 4.26). Methylation of ethanolamine by three SAM transfers yields phosphatidyl choline, also known as lecithin. Metabolism of dietary lecithin can also produce choline for acetylcholine synthesis. High plasma ethanolamine levels may reflect reduced synthesis of choline due to poor methylation status.

Brain: Human tissues (especially the brain) contain another form of lipid ethanolamine compound called plasmalogen, where an unsaturated fatty acid is attached to the first carbon of glycerol by an ether linkage. Recent studies have shown that ethanolamine plasmalogens confer special antioxidant properties on membranes.[461] Phosphoethanolamine is a precursor to acetaldehyde,[462] which can exert metabolic poisoning effects by irreversible binding to coenzyme A, especially in an individual with restricted intake of pantothenic acid (vitamin B_5). Mitochondrial inhibition by both ethanolamine and PE in vitro suggests that altered brain phospholipid metabolism may initiate depression and bipolar disorder.[463]

Parkinson's disease patients have elevated plasma phosphoserine and the CSF-plasma ratio of many amino acids is significantly lower in patients than those of controls. In contrast, Alzheimer's disease patients had lower CSF levels of phosphoserine than controls.[96] Both of these disorders may have origins in the dysfunction of transport of neutral and basic amino acids across the blood-brain barrier.[95]

DNA, Wound Healing and Tissue Regeneration: Ethanolamine is converted to phosphoethanolamine via a magnesium- and manganese-dependent reaction. High levels of ethanolamine combined with low levels of phosphoethanolamine may reflect functional deficiency of these elements. Ethanolamine exerts significant effects on DNA synthesis, which are independent of phosphoethanolamine utilization in membrane lipid formation.[464] At high concentrations, ethanolamine exerts inhibition of insulin-induced DNA synthesis. Patients with diseases that produce increased requirements for tissue replacement have increased demands for DNA synthesis. Assuring adequate cofactor status to prevent chronic elevated ethanolamine should assist such patients, such as those with chronic inflammatory bowel conditions.

In addition to possible physiological effects, high ethanolamine can be due to environmental exposure, shown in Table 4.15. Other names for ethanolamine that might appear on labels for chemicals are ethylolamine, monoethanolamine, β-aminoethyl alcohol, aminoethanol and 2-hydroxyethylamine. Although no data is available for effects of chronic exposure in humans, ethanolamine is irritating to the skin, eyes and lungs. At

TABLE 4.15 — ENVIRONMENTAL SOURCES OF ETHANOLAMINE

Agricultural chemicals	Cleansers
Corrosion inhibitors	Cosmetics
Detergents	Dry cleaning
Electroplating	Emulsifiers
Emulsion paints	Fuel oil additives
Hair-waving solutions	Pharmaceuticals
Polishes	Soaps
Wool treatment	

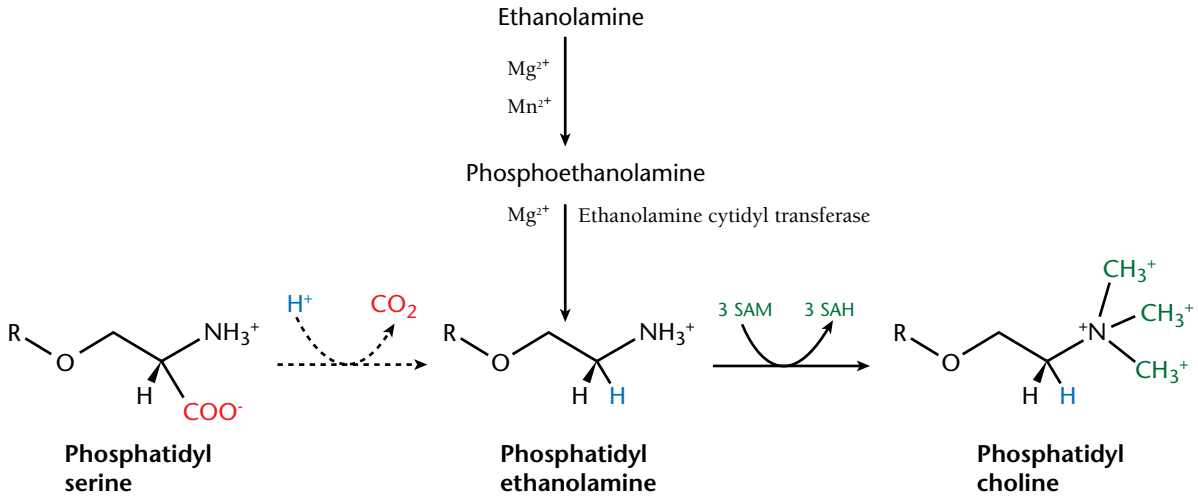


Figure 4.26 — **Metabolism of Phosphatidyl Ethanolamine**

The "R" group in these structures represents the diacylglycerol phosphate group. These derivatives of the amino acids are major components of cell membranes and they are present in virtually all whole foods. Recent evidence indicates that human tissues do not have the enzyme for direct phosphatidyl serine decarboxylation. The absence of this reaction suggests that dietary intake of phosphatidyl ethanolamine is of more importance to supply the large demand for cell membrane formation.

high concentrations, it causes central nervous system depression in exposed animals. Further information on the toxicity of ethanolamine may be found at the U.S. Occupational Safety & Health Administration Web site (www.osha.gov).

Interpretation and Treatment: Cytosolic phosphoethanolamine and ethanolamine have been shown to provide prognostic factors in breast cancer. Phosphoethanolamine is elevated in patients with high rates of breast tumor growth.[465] As the stage of the disease progresses, the values for phosphoethanolamine plus ethanolamine become higher. Increased plasma and urinary phosphoethanolamine is found in individuals with osteopenia associated with "adult" hypophosphatasia, along with subnormal circulating total alkaline phosphatase activity.[466]

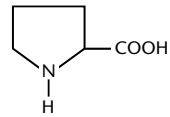
High PE may indicate functional impairment of acetylcholine synthesis. Functional vitamin B_{12} or folic acid deficiency, or disordered methionine metabolism (i.e., inhibition of methyl group transfer), may account for high PE levels. Therefore, high phosphoethanolamine may indicate a need for SAMe, vitamin B_{12}, folate and betaine.

Notes:

BONE COLLAGEN-SPECIFIC AMINO ACIDS

Collagen, the protein of bone, tendons, ligaments and basement membranes is the most abundant protein in the human body. Its unique structural properties are due to the recurrence of proline and glycine residues and its great strength and stability are due to cross-linking afforded by lysine side chains. Cells that are dedicated to collagen synthesis make large demands on proline and glycine supply.

Proline (Pro)



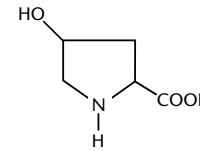
Proline is found in virtually every dietary protein except lactalbumin. Although proline is not classified as an essential amino acid, proline levels in plasma are dramatically reduced with a proline-free diet.[467,468] Proline is a major constituent of collagen and can be metabolized to α-KG, which is important in ammonia detoxification and citric acid cycle.

Proline is universally required for protein synthesis and is metabolized to hydroxyproline, an important component in connective tissue. Therefore, high levels may reflect inadequate connective tissue synthesis. Proline can also be oxidized to glutamic acid, requiring niacin as a cofactor precursor.

Interpretation and Treatment: Low plasma or urine levels of proline reflect low tissue supply of this amino acid and α-KG could be used to normalize the value. Low proline levels can indicate a low-protein diet and may prevent optimal connective tissue maintenance. Hyperprolinemia is a recognized genetic disorder characterized by renal and central nervous system dysfunction.[26]

Following on the earliest nutritional interventions for scurvy, vitamin C and iron are known cofactors in the conversion to hydroxyproline and may be useful if connective tissue symptoms are present. Acute or chronic deficiency of vitamin C produces a significant increase in the proline/hydroxyproline ratio in urine.[469] The relationship between proline and hydroxyproline urinary excretion has been used as an index of collagen catabolism.[470] In conclusion, high levels of proline and hydroxyproline may be lowered by supplementing vitamin C and iron.[471]

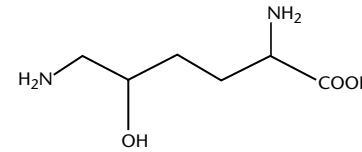
Hydroxyproline (HPro)



High levels in 24-hour urine or plasma correlate with the increased osteocalcin secretion that is characteristic of high bone turnover.[472] This marker may be as sensitive as the measurement of bone collagen-specific peptides.[473] Free plasma hydroxyproline in multiple myeloma is closely related to the extension of skeletal lesions at diagnosis and is useful in the follow-up of bone disease.[474] Plasma hydroxyproline also is highly correlated with serum alkaline phosphatase,[475] another marker of bone resorption.

Hydroxyproline, a component of collagen, is synthesized from proline using vitamin C and iron as cofactors. Vitamin C has been used to successfully treat certain types of collagen disorders and to stimulate collagen synthesis.[476]

Hydroxylysine (HLys)



A portion of the lysine of collagen is modified to hydroxylysine, which provides a marker for bone loss due to the turnover of collagen during bone resorption. Both hydroxylysine and hydroxyproline are indicators of liver disease. However, hydroxylysine has the strongest correlation, possibly because dietary intake of collagen can influence hydroxyproline levels.[477] Changes in hydroxylysine excretion with cadmium loading in experimental animals indicate that cadmium interferes with collagen synthesis.[478]

Urinary hydroxylysine provides a marker of response to growth hormone in GH-deficient children.[479] The hydroxylysine group of bone collagen is glycated and elevated urinary concentrations of galactosyl-hydroxylysine (GHYL) provides a specific indicator of bone metastases because of the greatly increased rate of degradation of bone collagen.[480] Glycosylation of hydroxylysine in bone collagen and has been proposed

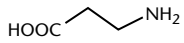
to explain the higher urinary GHYL in osteoporotic women.[481]

High plasma levels are indicative of connective tissue breakdown. Collagen is synthesized using the cofactors iron, α-ketoglutaric acid and vitamin C. Supplementation of these nutrients as well as chondroitin sulfate and manganese may be useful in reversing this condition.[482]

β-Amino Acids

β-Amino acids are so named because their amino groups are attached to the beta carbon. These compounds are not found in proteins. They serve physiological functions ranging from bile acid precursor and antioxidant to neurotransmitter and metabolic control. They can be acquired from the diet or synthesized de novo. Taurine is a β-amino acid, but was also discussed under the sulfur amino acids. Taurine and the other β-amino acids use the same carrier-mediated active transport into cells.

β-Alanine



β-Alanine is released from skeletal muscle during strenuous exercise and it occurs in food mainly as carnosine in red meats or anserine in poultry. The pyrimidines cytosine and uracil from DNA and RNA are degraded to β-alanine.

β-Alanine can become elevated in plasma or urine due to enzyme deficiency, dietary intake, intestinal microbial overgrowth, or high turnover of muscle tissue (Figure 4.27). β-Alanine has been used as an index of carnosine catabolism.[259]

Deficient activity of the enzyme β-alanyl-α-ketoglutarate transaminase in a 4-year-old girl was corrected by oral pyridoxine therapy in one reported case.[483] Intermittent seizures and lethargy were reduced. The biochemical pathway involved in this case is the conversion of β-alanine to α-ketoglutarate (not shown in Figure 4.27).

Vitamin B_6 deficiency generally causes lowered activity of the enzymes that degrade β-alanine, resulting in high urinary excretion.[484] High β-alanine is frequently associated with generalized β-aminoaciduria and concomitant loss of other amino acids such as taurine, due to impairment of renal tubular resorption. Low taurine levels may indicate taurine depletion by this mechanism. High levels of β-alanine are frequently accompanied by increases in 1- and 3-methyl-histidine, carnosine and anserine.[79]

Uptake of taurine occurs by a carrier-mediated active transport process specific for β-amino acids.[485] Because there is transporter competition for β-amino acid entry into cells, excessive taurine administration may cause elevated carnosine (resulting in muscle weakness) or elevated β-alanine.[486] Therefore, monitoring β-alanine levels can help the clinician appropriately adjust taurine supplementation. Use of taurine should be decreased when β-alanine is elevated. Excess excretion of taurine may indicate β-aminoaciduria. β-Alanine impairs renal tubular resorption of a variety of amino acids, including taurine, thus propagating amino acid deficiencies.

Notes:

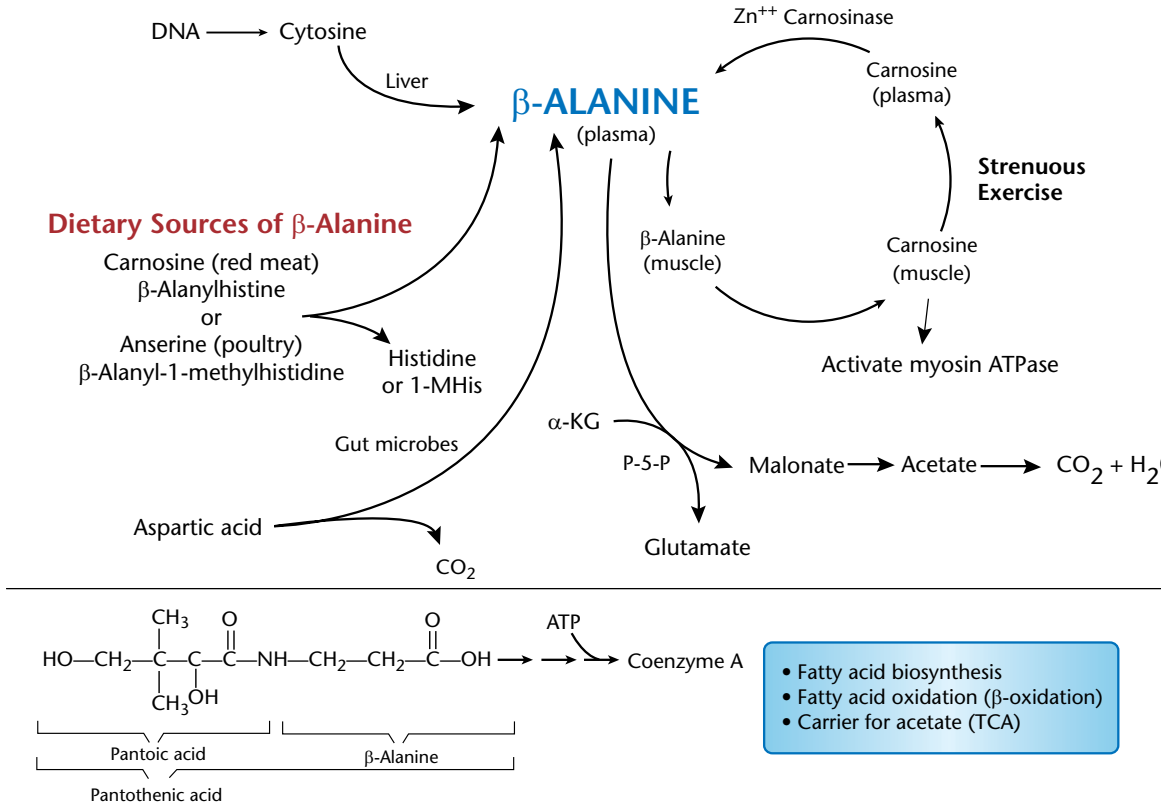


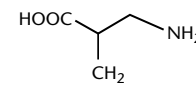
FIGURE 4.27 — β-Amino Acid Pathways

The origins and dispositions for β-alanine are quite different from those for alanine discussed previously. At cell death, DNA catabolism releases β-alanine from breakdown of cytosine. Dietary carnosine and anserine are normally hydrolyzed rapidly with release of β-alanine. β-Alanine is used for synthesis of muscle carnosine and for ubiquitously distributed coenzyme A that is required for multiple central energy pathways. Excess β-alanine is oxidized via conversion to acetate.

Epileptic patient treatment with the GABA transaminase inhibitor, vigabatrin, produces elevated β-alanine because the drug also blocks its breakdown.[487]

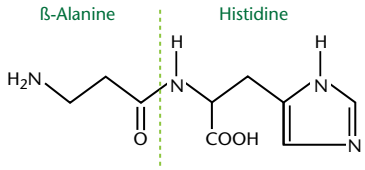
Intestinal bacteria and/or *Candida albicans* can also make β-alanine, which can raise plasma levels of β-alanine. With high β-alanine, check urinary indican or other dysbiosis markers as a measure of bowel dysbiosis. A bowel detoxification program may be appropriate with supplementation of a high-potency *Lactobacillus acidophilus* and *L. bifidus* products along with pre-biotics and a high-fiber diet to support growth of the favorable organisms (see Chapter 7, "GI Function"). Because of the competition of β-alanine for the taurine transporter, a bowel detoxification program to remove a major source (microbial overgrowth) of β-alanine can help to raise the kidney threshold to taurine spill and, therefore, help raise plasma taurine levels.

β-*Aminoisobutyric Acid*

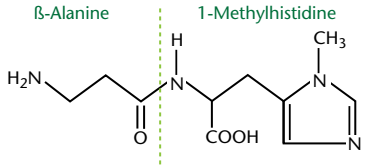


Thymine, released when RNA and DNA are degraded, enters a catabolic pathway that leads to β-aminoisobutyric acid (β-AIB). High β-AIB in urine is a benign metabolic polymorphism present in some human populations due to deficiency of hepatic β-AIB-pyruvate transaminase. Over 40% of some asian populations have this condition.[79] Excretion of β-AIB increases in cases of leukemia and x-ray radiation-induced destruction of DNA.[488] Parenteral administration of excessive folic acid also stimulates cell turnover that results in increased β-AIB.[489] If this amino acid is high, it may indicate a vitamin B_6 insufficiency.

Carnosine



Anserine



Carnosine and anserine are involved in muscle function through the regulation of muscle phosphorylase activity.[490] Carnosine and anserine are formed in skeletal muscle, where they activate the myosin ATPase enzyme.[491] Carnosine is a dipeptide containing histidine and β-alanine. There is an active turnover of carnosine normally, with exchange between muscle, plasma and liver. Anserine is structurally like carnosine except that the histidine ring contains a methyl group in the 1 position (see 1-methylhistidine). It has been shown that testosterone increases carnosine synthesis, whereas anserine synthesis increases with age, indicating the role of these amino acids in androgen-modulated changes in skeletal muscle.[492]

Both compounds are present in the muscle tissue of animals. Their concentrations are lower in humans than in most animals. Anserine predominates in poultry (especially hens) and carnosine is highest in pork.[493] Carnosine and anserine are hydrolyzed by carnosinase in human tissues, releasing histidine and 1-methylhistidine, respectively and β-alanine (Figure 4.27).

Dietary carnosine forms a tight complex with zinc, which greatly assists in the absorption of zinc[494] and acts as an antioxidant in ischemia[495] and in ethanol-induced injury.[496] Through this tight binding complex, carnosine can rescue neurons from zinc- and copper-mediated neurotoxicity and may be an endogenous neuroprotective agent.[497]

In mammalian mitochondria, anserine activates and carnosine inhibits Ca(2+) uptake.[498] Both anserine and carnosine increase the respiratory burst and interleukin-1 beta production of human neutrophils. They suppress apoptosis of human neutrophils in vitro, showing that carnosine and anserine have the capacity to modulate the immune response.[499] It has been shown that they potentiate the carcinogenic effects of Ni(II) by increasing the formation of oxidized nucleotides into DNA.[500] However, carnosine provides protection of rat brain endothelial cells against degenerative affects of β-amyloid[501] and against malondialdehyde-induced toxicity.[502]

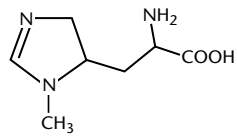
Genetic variants with deficient carnosinase activity in plasma show greatly increased urinary carnosine, but no detectable increase in plasma carnosine. Genetic carnosinase deficiency is a variable phenotype with presentations ranging from normal to severe psychomotor retardation, hypotonia and myoclonic seizures in the first year of life. Affected individuals excrete high amounts of carnosine and anserine.[503] The condition was found in a 12-year-old male with profound mental retardation, severe athetoid spastic quadriparesis, optic atrophy, sensory peripheral neuropathy and suprabulbar signs.[504] Reduced serum carnosinase activity is also found in patients with Parkinson's disease and MS and in patients following a cerebrovascular accident. Chronic high carnosine in plasma or urine is associated with mental retardation.[505] Inherited traits producing a deficiency of carnosinase, the enzyme that converts carnosine back to histidine plus β-alanine, are associated with progressive neurological problems and sensory polyneuropathy.[504,506]

Zinc is the cofactor for carnosinase and elevations of carnosine or anserine may be one effect of zinc deficiency. A patient with zinc deficiency who consumes a high-poultry diet would tend to show increased anserine excretion because of low carnosinase activity.

Notes:

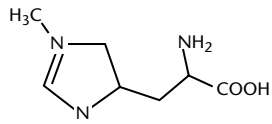
The Methylhistidines

1-Methylhistidine (1-MHis)



1-Methylhistidine is derived mainly from hydrolysis of the anserine of dietary meat, especially poultry. Carnosinase splits anserine into β-alanine and 1-methylhistidine (Figure 4.27) and since 1-methylhistidine is not normally metabolized in human tissues, most of it rapidly appears in urine.[507] Urinary excretion of 1-methylhistidine has been used as a marker to distinguish meat-eating individuals from vegetarians.[508] Vitamin E deficiency can lead to 1-methylhistidinuria from increased oxidative effects in skeletal muscle.[509]

3-Methylhistidine (3MHis)



Enzymes in skeletal muscle carry out the post-translational methylation of protein histidine residues forming 3-methylhistidine. High urinary excretion of 3-MHis indicates active catabolism of muscle and is a marker for skeletal muscle breakdown as in strenuous exercise.[510] The catabolic effects of cortisol and the opposing anabolic effects of testosterone on muscle catabolism are reflected in the level of 3-MHis in urine. Excretion of 3-MHis rises with cortisol and falls with administration of testosterone when cortisol is normal.[511]

3-MHis has been used as a marker in studies of clinical conditions associated with nitrogen loss, including trauma, infection,[512] and in uncontrolled diabetes.[513] The ratio of urinary 3-MHis to creatinine is increased in severe injury, thyrotoxicosis, neoplastic disease, prednisolone administration and sometimes Duchenne muscular dystrophy. In myxedema, osteomalacia and hypothermia, the ratio of 3-MHis to creatinine in urine was decreased.[514] Hypervitaminosis A in rats causes acceleration in myofibrillar protein breakdown revealed by increased urinary 3-methylhistidine.[515]

In cases of mild elevation of 3-methylhistidine, the contribution of dietary meat intake can be accounted for by checking 1-methylhistidine. Significant 1-methylhistidine excretion confirms high-meat intake.[507] This method has been used to achieve greater accuracy in the assessment of the catabolic effects of infection.[516] Hypothyroidism and reduced dietary protein can result in decreased urinary 3-MHis.[517] Twenty-four-hour urinary 3-MHis excretions can be applied for estimating skeletal muscle mass (SM) in healthy adults on a meat-free diet.[518]

Excessive muscle tissue breakdown can be associated with inadequate antioxidant protection in the muscle. Supplementation of the natural antioxidants—vitamins A, C, E and B_2 and selenium, β-carotene, lipoic acid and coenzyme Q_{10} (CoQ_{10})—may be useful in preventing excessive free radical pathology associated with this problem.[519] BCAAs have also been indicated to lower plasma levels of 3-MHis by preventing muscle proteolysis.[520]

The Polyamines

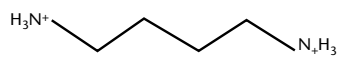
There is a relative paucity of information about clinical interventions to correct polyamine abnormalities, partly because laboratory evaluations of polyamines have not been routinely available. The greater availability of instrumental methods for their analysis and the key physiological roles of polyamines, however, make their inclusion in profiles used to screen for metabolic issues a likely eventuality.

Since they posses no carboxylic acid group, the polyamines are not considered amino acids. They will be discussed here, however, because they are derived from amino acids and are likely to be reported in amino acid profiles. The biologically active polyamines contain two (putrescene and cadaverine), three (spermidine), or four (spermine) amine groups. This means that they are highly basic molecules that become positively charged at neutral pH. Their special charge characteristics lead to their participation in ionic transfer phenomena, especially calcium ion (Ca^{++}) movements that are widely used for cellular regulation. The amine groups also make these compounds highly odiferous, producing the characteristic odor of putrefaction. Salivary cadaverine is associated with oral malodor independent of sulfur compound presence.[521]

Polyamine levels have not been available as routine assays from clinical laboratories, so little information is available on abnormal levels. Availability of precursor amino acids and pathway cofactors may be presumed

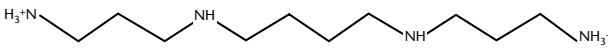
to have effects that could lead to lowered polyamine synthesis. Genetic polymorphic changes in the enzymes of the pathways also have potential to reduce rates of synthesis.

Putrescene



Putrescene is an intermediate in the formation of spermine. The conversion of putrescene into spermine consumes two SAM molecules in reactions that transfer propylamine groups (-$(CH_2)_3$-NH_2) rather than the methyl group from the methionine sulfur atom as occurs in methyl transfer pathways (Figure 4.28). Thus, the formation of spermine from putrescene results in a net loss of methionine. Putrescene accumulation can alter chromatin structure.[522] Cancer of blood and solid tissues, including stomach tumors induced by N-methyl-N-nitro-N'-nitrosoguanidine are associated with significant elevations of putrescine, spermidine and spermine.[523] However, the production of spermine from putrescene may be why it is required in cell culture growth media for mammalian cells.[524] Other evidence links putrascene with regulatoin of cell proliferation. Human HL-60 cells grown in culture show rapidly declining putrescene (but not spermine or spermidine) during apoptosis,[525] whereas proliferation due to chemically induced carcinogenesis is associated with increased putrescene.[526]

Spermine



Spermine is abundant in seminal plasma and brain tissue. Polyamines have long been known to be associated with male reproductive function and, in the mouse, growth and fertility require normal spermine synthesis.[527] Modulation of synaptic transmission by polyamines has been proposed as a reason for high brain levels of spermine. Uptake of choline and dopamine is inhibited by spermine.[528]

Spermine oxidation by copper-containing serum amine oxidase enzymes causes a necrotizing cytotoxic effect, in which phosphatidyl serine participates via its ability to signal phagocytic engulfment.[529] Oxidation of spermine is the mechanism that makes seminal plasma immunosuppressive.[530] Spermine also has been shown to be responsible for the T-lymphocyte suppressive effect of seminal fluid.[531]

In myometrium cell plasma membranes, spermine has a relaxing effect on smooth muscle cells. The effect is mediated by increasing Ca^{++} concentration.[532] Activity of Ca^{2+}, $Mg^{(2+)}$-ATPase was stimulated at very low concentrations (0.1–0.5 µM), but an inhibitory effect became dominant at higher concentrations.

In women, plasma spermine concentrations undergo distinct cyclic changes during the menstrual cycle. Peak spermine concentrations coincide with the first estradiol increase and the follicular phase, just prior to ovulation, during the period of rapid endometrial growth.[533] Since the biosynthesis of spermine results in net loss of methionine, the availability of this essential amino acid for methylation functions may drop as spermine rises.

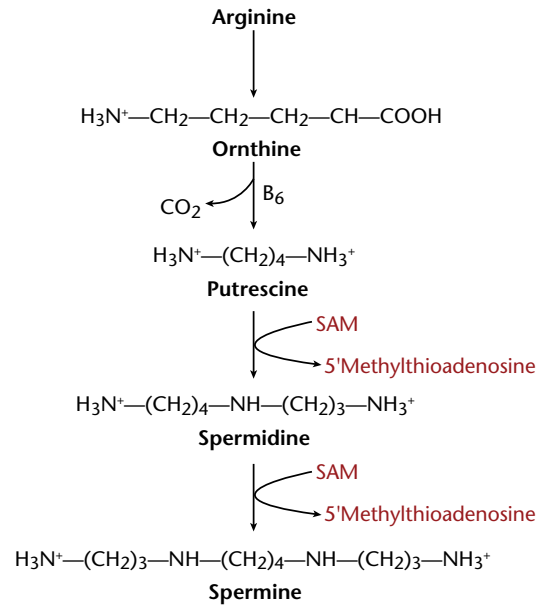
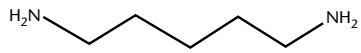


FIGURE 4.28 — Biosynthesis of Spermine from Arginine or Ornithine and SAM

Arginine decarboxylase or ornithine decarboxylase initiates the conversion of arginine or ornithine to putrescine, respectively. Two molecules of SAM are then required to transfer $–(CH_2)_3$-NH_2 groups from methionine, forming spermidine and spermine.

Cadaverine



Lysine decarboxylase is not present in human tissues, but some bacterial species possess the gene that allows the formation of cadaverine from lysine. (Figure 4.29) The production of cadaverine by *Escherichia coli* allows the organism to survive acid stress via inhibition of outer membrane proteins called porins.[534] Cadaverine induces compartmentalization of *Shigella* species to the phagolysosome of polymorphonuclear leukocytes. This response appears to be protective, contributing to the diminished ability of polymorphonuclear leukocytes to transmigrate across intestinal epithelia, carrying the infection to the tissues of the host.[535] Putrescine and cadaverine are the most concentrated polyamines in saliva, having highest concentrations of 33 and 18 µg/mL, respectively, immediately after awakening.[536] The growth of anaerobic organisms in bacterial vaginosis is accompanied by higher levels of putrescene and cadaverine that generate the fishy odor.[537,538]

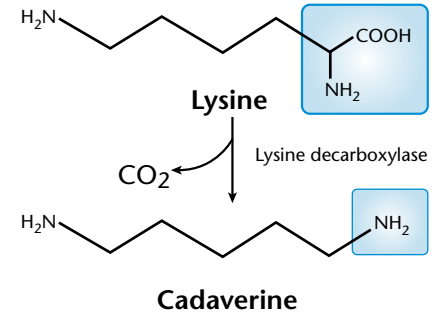


FIGURE 4.29 — **Conversion of Lysine to Cadaverine**

This reaction does not occur in human tissues, but bacteria expressing lysine decarboxylase can produce cadaverine from lysine in a single step.

FREE-FORM AMINO ACID SUPPLEMENT FORMULAS

The percentages of amino acids in a free-form amino acid base formula are set according to studies estimating optimum intake for maintenance and growth.[539] For the indispensable amino acids with reported levels below their low reference limits, additional amounts are added as calculated by formulas like that shown below, where the constant is adjusted to provide safe and effective increments to the base.The cofactors α-ketoglutarate and vitamin B_6 are added to assure interconversions leading to non-essential amino acids.

Added amount (g) =
(Low limit – Reported level) ÷ Low limit × Constant

Because of their free (non-peptide linked) form, the amino acids are very efficiently absorbed, even in the absence of stomach acid and pancreatic secretions. Some patients prefer encapsulation to avoid contact with taste buds, but in most cases, mixing with flavorful juices or food is sufficient. Effective adult dosage is 10 to 30 grams of the mixture daily.

The biochemistry, physiology and clinical impact for individual amino acids that have been covered in this chapter explain the extreme variety of favorable clinical responses to improved amino acid status. A common hypothesis among practitioners is that many cell functions can be restored by providing amino acids to assist neurotransmitter and hormone synthesis. The physical and immunologic barriers in the gut can be restored as the rate of secretory and structural protein production is increased. Blood glucose can be stabilized through better gluconeogenesis, whereas utilization of fatty acids for energy is enhanced by better lipoprotein synthesis and mitochondrial performance. Hepatic and gastrointestinal detoxification reactions are improved when amino acids required for conjugation reactions are available and the oxidative damage from such activity is reduced when sulfur amino acid supply is adequate to maintain glutathione.

Notes:

Correction of Abnormal Amino Acid Levels

Fasting plasma amino acid profiles reflect amino acid status in humans, as shown in the case of an 81-year-old female with maldigestion and muscle wasting. A serum chemistry profile revealed frank protein and amino acid deficiency as simultaneous low albumin and total serum protein in the presence of normal BUN-Creatinine ratio (Figure 4.30A). The protein deficiency state was evident in her plasma amino acid profile, which showed abnormally low values for all essential amino acids as well as the 4 non-essential amino acids reported. Thus, not only does she show signs of reduced rates of major protein synthesis, but there is also evidence that she is unable to sustain plasma amino acid concentrations for all anabolic or energy-yielding processes. The added dimension of relative concentrations from the amino acid profile is seen in the disproportionately low arginine and lysine, relative to leucine. Likewise, phenylalanine is only moderately low relative to tyrosine, which is severely depressed. The rate of fall for individual amino acids depends on specific non-protein demands, whereas urea and blood protein synthesis change uniformly as they represent processes involving all 20 protein-building amino acids.

Serum Chem-Screen

	Result		Reference Limit
BUN/Creatinine	15.6 g/dl		12.0–20.0
Protein, Total	5.1	L	6.4–8.4
Albumin	2.9	L	3.0–5.5

Plasma Amino Acid Profile

	Results		Reference Limit
Essential Amino Acids			
Arginine	17	L	50–160
Histidine	63	L	70–140
Isoleucine	28	L	50–160
Leucine	86	L	90–200
Lysine	57	L	150–300
Methionine	15	L	25–50
Phenylalanine	31	L	45–140
Threonine	41	L	100–250
Tryptophan	20	L	35–60
Valine	122	L	170–420
Essential Amino Acid Derivatives			
Glycine	145	L	200–450
Serine	50	L	80–200
Taurine	45	L	50–250
Tyrosine	59	L	140–360

Figure 4.30A — **Laboratory Results in Amino Acid Deficiency**

Chronic deficiency of amino acids produces low albumin and total protein with normal blood urea nitrogen (BUN-Creatinine) and concurrently lowered plasma amino acid concentrations. These markers show different aspects of the failure to supply nitrogen and essential amino acids.

Free-Form Amino Acid Supplementation

Conditions that manifest due to impaired protein synthesis, poor detoxification activity or low neurotransmitter status may improve with optimized amino acid supply. Low levels of EAAs in plasma provide the most

Example of an Individual Plasma Amino Acid Profile

Essential Amino Acids	Results		Reference Limit
Arginine	74		50–160
Histidine	72		70–140
Isoleucine	42	L	50–160
Leucine	73	L	90–200
Lysine	204		150–300
Methionine	26		25–50
Phenylalanine	42	L	45–140
Threonine	142		100–250
Tryptophan	40		35–60
Valine	141	L	170–420
Taurine	41	L	50–250

Example of an Individualized Amino Acid Supplement

Base Formula Amount: 253 gm	% of Base	Grams Added
Valine	11.1 +	11 gm
Leucine	12.9 +	14 gm
Isoleucine	9.4 +	9 gm
Phenylalanine	12.9 +	5 gm
Tryptophan	2.0 +	0 gm
Methionine	7.7 +	0 gm
Threonine	8.1 +	0 gm
Lysine	9.4 +	0 gm
Histidine	10.1 +	0 gm
Arginine	9.4 +	0 gm
Taurine	3.3 +	8 gm
Pyridoxal-5-Phosphate	.30 +	0 gm
Alpha-ketoglutaric acid	8.5 +	0 gm

Figure 4.30B — **Amino Acid Supplement Formula Based on Plasma Amino Acid Profile Results**

The laboratory results shown are used to design a customized formula of essential amino acids and cofactors to assure metabolic utilization. In order to more closely approximate the most effective individual balance, a calculation is used to adjust amounts of individual amino acids in the supplemental formula.

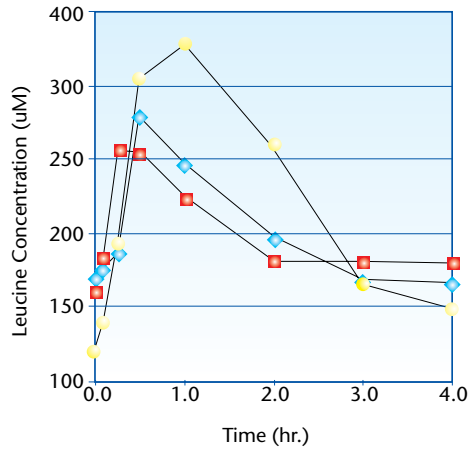


FIGURE 4.31 — Blood Spot Amino Acid Concentrations after Oral Dosing

Three healthy adult fasting males submitted blood spot specimens at the intervals shown after consuming a 10 g bolus of free-form amino acids containing 1.3 g of L-leucine. The three different symbols show an individual subject's results from the eight dried blood spot specimens collected over the 4-hour interval. Leucine concentrations were determined by quantitative LC-MS/MS analysis.

direct information regarding candidacy for amino acid therapy. Retesting after administration of an amino acid mixture allows assessment of efficacy.

There is abundant evidence from the medical literature to demonstrate a wide range of clinical responses to amino acid supplementation. Since no digestive function is needed for free-form amino acids and absorption is efficient, their assimilation is much quicker than for amino acids released from dietary protein. Oral amino acid supplementation attenuates the extent of ischemia-reperfusion injury.[540] Daily dosing of elderly adults with 18 grams of essential amino acids stimulates muscle protein anabolism.[541] The positive effect of arginine and lysine on osteoblast proliferation, activation and differentiation suggests a benefit of amino acids in prevention of osteoporosis.[542] Supplementation of a mixture of essential amino acids lowered blood sugar and reduced cataract incidence in rats with streptozotocin-induced diabetes.[295]

Symptom improvements have been reported after administration of amino acids in a large number of patients who presented with a broad range of chronic illnesses and children with developmental or behavioral problems.[550] The frequency and magnitude of favorable clinical responses to amino acid supplementation customized according to plasma levels in the manner described in this chapter was initially surprising. Since protein intake was generally near levels corresponding to nitrogen balance, the effects may be best explained by considering effects of transient elevated blood levels rather than total daily increase of amino acid intake.

An algorithm can be used to individually customize formulations based on fractional reductions of each amino acid below reference limits. Compounding pharmacies can produce the custom formulations. Figure 4.30B (top) shows an example of laboratory results from which the customized amino acid formula in Figure 4.30B (bottom) is designed. The variable (Patient Result – Low Reference Limit), is applied with appropriate multipliers to yield the values in the column labeled "Grams Added." The supplement formula is designed to yield 300 grams of blended powder. The supply normally lasts for 30 days, at the usual adult dosage of 1.5 tsp (5.0 g) twice daily, before meals. A base mixture is used as the starting point to which extra amounts of individual amino acids are added. The "% of Base" column shows the fixed composition of the base. To the 253 grams of base mixture shown in the example, additional amino acids are added in amounts shown in the "Grams Added" column. The resulting formula is enriched in those amino acids that the individual has the most difficulty sustaining. In this case the effect is to enhance primarily the branched-chain and aromatic amino acids. Critical cofactors, α-ketoglutarate and pyridoxal-5-phosphate helps to assure hepatic interconversions so that NEAAs may be formed as needed.

One explanation for the clinical improvements from such therapy is the breaking of vicious cycles formed when patients develop homeostatic set points of amino acid utilization that limit processes such as protein synthesis for formation of new healthy tissue, neurotransmitter synthesis, detoxification functions, or nuclear regulatory controls. After consumption of a 10 g oral dose of EAAs, plasma levels quickly rise, peaking at concentrations approximately twice the upper limit of fasting levels within 30 to 60 minutes (Figure 4.31). Figure 4.32 shows a hypothetical curve representing daily amino acid excursions following meals overlaid with the shorter duration supraphysiologic rises from oral doses of free-form amino acids. One hypothesis explaining the high percentage of patients reporting clinical improvements after starting such

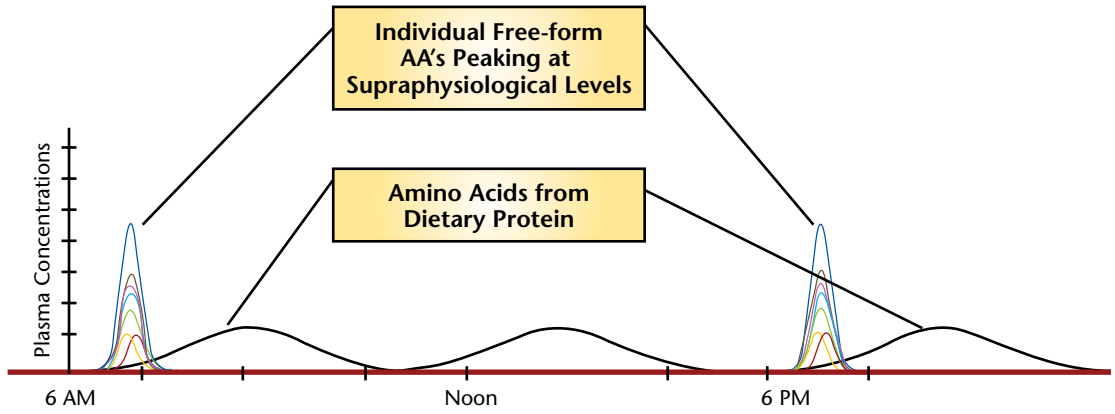


Figure 4.32 — Representation of Daily Plasma Amino Acid Levels

Schematic approximations are shown for plasma concentrations of essential amino acids from three meals of dietary protein compared with twice daily oral dosing with 5 grams of a free-form essential amino mixture. Although the supplement supplies only a fraction of total daily intake, the immediate delivery to absorptive mechanisms without need for protein digestion means that assimilation is rapid. Each amino acid (represented by colored lines) is delivered to the tissues at mild supraphysiologic concentration, allowing greater saturation of multiple biosynthetic and energetic processes.

supplementation routines is that free-form amino acids twice daily stimulate metabolism by significantly increasing plasma levels.

Cofactors

A major reason for impaired utilization of amino acids generating elevated fasting plasma levels is dietary deficiency of micronutrients such as zinc and B-complex vitamins. For example, plasma serine, threonine, glycine, alanine, tyrosine, lysine and histidine levels increase in vitamin B_{12} deficiency.[543] Restriction of flux out of plasma results from lowered utilization due to restricted vitamin B_{12} supply. Activities of the major transminase enzymes discussed under glutamine and alanine are lowered by zinc deficiency.[544] This effect tends to slow down the rate of interchange of many amino acids, producing higher levels in plasma due to lower utilization rates. Table 4.16 summarizes some specific nutrient indications from amino acid profile results.

If blood amino acids are initially in the low-normal ranges and micronutrients are added, then follow-up testing may show even lower amino acid concentrations because the rates of utilization have increased. Extra zinc and B-complex vitamins, for example, can increase the rate of protein synthesis in multiple tissues. Such responses can result in plasma amino acid concentrations being lower on follow-up testing, even though amino acids are being supplemented. Thus, multiple factors must be considered for interpretation changes seen on follow-up testing of amino acids.

Table 4.16 — Vitamin and Mineral Indications from Amino Acid Reports

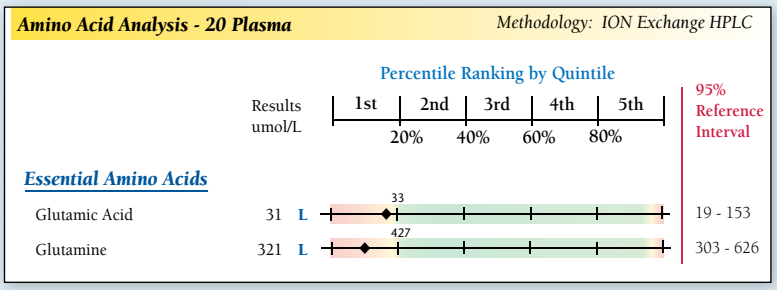
Amino Acids	Specimen	High/Low	Nutrient	Reference
Multiple High EAAs	Plasma or whole blood	High	Vitamin B_6	545, 546
His	Plasma	Low	Folic acid Zinc	14 547
Lys	Plasma	Low	Carnitine	311
Pro/HPro	Urine	High	Vitamin C	469
Sarcosine, Gly	Plasma	High	Vitamin B_{12}, Folate	548
Phe/Tyr	Plasma	High	Tetrahydrobiopterin Iron	549 322

Case Illustrations

Case Illustration 4.1 — Metabolic Fragility from Insufficient Glu & Gln

History: Four years prior to the testing date, this 30-year-old clinician had recurrent urinary tract infections for several months, treated with antibiotics. She says, "Then suddenly my body just broke down. My immune system just collapsed and I had infections everywhere: lungs, ears, nose, bladder, etc. I got allergic to everything I ate."

Current diagnosis and symptoms: Interstitial cystitis, frequent upper respiratory infections, constipation and bloating.



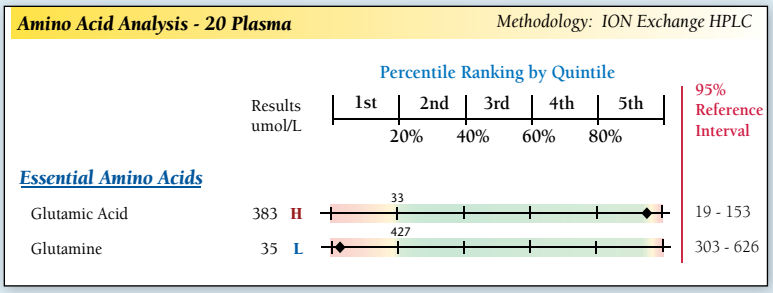
The amino acid profile was measured on a plasma specimen. The results show concurrent low Glu and Gln. Although neither amino acid is below the 95th percentile ranges, the dependent nature of this pair makes the results quite unusual. The physiological state might be described as one of metabolic fragility because of difficulty in responding to systemic pH and ammonia production shifts.

She reports that her bladder and general body pain is much worse in the morning, a time when systemic pH is under stress from cortisol-stimulated organic acid and ammonia formation.

The most common amino acid-based treatment in such cases is to use customized free-form essential amino acid mixtures formulated as described in this chapter. Such low glutamic acid and glutamine patterns are very frequently accompanied by multiple imbalances in utilization of EAA. ❖

Case Illustration 4.2 — High Glu/Gln Ratio in a Patient with Autism

A routine amino acid profile was orderd along with other metabolic testing on a severely autistic 3-year-old girl. Her plasma glutamate was very elevated, 383 µM (19–153), while glutamine was only 35 µM (303–626).



Extreme elevations of multiple organic acids were found in urine, consistent with acidemia and calcium was very high in erythrocytes, indicating difficulty maintaining the membrane calcium pumping system. Her plasma fatty acid profile showed an extremely high AA/EPA ratio that is associated with the pro-inflammatory state and her urine contained very high quinolinate and kynurenate levels, indicating interferon-γ-stimulated macrophage inflammatory response. Since her urinary p-hydroxybenzoate was very elevated, one area of suspected etiology is a severe overgrowth of bacteria or parasites producing an inflammatory response in the gut.

This case has multiple metabolic disturbances that can be affecting systemic pH and brain chemistry. Her situation is exacerbated by the loss of control over conversion of glutamate to glutamine. There is the possibility of genetic impairment of hepatic glutamine synthetase, but correction of potential dependent metabolic issues could be undertaken before attempting to confirm such diagnosis. ❖

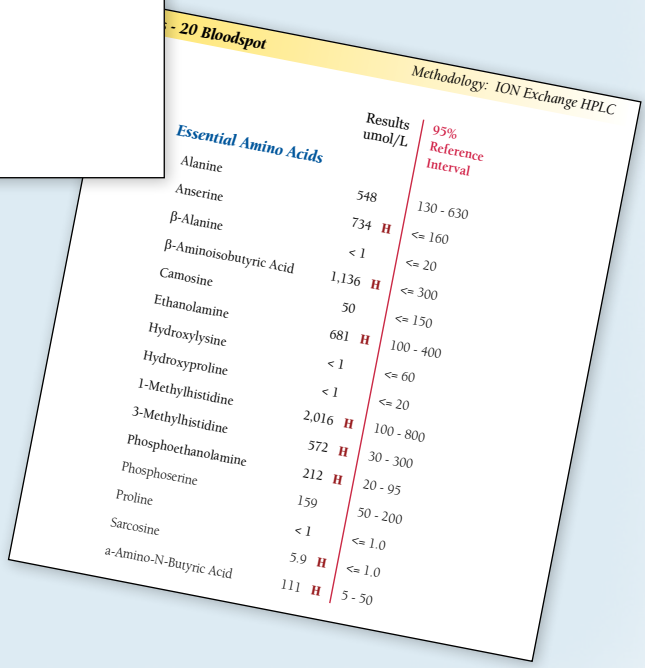
CASE ILLUSTRATION 4.3 —
Apparent Ornithine Transcarbamylase Deficiency

Amino Acid Analysis - 40 Urine — *Methodology: ION Exchange HPLC*

	Results umol/L		95% Reference Interval
Essential Amino Acids			
Arginine	21		10 - 70
Histidine	2,182	H	460 - 2,100
Isoleucine	37		10 - 70
Leucine	95		15 - 110
Lysine	179		75 - 600
Methionine	38		15 - 95
Phenylalanine	163		30 - 190
Threonine	344		90 - 490
Tryptophan	98		30 - 150
Valine	53		10 - 70
Essential Amino Acid Derivatives Neuroendocrine Metabolism			
Gamma-Aminobutyric Acid	66	H	<= 30
Glycine	1,099		500 - 2,000
Serine	376		160 - 710
Taurine	79		350 - 1,850
Tyrosine	198		40 - 290
Ammonia/Energy Metabolism			
α-Aminoadipic Acid	262	H	30 - 85
Asparagine	234		70 - 500
Aspartic Acid	556	H	135 - 275
Citrulline	15		<= 30
Glutamic Acid	58		20- 95
Glutamine	1,266	H	300- 800
Ornithine	124	H	10 - 100

This 62-year-old male exhibits manifestations of ornithine transcarbamylase (OCT) deficiency, one of the most prevalent inherited disorders. Estimates of urea cycle polymorphmism incidence, including those beyond the childhood years are as high as 1 in 20,000.

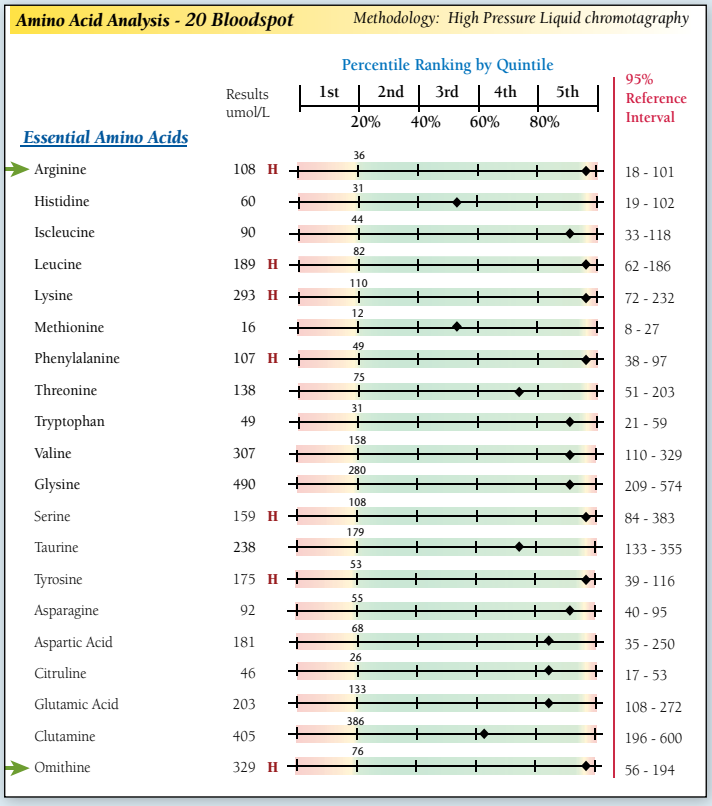
A urine amino acid pattern reveals very high ornithine with low-normal citrulline (see arrows). In addition, the amine-transporting amino acids, glutamine and aspartic acid, are extremely elevated, indicating a general metabolic acidosis with nitrogen transport impairment. These products may spill into urine to reduce the nitrogen (ammonia) load on the urea cycle (see Figure 4.9). Urinary histidine has one of the highest upper reference limits, indicating the ease of histidine loss.



The follow-up blood spot amino acid pattern supports the conclusion that this patient is exhibiting an OCT deficiency. Ornithine is extremely elevated as is the urea cycle intermediate, arginine, further indicating a metabolic backup due to lack of urea cycle activity. Many other amino acids are in their high-normal or moderately elevated ranges. The passage of histidine, glutamine and aspartic acid into urine keeps their blood levels within normal limits.

Continued on following page…

CASE ILLUSTRATION 4.3 (CONTINUED) — APPARENT ORNITHINE CARBAMOYL TRANSFERASE DEFICIENCY



Amino Acid Analysis - 20 Bloodspot — *Methodology: High Pressure Liquid chromotography*

Essential Amino Acids	Results umol/L		Percentile Ranking by Quintile (1st 2nd 3rd 4th 5th; 20% 40% 60% 80%)	95% Reference Interval
Arginine	108	H	36	18 - 101
Histidine	60		31	19 - 102
Iscleucine	90		44	33 -118
Leucine	189	H	82	62 -186
Lysine	293	H	110	72 - 232
Methionine	16		12	8 - 27
Phenylalanine	107	H	49	38 - 97
Threonine	138		75	51 - 203
Tryptophan	49		31	21 - 59
Valine	307		158	110 - 329
Glysine	490		280	209 - 574
Serine	159	H	108	84 - 383
Taurine	238		179	133 - 355
Tyrosine	175	H	53	39 - 116
Asparagine	92		55	40 - 95
Aspartic Acid	181		68	35 - 250
Citruline	46		26	17 - 53
Glutamic Acid	203		133	108 - 272
Clutamine	405		386	196 - 600
Omithine	329	H	76	56 - 194

These profiles provide evidence that blood spot amino acid analyses are well suited for monitoring amino acid status in this condition. The organic acid pattern should reveal an ammonia overload pattern. For instance, orotate should be elevated (see Chapter 6, "Organic Acids") and could be used to monitor resolution of the problem.

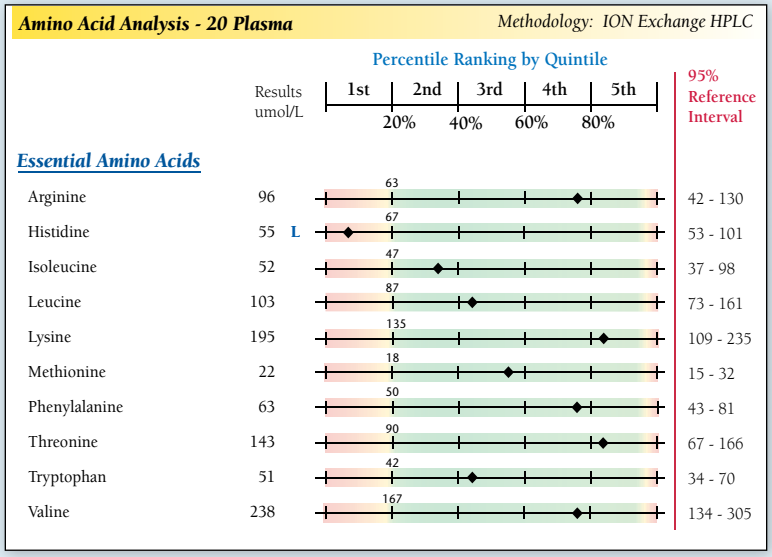
The patient may need a carefully controlled low-protein diet to reduce ammonemia and the symptoms may largely stem from ammonemia. A blood ammonia evaluation could be used to confirm. The value of supplemental arginine depends on the degree of structural impairment of the enzyme. ❖

Notes:

CASE ILLUSTRATION 4.4 —
Plasma Histidine in Major Cognitive Failure

This profile was found in a 66-year-old female with very poor short-term memory and difficulty following simple action sequences. She indicates a sense of enhanced well-being on high doses of 5-hydroxytryptophan. Her plasma amino acid profile reveals normal EAA except a for low histidine level.

Chronic stimulation of histidine conversion to histamine can lead to depleted total-body histidine. In addition to reduced histamine production capacity having effects on brain and immune responses, her hematopoietic system may be under metabolic stress due to histidine insufficiency. Low hematocrit and hemoglobin would confirm such effects. The obvious action of using supplemental histidine should be effective. As ususal, using an essential amino acid formula enhanced with histidine can prevent induced imbalances and assist histidine utilization. ❖



Amino Acid Analysis - 20 Plasma *Methodology: ION Exchange HPLC*

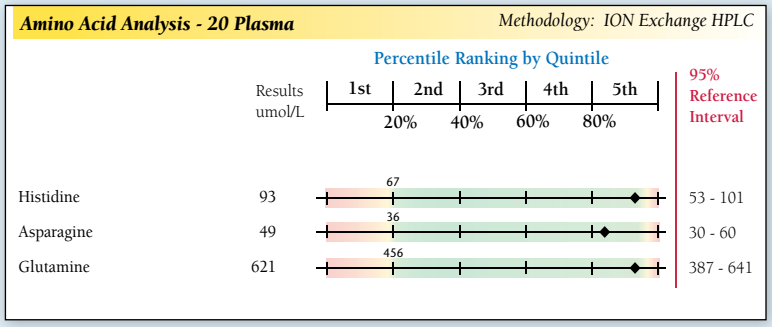
Percentile Ranking by Quintile

	Results umol/L	1st (20%)	2nd (40%)	3rd (60%)	4th (80%)	5th	95% Reference Interval
Essential Amino Acids							
Arginine	96		63				42 - 130
Histidine	55 L		67				53 - 101
Isoleucine	52		47				37 - 98
Leucine	103		87				73 - 161
Lysine	195		135				109 - 235
Methionine	22		18				15 - 32
Phenylalanine	63		50				43 - 81
Threonine	143		90				67 - 166
Tryptophan	51		42				34 - 70
Valine	238		167				134 - 305

CASE ILLUSTRATION 4.5 —
Indication of Impaired SN1-SN2 Transporter

A portion of the plasma amino acid profile report from a 15-year-old boy with severe behavioral problems and addiction to marijuana is shown.

The results of concurrent 5th quintile levels for histidine, asparagine and glutamine indicate a possible defect in the SN1-SN2 transporter system. This system is required for transport of these three amino acids away from blood in liver and brain, preventing circulating levels in fasting plasma from being elevated. ❖



Amino Acid Analysis - 20 Plasma *Methodology: ION Exchange HPLC*

Percentile Ranking by Quintile

	Results umol/L	1st (20%)	2nd (40%)	3rd (60%)	4th (80%)	5th	95% Reference Interval
Histidine	93		67				53 - 101
Asparagine	49		36				30 - 60
Glutamine	621		456				387 - 641

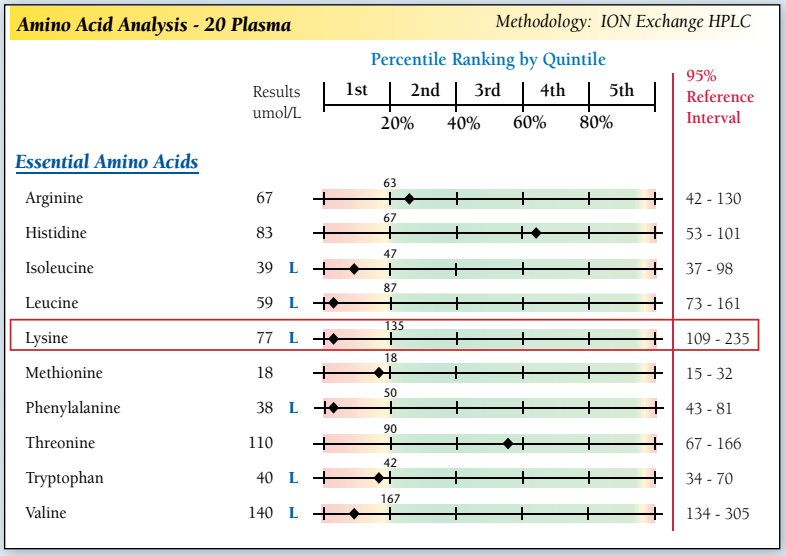
Notes:

Case Illustration 4.6 — Essential Amino Acid Support in Schizophrenia

This data is from a woman who had her first psychotic episode at 21-years-old. She presented for this metabolic profile 12 years later, exhibiting a severe form of schizophrenia. This data is presented not to explain the mental disturbance, but rather that the essential amino acid insufficiency often resulting from such disorders is identified by laboratory testing.

Her plasma amino acid profile shows very low lysine along with low values for the BCAA. A urinary organic acid profile done at the same time showed positive biochemical markers for carnitine insufficiency.

The results justify essential amino acid therapy with a custom formula that enhances lysine and BCAA levels. Initial use of carnitine can help to assure metabolic demand for energy production from fatty acid oxidation is met while lysine status is enhanced gradually by twice daily dosing of free-form amino acids. ❖



Amino Acid Analysis - 20 Plasma — Methodology: ION Exchange HPLC

Percentile Ranking by Quintile

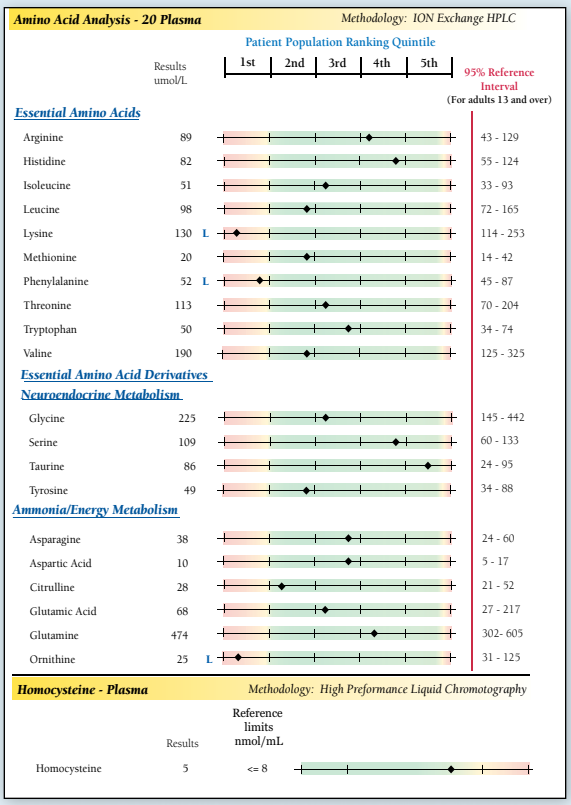
	Results umol/L		1st (20%)	2nd (40%)	3rd (60%)	4th (80%)	5th	95% Reference Interval
Essential Amino Acids								
Arginine	67		63					42 - 130
Histidine	83		67					53 - 101
Isoleucine	39	L	47					37 - 98
Leucine	59	L	87					73 - 161
Lysine	77	L	135					109 - 235
Methionine	18		18					15 - 32
Phenylalanine	38	L	50					43 - 81
Threonine	110		90					67 - 166
Tryptophan	40	L	42					34 - 70
Valine	140	L	167					134 - 305

Case Illustration 4.7 — Anxiety Correction by Lysine

This patient presented with various physical complaints and significant anxiety and sleep disturbance. Test Results: Although most of the essential amino acids were in healthy range, lysine was very low, along with 1st quintile phenylalanine.

Recommendations: In cases of anxiety, sleep disturbance, or irritable bowel, consider lysine deficiency as a possible moderator for serotonin hypersensitivity. Lysine at several hundred mg/d is the normal dosing.

Comments: In follow-up discussions the testing physician indicated a remarkable, immediate reduction in severity and frequency of anxiety after supplementing lysine and phenylalanine. ❖



Amino Acid Analysis - 20 Plasma — Methodology: ION Exchange HPLC

Patient Population Ranking Quintile

	Results umol/L		1st	2nd	3rd	4th	5th	95% Reference Interval (For adults 13 and over)
Essential Amino Acids								
Arginine	89							43 - 129
Histidine	82							55 - 124
Isoleucine	51							33 - 93
Leucine	98							72 - 165
Lysine	130	L						114 - 253
Methionine	20							14 - 42
Phenylalanine	52	L						45 - 87
Threonine	113							70 - 204
Tryptophan	50							34 - 74
Valine	190							125 - 325
Essential Amino Acid Derivatives								
Neuroendocrine Metabolism								
Glycine	225							145 - 442
Serine	109							60 - 133
Taurine	86							24 - 95
Tyrosine	49							34 - 88
Ammonia/Energy Metabolism								
Asparagine	38							24 - 60
Aspartic Acid	10							5 - 17
Citrulline	28							21 - 52
Glutamic Acid	68							27 - 217
Glutamine	474							302- 605
Ornithine	25	L						31 - 125

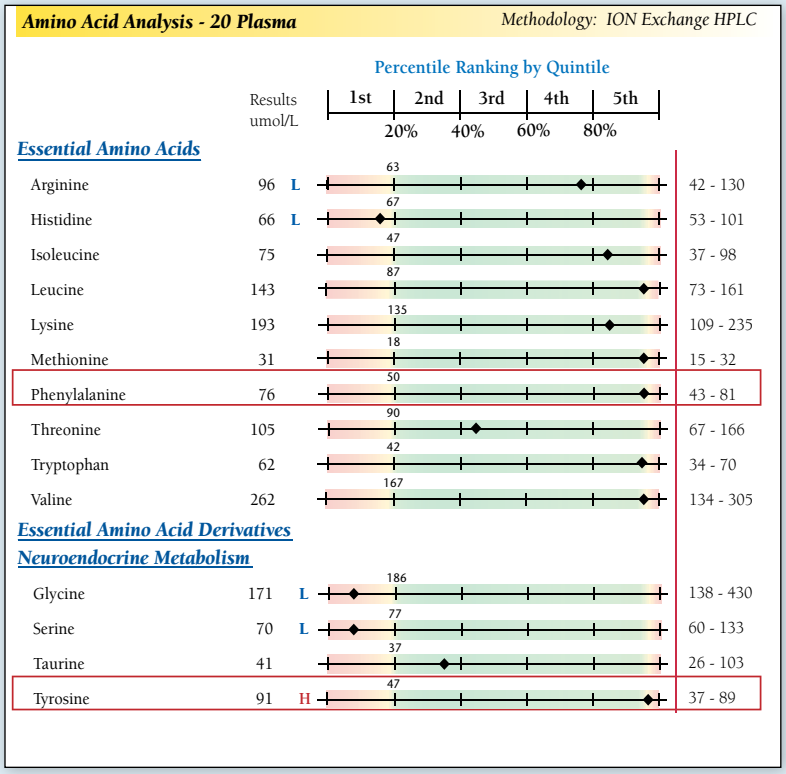
Homocysteine - Plasma — Methodology: High Preformance Liquid Chromotography

	Results	Reference limits nmol/mL
Homocysteine	5	<= 8

CASE ILLUSTRATION 4.8 —
Tyrosine Utilization Cofactors in Bronchospasms

Plasma amino acid analysis revealed elevated tyrosine in a man suffering from adult-onset, cold-induced bronchospams. The cofactors converting tyrosine to epinephrine, a bronchodilator, include vitamin C and copper, among others. The patient tested low for copper.

When copper was added to his nutrient supplementation regimen, the bronchospams ceased to occur, giving clinical evidence that tyrosine metabolism to catecholamines had been restored. ❖



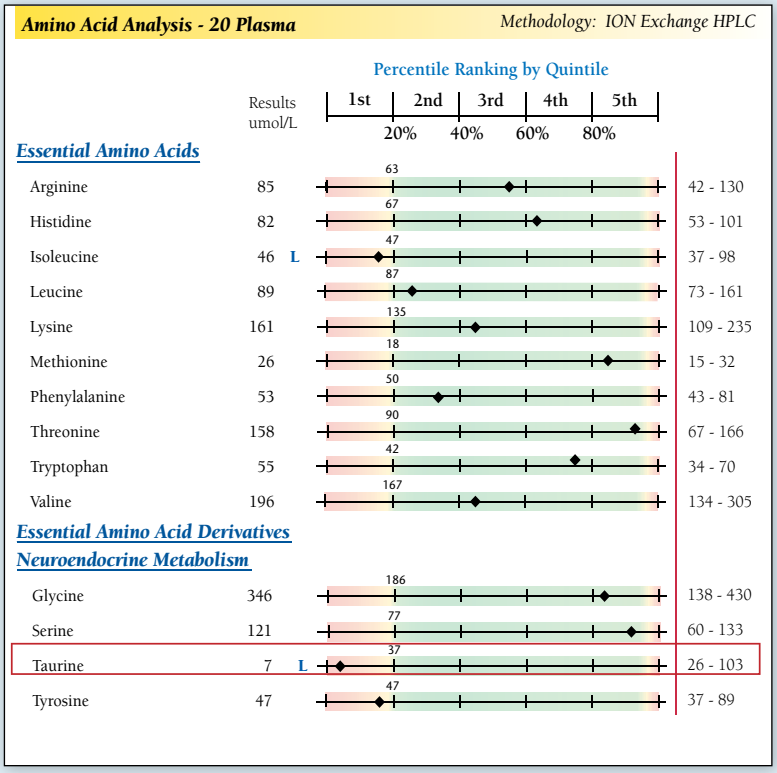
Notes:

CASE ILLUSTRATION 4.9 — APPARENT DIET-INDUCED TAURINE DEFICIENCY WITH CARDIOVASCULAR CONSEQUENCES

A 59-year-old female physician who had been vegetarian for several years presented with hypertension and paroxysmal supraventricular tachycardia. She had started taking the usual multiple supplemental nutrients with little effect.

The plasma amino acid laboratory report revealed extremely low taurine in the presence of upper-normal methionine, indicating a problem with the pathway from homocysteine to taurine. The multiple amino acid levels in the 5th quintile suggest an insufficiency of vitamin B_6, possibly exacerbated by iron deficiency that is common in vegetarians.

Further testing can help identify these deficiencies. If correction of micronutrient insufficiencies does not resolve the low taurine, genetic impairment may be present, requiring sustained taurine supplementation. ❖



Notes:

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Chapter 5

Fatty Acids

Richard S. Lord and J. Alexander Bralley

CONTENTS

Notes:

TABLE 5.1 — SUMMARY OF FATTY ACID ABNORMALITIES

No.	*	Name		Potential Responses	Metabolic Association
Omega-3 Polyunsaturated					
1	18:3	Alpha linolenic (ALA)	L	Flax and/or fish oils	Essential fatty acid
2	20:5	Eicosapentaenoic (EPA)	L	Fish oils	Eicosanoid substrate
3	22:5	Docosapentaenoic	L	Fish oils	Nerve membrane function
4	22:6	Docosahexaenoic (DHA)	L	Fish oils	Neurological development
Omega-6 Polyunsaturated					
5	18:2	Linoleic (LA)	L	Sunflower oil, seeds	Essential fatty acid
6	18:3	Gamma linolenic (GLA)	L	GLA source**	Eicosanoid precursor
7	20:2	Eicosadienoic	H	Zinc, 50 mg/d; B_6, 50 mg/d; iron, 15 mg/d	Desaturase inhibition
8	20:3	Dihomogammalinolenic (DGLA)	L	Zinc, 50 mg/d; B_6, 50 mg/d; iron, 15 mg/d	Eicosanoid substrate
9	20:4	Arachidonic (AA)	L	Corn	Eicosanoid substrate
			H	Reduce red meats	Pro-inflammatory responses
10	22:2	Docosadienoic	H	Copper citrate, 6 mg	Copper deficiency
11	22:4	Docosatetraenoic	H	Glycemic control	Increase in adipose tissue
Omega-9 Polyunsaturated					
12	18:3	Mead acid	H	High PUFA oils	Essential fatty acid deficiency
Monounsaturated					
13	12:1	Vaccenic	L	Biotin, 500 µg BID	Biotin deficiency
14	14:1	Myristoleic	H	High PUFA oils	General EFA deficiency
15	16:1	Palmitoleic	H	High PUFA oils	Essential fatty acid deficiency
16	18:1	Oleic	L	Olive oil	Membrane fluidity
17	20:1	11-Eicosenoic (Gondoic)	H	High PUFA oils	
18	22:1	Nervonic	L	Fish or canola oils	Neurological development
19	24:1	Erucic	L	High erucate seed oils (canola)	Nerve membrane function
Saturated					
20	10:0	Capric Acid	H	Riboflavin, 50 mg TID	MAD^^
21	12:0	Lauric	H	Riboflavin, 50 mg TID	MAD^^
22	14:0	Myristic	H	Riboflavin, 50 mg TID	MAD^^
23	16:0	Palmitic	H	Reduce saturated fats; add niacin	Cholesterogenic
24	18:0	Stearic	H	Reduce saturated fats; add niacin	Elevated triglycerides
			L		Cancer marker ratio (see below)
25	20:0	Arachidic	H	Check eicosanoid ratios	Δ^6 desaturase inhibition
26	22:0	Behenic	H	Peroxisomal insufficiency	Elongation stimulation
27	24:0	Lignoceric	H	Consider rape or mustard oils	Nerve membrane function
28	26:0	Hexacosanoic	H	Peroxisomal insufficiency	Elongation stimulation
Odd-numbered					
29	15:0	Pentadecanoic	H	Biotin, 1000 µg TID; Vitamin B_{12}, 1000 µg TID; Carnitine, 500 mg TID; Dysbiosis	Propionate accumulation
30	17:0	Heptadecanoic	H		
31	19:0	Nonadecanoic	H		
32	21:0	Heneicosanoic	H		
33	23:0	Tricosanoic	H		

Table 5.1 continued on following page...

Table 5.1 continued from previous page...					
No.	*****	**Name**		**Potential Responses**	**Metabolic Association**
***Trans*-fatty acids**					
34	16:1t	Palmitelaidic	H	Eliminate hydrogenated oils	Eicosanoid interference
35	18:1t	Elaidic	H	Eliminate hydrogenated oils	Eicosanoid interference
Ratios and Indexes					
36		LA / DGLA	H	GLA source**	Δ^6 desaturase enzyme
37		DGLA/ EPA	L	Black currant oils	Eicosanoid imbalance
			H	Fish oil	Eicosanoid imbalance
38		AA/EPA	H	Fish oils	Inflammatory marker
39		Triene/Tetraene	H	Essential oils	Essential fatty acid deficiency
40		Vaccenic/Palmitoleic	L	Biotin	Elongase – desaturase inhibition
41		Stearic/Oleic (rbc)	L	Cancer therapeutic efficacy	Metabolic effect of malignancy
42		Total fatty acids (plasma)	H	Evaluate hyperlipidemias	Serum triglyceride (VLDL)

** Number of carbons: Number of double bonds. Main grouping by degree of desaturation is used, with members within each group ordered by chain length. The abnormality pattern is shown as L or H for levels below or above reference limits, and potential clinical responses to correct the abnormalities are shown as adult daily dosages. ** Sources of GLA: Evening primrose oil or black currant oil. ^^ Multiple Acyl-coenzyme A Dehydrogenation disorders.*

Introduction

Over the past few decades the relationship between dietary fat and disease has been the subject of much controversy and confusion. Recognition of the importance of specific physiological and toxicological roles for the individual fatty acids that largely constitute dietary fat has been a significant advance. Health issues associated with fatty acids are largely traced to modern dietary habits of low intake for fish, vegetables, whole grains, nuts, and seeds and high intake for processed and manufactured dietary fats. Long-term restriction of total dietary fat exacerbates essential fatty acid (EFA) insufficiencies and has been related to several disease conditions, though excess animal fats have been strongly correlated to increases in chronic diseases. Fatty acid levels are determined primarily by dietary intake and genetics, though estrogen levels and parity are factors that contribute to EFA status among women[1, 2], and variations in testosterone levels have a significant influence on fatty acid status in men.[3, 4] In clinical practice the need for laboratory evaluations of fatty acid status is helpful for making decisions about dietary modification and/or supplementation with EFAs and/or other nutrients involved in fatty acid metabolism.

Common abbreviations used in discussing fatty acids that will be used throughout this chapter are found in the list following the index in this book.

Fatty Acid Status and Disease

Fatty acids and their metabolic effects have been implicated in the pathogenesis of many diseases, including obesity, diabetes, cancer, heart disease, genetic diseases such as cystic fibrosis, and autoimmune disorders such as rheumatoid arthritis and multiple sclerosis.[5-19] Attempting to determine the relationship of dietary fatty acids and cardiovascular disease has been difficult, and further complicated by the synergistic effect of obesity and diabetes.[20-23] The specific effect of dietary fatty acids on obesity has also been long-debated.[24-32] These three disorders have been proposed to be related through chronic inflammation that can be modulated by intake of EFAs.[33-35]

Dietary fat intake has also been found to be related to several forms of cancer, though its relationship is complicated by genetic factors and micronutrient status.[36, 37] Insufficiencies of EFAs were found to increase morbidity in recovering cancer patients.[38] Low serum triglyceride levels may be a general feature in many autoimmune disorders,[39] and may explain why supplementation of EFA has been shown to be effective in decreasing symptoms.[40, 41]

The nervous system, due to its high fatty acid content and the critical dependence of nerve signal conduction on membrane status, is highly susceptible to pathologies of fatty acid deficiency. Lack of EFAs may contribute to conditions such as Alzheimer's disease (AD)

and seizure disorders. The major pathological feature of AD, increased deposition of amyloid plaques, is preceded by increased lipid peroxidation in animal models.[42] Oxidation of neuronal membrane polyunsaturated fatty acids (PUFA) can create products that can lead to neurodegeneration and may serve as biomarkers of AD in the future.[43, 44] Platelet membrane fluidity has been proposed as an adjunct marker of in vivo AD diagnosis.[45] Membrane fatty acid composition affects the function of neurons by changing membrane fluidity and function, by altering local signaling by means of eicosanoid/docosanoid synthesis, or by altering gene expression/transcription by means of PPARs.[46,19, 47]

Membrane Fluidity Changes

Multiple clinical effects of fatty acids are mediated, at least in part, by means of membrane fluidity changes.[48] In biological membranes, lipids and many membrane proteins within the bilayer are constantly in motion. Although bacteria regulate the fluidity of their membranes by varying the number of double bonds and the length of their fatty acyl chains, cholesterol is an additional key regulator of membrane fluidity in animals.[49] Pathogeneses of several diseases, such as, cancer, blindness and numerous behavioral disorders have been shown to share membrane fluidity components that mediate the activity of protein receptor complexes within cell membranes.

In the brain, synaptic transmission requires the fusion of pre-synaptic vesicles with cell membranes for exocytosis of neurotransmitters. Membrane fusion is affected by fluidity. In this process vesicular fatty acids become recycled into the cell membrane for re-extraction.[50] It is not suprising then that a deficiency of n-3 fatty acids was found to alter the release of neurotransmitters in the rat brain. Animal studies have also found that rats fed *cis*-alpha linolenic acid (ALA), compared to those fed *trans*- corrected dopamine concentrations in the hippocampus.[51] These changes may be due to the known lowering of membrane fluidity by trans fatty acid intake.

Mitochondrial membranes are especially susceptible to oxidative damage because of their proximity to the intense oxygen-radical activity of the electron transport system. Age associated mitochondrial membrane changes include increases in membrane rigidity, cholesterol, phosphatidylcholine, n-6 fatty acids, and 4-hydroxy-2-nonenal, and decreases in n-3 fatty acids and cardiolipin. By means of such mechanisms, fatty acid status determines responses to stimuli; mood; ability to remember, make decisions, and perform calculations; and the coordination of motor function.

Membrane receptors are less exposed to the external environment as membrane fluidity increases.[45] Short-term (4-week) dietary supplementation of fish oils altered macrophage membrane fluidity, and decreased TNF binding affinity, GTPase activity, and cAMP production. After a 4-week period, fluidity was lowest and highest in animals fed corn and fish oil, respectively. After 8 weeks, there was a general enhancement of cAMP production, and further differences became apparent.[47] These data show the influence of membrane fatty acid composition on the function of the all-important class of membrane-resident receptors that modulate neuronal activity.

Fatty Acid Structure and Metabolism

Michel Eugène Chevreul (1786–1889), a French chemist, discovered the first fatty acid while studying potassium soap made from pig fat. He named several fatty acids, including oleic, butyric, caproic and capric acids, and stearic acid. Chevreul also demonstrated that all fatty acids are composed of a carboxylic acid (-COOH) with a long carbon tail (4–24 carbon atoms in length). Additional members of the fatty acid family, including those that make up human lipids, were gradually identified over the 100 years following Chevreul's death. With advancements in chromatographic resolution, additional names and structures were added to this list of closely related compounds.

There are about 40 physiologically significant fatty acids, not including derivatives such as hydroxy- and branched fatty acids. Compared with other classes of molecules in living cells, fatty acids have simple structures and the variations among them are relatively small. Those slight structural differences, however, can cause profoundly different effects on the function of cells. For example, one 20-carbon fatty acid, arachidonic (AA), can increase the risk of heart disease, whereas another, eicosapentaenoic (EPA), which contains a similar straight chain of 20 carbon atoms, can help prevent it. The structural difference consists in the simple removal of a few atoms of hydrogen. The cellular effects arise from

the vast quantity of fatty acid in the membranes that surround the cells and subcellular compartments. The nature of those membranes governs the flow of energy and information that impacts the functions of cells and the tissues in which they are comprised. Subtle differences in bond positions can change this flow of energy and information in a way that determines the progression of major diseases, defines thresholds to pain, and directs responses of the immune system.

Naming Fatty Acids

The majority of biologically significant fatty acids have been given common names that are based upon the individual who discovered the fatty acid (e.g., Mead, Osbond) or a common food source of the fatty acid (e.g., oleic in olive oil, vaccenic from the Latin *vacca* for cow, as it is found in milk fat).

To more precisely describe fatty acids, naming conventions have evolved based upon three key features of fatty acid structure—the number of carbon atoms, the number of double bonds, and the location of the double bonds. One such system uses Greek number descriptors whereby each name begins with the number of carbon atoms (e.g., octadeca = eighteen) followed by the number of double bonds (e.g., tri = three). Hence octadecatrienoic acid describes more precisely a fatty acid that has eighteen carbon atoms and three double bonds. To further distinguish between fatty acids that have that structure, numbers that describe the positions of the double bonds are prefixed to the Greek descriptors. For example, counting atoms from the carboxyl end of the fatty acid molecule, ALA has double bonds at positions 9, 12, and 15, and it is described as 9, 12, 15-octadecatrienoic acid. Gamma linolenic (GLA) is also an eighteen-carbon fatty acid with three double bonds, yet its double bonds are at the 6, 9, and 12 positions, so its scientific descriptor is 6, 9, 12-octadecatrieonic acid.

This system has been simplified by the substitution of Arabic numerals for the Greek syllables. Hence octadecatrieonic acid becomes 18:3. The positions of the double bonds are designated by the delta system, whereby the Greek letter Δ is superscripted with the numbers that specify the position of the double bonds, again counting the atoms from the carboxyl end of the molecule. Hence, ALA is written $18{:}3\Delta^{9,\,12,\,15}$ and GLA is $18{:}3\Delta^{6,\,9,\,12}$.

An even simpler naming system—the omega system—identifies the position of the double bonds by counting the atoms from the methyl end of the molecule. Since, in humans, three carbon atoms always separate fatty acid double bonds, knowing the position of the first gives the position of all the others. This naming system uses the Arabic numerals to designate the number of carbon atoms and double bonds (e.g., 18:3) followed by ω, w or n (these are synonymous) followed by the position of the first double bond from the methyl end of the molecule. Hence, ALA is 18:3n3 and GLA is 18:3n6. The specificity and simplicity of this system has made it the most often used fatty acid scientific nomenclature in recent years. The common names are still frequently used when the specific descriptors are not necessary. Families of structurally related, straight-chain fatty acids are defined according to the number of carbons in the chain, such as "short chain" (fewer than

Notes:

10 atoms), "medium chain" (10 to 14 atoms), "long chain" (16 to 20 atoms), and "very-long chain" (20 or more atoms). These families may be abbreviated as SCFA, MCFA, LCFA and VLCFA, respectively.

GROUPING BASED ON DOUBLE BONDS

Using the omega system, the polyunsaturates are grouped according to the position of the first double bond. Hence the omega-3 fatty acids (n-3 fatty acids) share the structural characteristics of having the first double bond begin at the third atom from the methyl end of the molecule; omega-6 (n-6 fatty acid) at the sixth atom; and omega-9 (n-9 fatty acid) at the ninth atom. The monounsaturates have only one double bond. Saturated fatty acids have no double bonds; their carbon atoms are in straight chains and, as a result, can be packed together very tightly, allowing living organisms to store chemical energy very densely. Odd-numbered fatty acids are also saturated. Figure 5.1 reviews the naming conventions of unsaturated fatty acids.

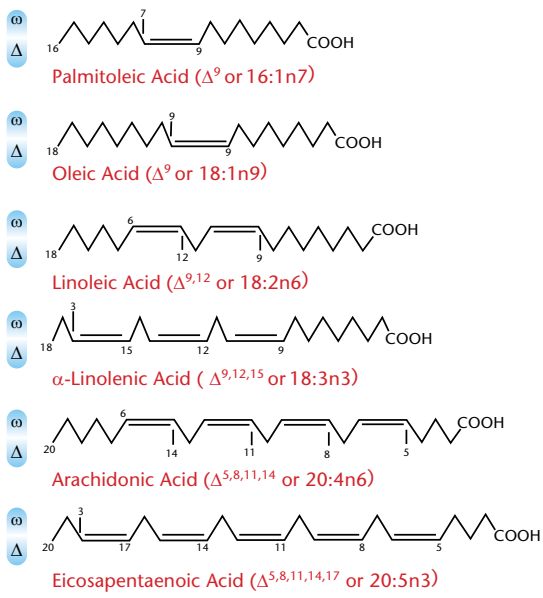

FIGURE 5.1 — **Naming Conventions for Unsaturated Fatty Acids**

The omega (ω or n) and delta (Δ) schemes are shown. The small numbers adjacent to the structures label the double bond positions in the carbon chains counting from the methyl (ω or n) and carboxy (Δ) ends, respectively. Common names and Δ-nomenclature shorthand designations are shown below each fatty acid structure.

In most naturally occurring fatty acids the carbon atoms on either side of a double bond are on the same side of the molecule (*cis*-geometry). Trans fatty acids represent a special group of molecules wherein the carbon atoms are on opposite sides of the molecule (*trans*-geometry) . Most of these are not found in nature and result from the man-made process of chemical hydrogenation of plant-based oils. This causes their shape and function to be similar to the straight-chained saturated fatty acids. The omega nomenclature system uses a "t" to designate a trans fat. Hence, 18:1t-n7 indicates that the single double bond at the omega-7 position is in the *trans*- configuration. This is the formula for elaidic acid, the most abundant *trans*-fatty acid in most dietary hydrogenated oils.

CONTROL OF FATTY ACID SUPPLY AND DISTRIBUTION

Fatty acids are present in the diet in the form of triglycerides in solid fats or liquid oils, and as phosphatides in the cell membranes of whole foods. They are rarely present in nature in the form of free fatty acids.

Bile acids carry out the first phase in digestion of dietary fat by causing the dispersal of particles of fatty tissue or dietary oils into fine droplets. Lipase enzymes then break the ester bonds releasing glycerol and free fatty acids that pass into intestinal epithelial cells along with cholesterol. The enterocytes re-esterify them, forming triglycerides that are associated with proteins to form chylomicrons (see Figure 5.2). The chylomicrons are passed into the lymphatic system to flow directly through the heart into systemic circulation without action by hepatic enzymes. Enzymes present in capillary cell walls cause the transfer of fatty acids from the chylomicrons, which, after undergoing a great reduction in size, return for uptake by the liver as chylomicron remnants.

Because of their critical life-support function in forming cell membranes and in supplying energy sources and hormone controls, there are mechanisms for assuring that the supply of fatty acids will be continuous, even during short intervals between meals. In the fasting state, fatty acids are mobilized from adipose stores or are produced from either carbohydrate or protein. The liver handles the role of supply depot by forming the class of lipid-protein particles called VLDL. These particles deliver fatty acids and cholesterol to the tissues by mechanisms similar to those for chylomicrons, except

that the entire remnant of LDL particles is taken up by binding to receptor sites on extrahepatic cell surfaces. By ensuring a constant supply of fatty acids, the body controls material that is most critical in carrying out the various life-sustaining functions of growth and repair. In pregnant women, for example, maternal red cells may serve as a reservoir of AA and DHA (docosahexaenoic) for utilization by the developing fetus.[52]

Though a small but significant fraction of fatty acids are present in plasma in the unesterified, or free form, bound to albumin, the majority of fatty acids are in the esterified form in lipoproteins. When fatty acid profiles are measured on plasma specimens, the results primarily reflect the fatty acid composition of lipoproteins. Since serum triglyceride measurements also reflect the total lipoprotein triglyceride content, there is a significant

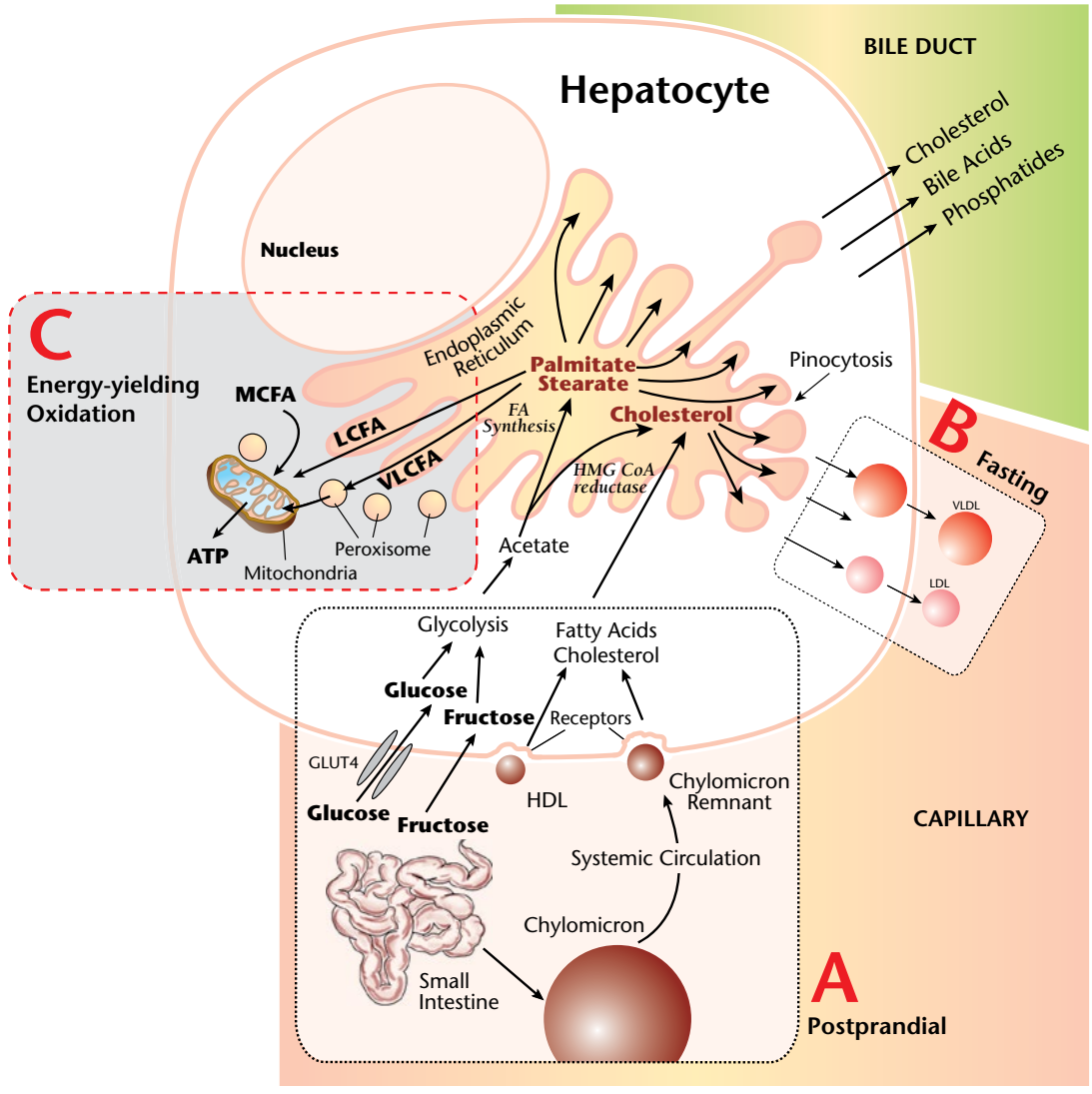

FIGURE 5.2 — **Hepatocyte Regulation of Blood Lipids**

In the postprandial state (A), fatty acids are cleared from blood by hepatocyte receptors that bind chylomicron remnants. Simple sugars (glucose, fructose) are simultaneously cleared, supplying carbon for fatty acid and cholesterol synthesis. During the fasting state (B), blood levels of lipoproteins are sustained by fatty acid synthesis and export from the endoplasmic reticulum. Meanwhile, the hepatocyte derives energy from peroxisomal and mitochondrial oxidation of fatty acids (C). Before entering mitochondria, very long chain fatty acids first undergo chain-shortening in peroxisomes or via the mitochondrial inner membrane cycle depicted in Figure 5.4.

relationship between triglyceride and total plasma fatty acid levels. The primary endogenously synthesized fatty acid, palmitic acid, is most strongly associated with serum triglycerides, as shown in Figure 5.3. From the regression line, the following formula can be constructed for estimating serum triglyceride values from plasma palmitate:

Triglycerides (serum) level =
(Palmitate (plasma) level x 0.12) + 145

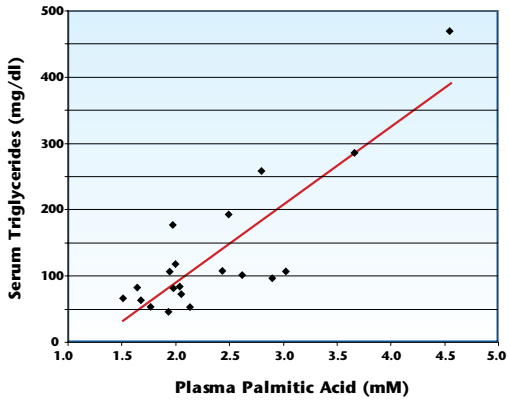

FIGURE 5.3 — **The Serum Triglyceride—Plasma Palmitate Relationship**

Hypertriglyceridemia is related to endogenous fatty acid synthesis and clearance. As the principal product of the fatty acid biosynthetic pathway, palmitate represents endogenous synthesis, and the level of palmitate in plasma reflects serum triglyceride levels. The trend line shows a strong linear relationship.

FATTY ACID METABOLISM

The center of total body fatty acid regulation is the liver, where the balance of fatty acid import, export and biosynthesis is constantly shifting as the cycle of eating and fasting causes the rising and falling of dietary lipid levels in portal blood. In order to assure constant delivery of blood lipids and to take advantage of energy surplus, activities of enzymes for fatty acid oxidation or synthesis are alternately stimulated and inhibited to accommodate the shifting demands. Fatty acid metabolism involves free fatty acid interconversions, membrane phospholipid dynamics, and multiple organelle activities that are under complex control by nuclear regulatory sites. Rates of protein synthesis are adjusted by nuclear regulatory factors resulting in constant variation in the number of organelles available for processing fatty acids. Specific steps in fatty acid metabolism occur in the endoplasmic reticulum (ER), whereas others require movement of fatty acids into microsomes or peroxisomes. In many tissues, the cellular contents of both of the ER and the peroxisomes are highly variable. In response to changes in demand for fatty acid metabolism, hepatic ER can double in surface area within a few days.[53] Peroxisomal metabolism is of such importance that it constitutes a separate field of study in which concepts of nuclear regulation must be invoked in order to explain the effects. Factors involved in the proliferation of peroxisomes are discussed below.

Transport and Oxidation

Fatty acids that arrive at tissue sites in blood lipoproteins or as free fatty acids bound to albumin must first be transported across cell and mitochondrial membranes before they can enter the sequence of reactions that produces ATP, with oxidation of the fatty acid structures to carbon dioxide and water. Cell membrane-bound transport proteins, along with carnitine, are required to move long-chain fatty acids into the cell, whereas medium-chain fatty acids may diffuse to cell interiors (Figure 5.4). Both medium- and long-chain fatty acids must be esterified to coenzyme A before mitochondrial entry can occur. Before entering the primary spiral oxidation pathway, long-chain fatty acids are shortened to lengths below C14 by inner membrane-resident enzymes that bind only the long-chain fatty acyl carnitine esters. Once shortened, the fatty acids are passed to the final degradation pathway that ends when the final four carbon atoms are split into two acetyl-CoA molecules, ready for citric acid cycle processing.

This chain length dependent metabolic system leads to multiple molecular origins of clinical symptoms deriving from failure to oxidize fatty acids. Disruptions tend to produce symptoms such as excessive fatigue on exertion, weakness, or myoglobinuria. Carnitine insufficiency affects all families of fatty acids, generating energy deficits affecting all organs, though symptoms tend to appear in the energy-demanding skeletal muscles. A childhood form called myopathic carnitine deficiency produces generalized limb weakness, sometimes involving limb, trunk, and facial muscles.[54] Medium-chain acyl transferase deficiency diseases are discussed in the section on genetic disorders in this chapter. Polymorphism

in the long-chain fatty acid transport proteins causes interference with this specific class. These patients are managed with low-fat diets supplemented with medium-chain triglycerides to bypass the transporter requirement.[55] Finally, the very-long-chain fatty acids are handled by a third set of transformations involving peroxisomal enzymes as discussed later in this chapter.

Genetic effects manifesting in that pathway produce another type of fatty acid oxidation difficulty.

Elongation and Desaturation

Fatty acids can be modified by desaturation enzymes that introduce double bonds, and are lengthened by elongation reactions that add 2-carbon units to the

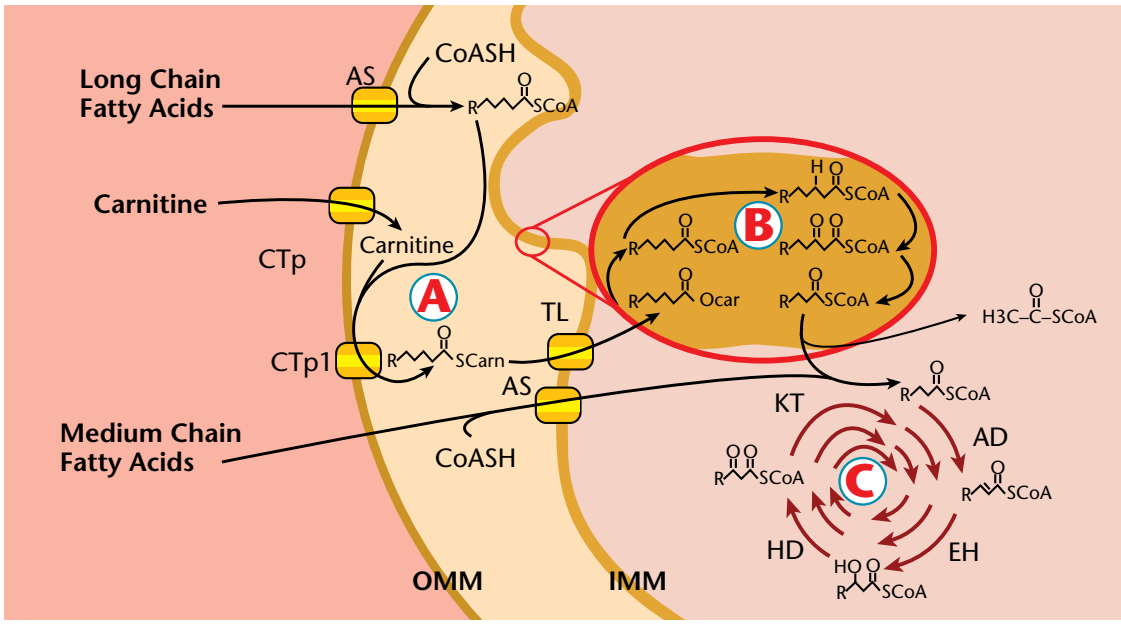

FIGURE 5.4 — **Intracellular Fatty Acid Transport and Oxidation**

Plasma carnitine gains entry to cells via a specific carnitine transport protein (CTp). The carnitine cycle **(A)** that operates in the *trans*-membrane space causes formation and breakage of fatty acyl carnitine. Acylcarnitine synthetase (AS) catalyzes the attachment of coenzyme A to the fatty acid. Then the translocase enzyme (TL) acts as a gate keeper for entry of LCFAs passing them to enzymes attached to the inner mitochondrial membrane. There, the inner membrane cycle **(B)** carries out two-carbon chain length reductions, producing acetyl-CoA with each chain-length reduction. When the chain length drops below 14, the fatty acyl-CoA product enters the beta oxidation cycle **(C)** carried out by acyl dehydrogenase (AD), enoyl hydratase (EH), hydroxyacyl dehydrogenase (HD) and ketoacyl thiolase (KT) enzymes. The riboflavin-dependent step is carried out by the FAD-requiring enzyme, AD. Medium chain fatty acids do not require the carnitine cycle since their chain length allows them to enter the b-oxidation cycle directly as acyl-CoA esters.

Fatty acid Transport: Activation of long-chain fatty acids and their transport into the transmembrane space is done via long-chain acyl-CoA synthetase (AS) located in the outer mitochondrial membrane (OMM).[290] Once inside the transmembrane space they cannot readily traverse the inner mitochondrial membrane and must be coupled to carnitine. The carnitine cycle **(A)** causes formation and breakage of fatty acyl carnitine. Carnitine acylcarnitine translocase (TL) shuttles the acylcarnitine through the inner mitochondrial membrane (IMM), where enzymes located on the matrix side of the membrane couple the acyl moiety to carnitine and regenerates acyl-CoA. Medium-chain fatty acids do not require such transport for mitochondrial import. They are attached to CoA and passed directly into the mitochondrial matrix via medium-chain acyl-CoA synthetases (AS).[291]

Fatty acid Oxidation: (B) Long-chain fatty acid oxidation takes place in the inner membrane-bound complex, and carries out two-carbon chain length reductions, producing acetyl-CoA with each chain-length reduction. When the chain length drops below 14, the acyl-CoAs are further oxidized by the specific enzymes in the mitochondrial matrix system. (C) Medium- and short-chain fatty acids are degradaded in the matrix system.[292-294] β-Oxidation degrades fatty acids completely to acetyl-CoA, which is then oxidized by the citric acid cycle or, during starvation, condensed into ketone bodies.

carboxyl end. Alternating desaturation and elongation sequences produce families of products derived from a common precursor. Linoleic acid (LA) (18:2n6) is desaturated to form GLA (18:3n6), which may then be lengthened (elongated) by two carbon atoms to form DGLA (20:3n6). DGLA can then be further desaturated to form AA (20:4n6) (see Figure 5.5). The n-6 and n-3 classes of fatty acids must be provided from dietary PUFAs as precursors for desaturation and elongation sequences. ALA (C18:3n3) and LA (C18:2n6) are considered dietary EFAs.

Members of the PUFA classes cannot be interchanged because the position of the double bond that defines them, n-3 or n-6, cannot be modified by human enzymes. The desaturation and elongation reactions can be repeated in various combinations, giving an array of saturated and unsaturated fatty acids for use in the essential functions of tissue maintenance. The sequence of reactions used to convert LA into AA is shown in Figure 5.5.

The enzymes used in saturated fatty acid biosynthesis and those used in their subsequent elongations are similar in their activities. Fatty acid elongation enzymes are membrane bound, localized on the outer surface of the endoplasmic reticulum. In elongation steps, two carbon atoms are introduced at the carboxyl end of a fatty acid by the condensation of a fatty acyl-CoA with acetyl or malonyl-CoA. The product is a β-keto chain that is longer by two carbon atoms. A three-step process of reduction and dehydration reactions converts the keto group to a methylene (-CH2-) group to yield the final elongated product. Yeast mutants lacking the elongase system gene have reduced VLCFA (very long chain fatty acid) synthesis along with the accumulation of medium-chain membrane phospholipid classes.[56]

Notes:

The 9-carbon-atom maximum span from carboxyl group to the position of insertion of a double bond is achieved by Δ^9 desaturase (Δ^9d). Counting from the other end, the position varies, depending on the length of the fatty acid. Thus, from stearic acid with its 18 carbon atoms, Δ^9 desaturation forms oleic acid with a double bond 9 carbon atoms from the carboxyl end, which is also 9 carbon atoms from the methyl end (18 – 9 = 9). Figure 5.6 illustrates the action of Δ^9d enzymes on several monounsaturated fatty acids. However, Δ^9 desaturation of palmitic acid (16:0) yields palmitoleic acid

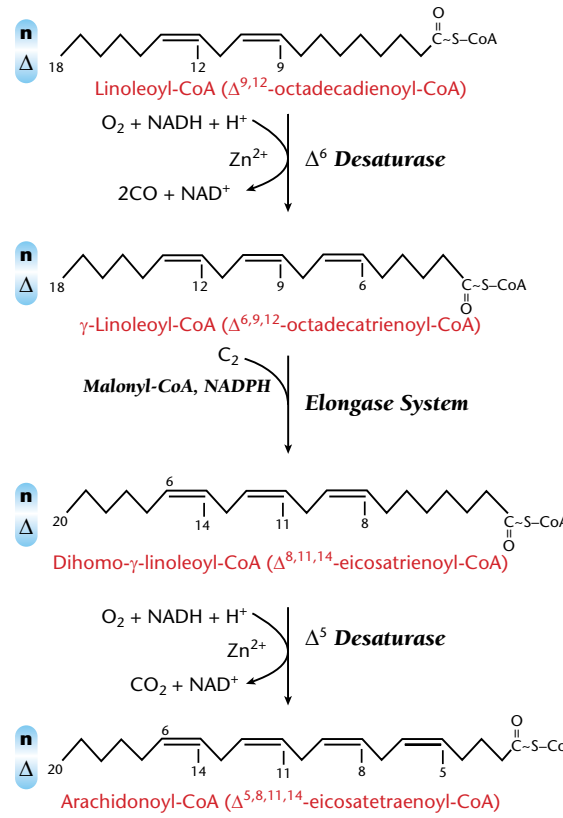

FIGURE 5.5 — Microsomal Conversion of Linoleic to Arachidonic Acid

After attachment to coenzyme A, a double bond is inserted at the Δ^6 position of linoleic acid to form γ-linoleic acid (GLA). The elongase system adds a two-carbon unit at the carboxyl end, and another desaturation step inserts the fourth double bond at the Δ^5 position to form arachidonic acid. All metabolic conversions of fatty acids act upon the acyl-S-CoA esters as shown here. Other figures may show the carboxyl group for simplicity.

(16:1n7), in which the double bond is only 7 carbon atoms from the methyl end. The position of the double bond is important, because it determines the class of eicosanoid hormones that can be formed. Eicosanoids will be discussed in later sections of this chapter.

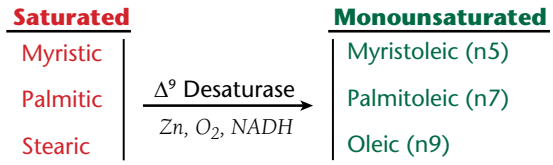

FIGURE 5.6 — Desaturation of Saturated Fatty Acids

The desaturase enzyme can operate on fatty acids of various chain lengths, placing double bonds up to nine carbons away from the carboxyl group. The first carbon atoms in the double bonds are at varying positions from the methyl ends of the monounsaturated fatty acid products resulting in the various n designations. Note that the n and omega (ω) designations (not shown) are synonymous. While other nutrients can affect changes in desaturation rates, the desaturase enzyme directly requires only the niacin-derived NADH and zinc. Of these, the relatively loosely bound zinc is far more likely to become the rate-limiting factor.

The activities of the desaturase enzymes are critical for maintaining the ratio of saturated and unsaturated components in cell membranes. Saturated and unsaturated fatty acids of various chain lengths can be acted on by this enzyme. Coenzyme A (derived from pantothenic acid) is used as a carrier for all of these reactions. Since the properties of the human desaturase enzymes will not allow insertion further than 9 carbon atoms from the carboxyl group (Δ^9), LA ($\Delta^{9,12}$) and ALA ($\Delta^{9,12,15}$) cannot be synthesized in humans.

Foods vary greatly in their content of PUFAs (see Table 5.3). The n-6 fatty acid family is abundant in vegetable seed oils, whereas the n-3 family is high in seafood and in vegetable leaves and modest in soybean oil.[57] Although modern diets tend to contain few foods with appreciable ALA content, they are high in LA from corn oil used for frying. Other common fat sources contain largely saturated or monounsaturated fatty acids. Foods that contain a high proportion of saturated fatty acids include butter, lard, coconut oil, palm oil, dairy products, and meat. Saturated fatty acids are the main dietary culprit in the elevation of blood cholesterol.

They have higher energy or caloric yield than corresponding unsaturated fatty acids. In a diet that supplies adequate LA and ALA, they may be used as precursors to form the longer chain-length PUFAs.

The Δ^6d enzyme, acts as a gateway for the flow of fatty acids through the desaturation and elongation pathway. Although it can act on any long-chain fatty acid, the substrate binding affinity increases greatly with the number of double bonds. Thus, ALA (18:3) binds stronger than LA (18:2), which in turn binds stronger than oleic acid (18:1). Thus, individuals eating diets with high levels of ALA and LA will have adequate amounts of the downstream fatty acids, AA (20:4) and EPA (20:5). Although intake of oleic acid may be significantly higher than the PUFAs, it is slowly converted into 20:3n9 (mead acid). When the essential fatty acids are deficient, however, mead acid accumulates because the desaturase is free to act on oleic acid. Figure 5.7 shows how the operations of the enzyme systems produce families of fatty acids from common precursors and the competition for the desaturase enzymes.

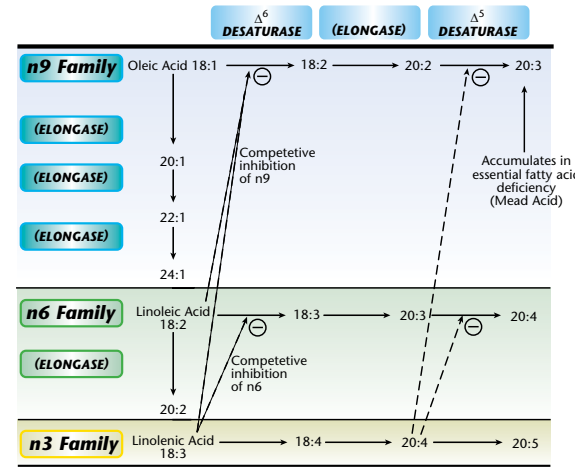

FIGURE 5.7 — Elongation and Desaturation Sequences

For fatty acids containing 18 or more carbon atoms, human enzymes cannot insert double bonds in the n6 or n3 positions, but they can elongate or desaturate at Δ5 and Δ6. The sequences of reactions produce families of fatty acids of chain lengths of 18 to 20 carbons containing one to six double bonds. The position of the first double bond from the methyl end defines the family of each product as n9, n6, or n3. Linolenic, linoleic, and oleic acids compete for the active site of desaturase enzymes in decreasing order of binding affinity.

TABLE 5.2 — LONG CHAIN FATTY ACID COMPOSITION OF SEEDS AND SEED OILS*

		% Fatty Acids in Total Oil and (% in Whole Seeds)					
	Total Fat (%)	ALA 18:3n3	GLA 18:3n6	LA 18:2n6	Oleic 18:1n9	Stearic 18:0	Palmitic 16:0
Nut or Seed							
Almond	54			17 (9)	78 (42)	5 (3)	
Avocado seed	12			10 (1)	70 (8)	20 (2)	
Avocado flesh		(1)		(27)	(36)		
Borage (starflower)			22				
Brazil	67			24 (16)	48 (32)	24 (16)	
Calendula (marigold)		(3)		(14)	(6)		
Cashew	42			6 (3)	70 (29)	18 (8)	
Chia	30	30 (9)		40 (12)			
Coconut	35			3 (1)	6 (2)		91 (32)
Corn	4			59 (2)	24 (1)	17 (1)	
Cottonseed	40			50 (20)	21 (8)	25 (10)	
Evening primrose	17		9 (2)	81 (14)	11 (2)	2	6 (1)
Fig		(13)		(10)	(5)		
Filbert	62			16 (10)	54 (34)	5 (3)	
Flax (linseed)	35	58 (20)		14 (5)	19 (7)	4 (1)	5 (2)
Grape	20			71 (14)	17 (3)	12 (2)	
Hemp (cannabis)	35	20 (7)	23 (8)	60 (21)	12 (4)	2 (1)	6 (2)
Hickory	69			17 (12)	68 (47)	9 (6)	
Kukul (candlenut)	30	29 (9)		40 (12)			
Macadamia	72			10 (7)	71 (51)	12 (9)	
Neem	40	1		20 (8)	41 (16)	20 (8)	
Noog oil		2		61	23	7	8
Olive	20			8 (2)	76 (15)	16 (3)	
Palm kernel	35			2 (1)	13 (5)		85 (30)
Peanut (groundnut)	48			29 (14)	47 (23)	18 (9)	
Pecan	71			20 (14)	63 (45)	7 (5)	
Pistachio	54			19 (10)	65 (35)	9 (5)	
Primrose oil			9	72	2	3	17
Pumpkin	47	8 (4)		50 (24)	34 (16)	0	9 (4)
Rape (canola)	30	7 (2)		30 (9)	54 (16)	7 (2)	
Rice bran	10	1		35 (4)	48 (5)	17 (2)	
Safflower	60	3 (2)		75 (45)	13 (8)	12 (7)	
Sesame	49			45 (22)	42 (21)	13 (6)	
Soybean	18	7 (1)		50 (9)	26 (5)	6 (1)	9 (2)
Sunflower	47			65 (31)	23 (11)	12 (6)	
Walnut	60	6 (4)		51 (31)	28 (17)	5 (3)	11 (7)
Wheat germ	11	5 (1)		50 (6)	25 (3)	18 (2)	

** The first value shown is the content in the seed oil, and value in parentheses is whole seed content. All pure oils are near 100% total fat content.*

Desaturation enzymes can be affected by many conditions. Delta-6 desaturase enzymes require a zinc ion for activity. Therefore, in a patient with low EPA, adding ALA-rich flax oil will do little good if there is a zinc deficiency. The changes in body growth, organ weight, and lipid concentrations of plasma and liver produced by zinc deficiency are reversed by addition of ALA, as contained in primrose oil, but not by addition of LA, as contained in safflower oil,[58] illustrating the role of zinc in the Δ^6d enzyme that inserts the extra double bond between carbon atoms 6 and 7 of linoleic acid. This enzyme is also inhibited by magnesium deficiency, elevated insulin, and high concentrations of saturated, monounsaturated, and *trans*-fatty acids. *Trans*-fatty acids, in competing for the binding site on the enzyme, yield products that are of less significance to the cell.

The metabolism of PUFAs is also affected by vitamin B_6 deficiency, the most pronounced effect being a decrease in the production of DHA.[59, 60] Mice made vitamin B_6 deficient and fed a diet high in ALA show impaired activity of Δ^6 dehydrogenase and of acyl-CoA oxidase, which is a gateway to peroxisomal fatty acid oxidation.

Organelle-Specific Steps

In the discussion of fatty acid metabolism, it is necessary to consider the movement of fatty acids between organelles. The fatty acid synthase system, along with the desaturase and elongase enzymes is located in or on the smooth endoplasmic reticulum (ER) and microsomes. The conversion of EPA to DHA, however, requires additional enzymes that are present only in peroxisomes. For this conversion, EPA must undergo two further elongation steps before the final Δ^6d step can occur, yielding 24:6n3. This very-long-chain, polyunsaturated fatty acid is then transported to the peroxisome. There, a β-oxidation sequence occurs, removing two carbon atoms from the carboxyl end of 24:6n3 to finally produce 22:6n3, DHA. In the liver, peroxisomal DHA must be reintroduced to the ER for packaging and export with LDL.

Peroxisomal oxidation was first observed in experiments with the blood lipid lowering drug, clofibrate. These experiments showed that rats treated with clofibrate exhibited an increased rate of fatty acid oxidation and a large increase in the number of peroxisomes in their liver cells.[61] Subsequent work demonstrated that peroxisomes, as well as mitochondria, can oxidize fatty acids. In fact, peroxisomes now appear to be the dominant site of fatty acid oxidation in many tissues. Very-long-chain fatty acids (those containing more than about 20 carbon atoms) are degraded only in peroxisomes in mammalian cells. Peroxisomal beta-oxidation is required to shorten very-long-chain fatty acids so that they can be degraded in the mitochondrion.

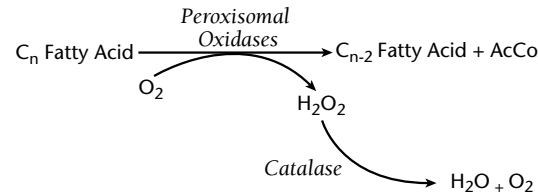

FIGURE 5.8 — Peroxisomal Fatty Acid Oxidases

Peroxisomes do not contain the elaborate electron transfer system for capture of energy released with large shifts in oxidation potential. Peroxisomal oxidases transfer electrons directly to molecular oxygen, forming hydrogen peroxide (and subsequent free radicals). The fatty acid is shortened by two carbons as a new acetyl-CoA molecule is released. The hydrogen peroxide byproduct must be converted to water and oxygen by catalase.

In contrast to mitochondrial fatty acid oxidation, peroxisomes contain enzymes that use molecular oxygen as an electron acceptor, forming hydrogen peroxide, which is then degraded by catalase (see Figure 5.8). Although the enzymes of peroxisomal degradation of fatty acids are similar to those found in mitochondria, peroxisomes lack the electron transport chain. In peroxisomes, electrons from the FADH2 and NADH produced during the oxidation of fatty acids are immediately transferred to O_2, forming H_2O_2. To prevent the toxic effects of H_2O_2 from causing oxidative degradation of peroxisomal and cytosolic enzymes, catalase quickly decomposes it to H_2O and O_2. Without an electron transport chain, peroxisomes cannot link the oxidation of fatty acids to ATP formation, and the released energy is converted to heat. The acetyl-CoA produced is transported away from peroxisomes for synthesis of fatty acids, cholesterol, and other metabolites.

The ability of fatty acids to move from endoplasmic reticulum to peroxisomal compartments provides an explanation of the sluggishness of conversion of ALA into DHA. The sequence of desaturation and elongation repeats in a staggered fashion, because the double bonds must be inserted at every third carbon,

whereas the elongation always adds two carbon atoms. This mismatch of positioning explains why two different desaturase enzymes, Δ^5d and Δ^6d are required to generate EPA from ALA. To further convert EPA to DHA with a similar pair of elongation and desaturation steps, however, would require a Δ^4desaturase. Such an enzyme was presumed to exist for many years, and the elongation sequence in earlier books indicates a Δ^4d step. More recent evidence indicates that human hepatocytes show negligible, if any, Δ^4d activity. Formation of DHA from EPA requires, rather, a multistep process, as illustrated in Figure 5.9. The rate of conversion is greatly slowed by the requirement of transfer between the endoplasmic reticulum and the peroxisome.

Lack of peroxisomes or peroxisomal enzymes is a characteristic feature of Zellweger syndrome, a rare hereditary disorder affecting infants. Clinical features include enlarged liver, high levels of iron and copper in the blood, and vision disturbances. In patients with Zellweger syndrome, peroxisomal retro-conversion of 24:6n3 to 22:6n3 (DHA) is deficient in cultured fibroblast,[62] and DHA and other desaturation fatty acid products are deficient.[63]

Because of the multiorganelle passage requirement, the rate of conversion from ALA to DHA in human hepatocytes is much lower than that of conversion from ALA to EPA. This disparity provides an explanation for the failure to observe a significant rise in DHA in pregnant and lactating women who are supplemented with ALA, even though their EPA rises in parallel to their plasma ALA.[64] This situation may not prevail throughout mammalian species, since ALA was found equally effective as DHA supplementation for raising brain DHA in Wistar rats.[65] The subject has enjoyed a high level of research

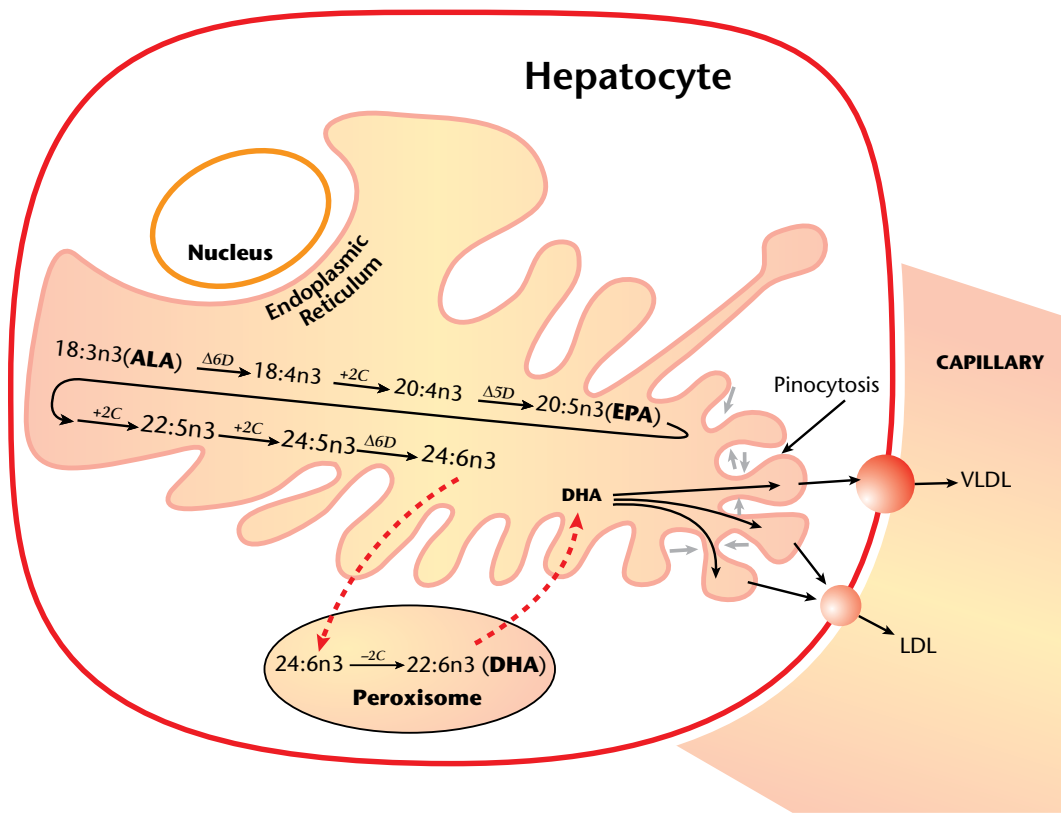

FIGURE 5.9 — From ALA to DHA

Since there is no appreciable Δ^4d activity in human endoplasmic reticular compartments, the final desaturation to DHA can not be done by a simple elongation and desaturation because the double bond would have to be inserted at the number four carbon. The available Δ^6d will do the job, but only after two elongation steps move the carboxyl group out by four more carbons. Then the product is two carbons too long, so it must be exported to the peroxisome where chain shortening can occur. In order to contribute to blood lipids, the resulting DHA must be passed back into the endoplasmic export system.

attention because of the critical need for DHA in early childhood central nervous system development.[46, 66]

The peroxisome also serves as the site for synthesis of a special class of phospholipids called plasmalogens. Plasmalogens, rich in the membranes of the CNS, exhibit two unique structural features: (1) attachment to the 1-position of glycerol occurs by means of ether bonds instead of ester bonds, and (2) insertion of a double bond at carbon Δ^2. The juxtaposition of a double bond immediately next to the ether linkage produces a form of rigid geometry that protrudes from membrane surfaces and that confers increased adherence properties when two cell membranes are adjacent.[67]

Peroxisomal fatty acid oxidation and desaturation, therefore, plays a large role in metabolism of fatty acids important to the nervous system. In addition, hepatic peroxisomes are involved in synthesis of bile acids, cholesterol, and plasmalogens, as well as in metabolism of amino acids and purines. Numerous genetic disorders with neurological consequences result from defects in peroxisomal enzymes or in regulation of peroxisomal proliferation.[68] The abnormalities in fatty acids detected by laboratory profiles of plasma or red blood cells can detect many such disorders.[69] In a much broader sense, the effect of nutritional status and toxicant exposure on peroxisomal regulation may be of significance in many of the major chronic diseases such as the metabolic syndrome.[70]

Phosphatides, Phospholipases, and Membrane Turnover

The dominant form of fatty acids in living organisms is the phospholipid, in which two fatty acids and a polar "head" group are attached to a molecule of glycerol. These compounds are collectively known as phosphatides, and they are the basic structural unit of cell membranes. Substrate specificity for the esterase enzymes that form the fatty acyl glycerol ester bonds results in saturated fatty acids predominantly in the 1-position, and unsaturated fatty acids in the 2-position. The membrane composition of phosphatides is constantly changing as a result of the action of phospholipase enzymes. Phospholipase A2 (PLA2) is a membrane-bound complex that extracts fatty acids from the middle or 2-position of phosphatides. The actions of two different forms of this enzyme cause a dynamic circulation of fatty acids from both the inner and outer layers of membranes. The resulting phosphomonoglycerides with saturated fatty acids in the 1-position are called lysophosphatides. The unsaturated fatty acids liberated from the 2-position may move into either the plasma fatty acid pool or the intracellular pool available for the metabolic actions described below. When PLA2 activities are linked with those of acyltransferases, a trafficking of fatty acids known as the "deacylation-reacylation cycle" occurs. The shifting of this dynamic balance produces waves of increasing or decreasing flow from membrane phosphatides to COX and LOX enzyme systems for signaling functions. Since the release of fatty acids from the 2-position of phosphatides is necessary for the formation of eicosanoids, agents that regulate their activities are of great interest to anti-inflammatory drug development.

There are more than 19 different isoforms of PLA2 in the mammalian system, but recent studies have focused on three major groups, namely, group IV cytosolic PLA2, group II secretory PLA2 (sPLA2), and group VI Ca(2+)-independent PLA2. By means of these PLA2s, the release of AA and the synthesis of eicosanoids are linked with receptor agonists, oxidative agents, and pro-inflammatory cytokines involved in a complex network of signaling pathways.[71] Natural modulators of PLA2 activity include angiotensin (stimulation), bradykinin (inhibition), epinephrine, and thrombin.[72-75]Abnormal PLA2 activities have been implicated in the pathology of a number of neurodegenerative diseases, including cerebral ischemia, Alzheimer's disease, and neuronal injury due to excitotoxic agents. Group I, II, IV, V, and VI PLAL2 have been shown to be constitutive enzymes of spinal cord.[76] Using an astrocytoma cell line, inflammatory/injury stimuli have been shown to activate PLA2 in the brain, initiating the AA cascade.[77] Unique catecholamine actions in cardiac tissue are also modulated by means of the PLA2 release of fatty acids.[78]

Notes:

The shift of modern diets away from high PUFA intakes, specifically n-3 fatty acids, is thought to be a primary contributor to the metabolic and hormonal factors impacting fatty acid desaturase and PLA enzymes, leading to peroxisome plasticity.

PUFA-Derived Cell Regulation Products

Eicosanoids — The overall function of the desaturation and elongation sequence is to maintain levels of DGLA, AA, and EPA for incorporation into biological membranes. These three PUFAs are the parent compounds of the 1-, 2-, and 3-series prostanoid (prostaglandin and thromboxane) and leukotriene pathways. The derivatives are collectively called "eicosanoids" (from the Greek eikosa, meaning twenty) because they all contain 20 carbon atoms. Eicosanoids may carry out extremely potent biological activities, including regulation of blood vessel leakage, lipid accumulation, immune cell behavior, and contribution to the initiation and progress of heart and blood vessel disease.[79] Eicosanoids show effects in amplifying and balancing signals to the brain, in the blood clotting system, and in the immune system. Various cytokines initiate the sequence by eliciting local cell response through a process starting in the cell membrane of affected cells. These various stimuli cause phospholipase A2 to cleave fatty acids (mainly AA) from the 2-position of membrane phosphodiglycerides (see Figure 5.10). Lipoxygenase and cyclooxygenase enzymes within the cell then convert the released DGLA, AA, and EPA into the various classes of eicosanoids. Leukotrienes, lipoxins, and prostaglandins are classes of eicosanoids within which are the subclasses, called series 1, 2, and 3, defined by the number of double bonds they possess. Note that the number of double bonds in the parent fatty acid always exceeds by two the number of double bonds present in the products indicated by their respective 1, 2, or 3 subscripts. Thus, PGE1, PGE2 and PGE3, though similar compounds, differ in that they possess 1, 2, or 3 double bonds, because they are derived from fatty acids containing 3, 4, or 5 double bonds. Two of the fatty acid double bonds are lost in the formation of the derivatives by the enzymes. Prostaglandins are pivotal in many primary functions. For example, prostaglandin D2 (PGD2) is uniquely important in sleep, as shown in studies where REM sleep is totally abolished by inhibitors of PDG2 formation.[80] This 2-series prostaglandin is formed from AA. Thus sleep requires adequate nerve membrane AA for extraction by PLA2 to supply the cyclooxygenase pathway.

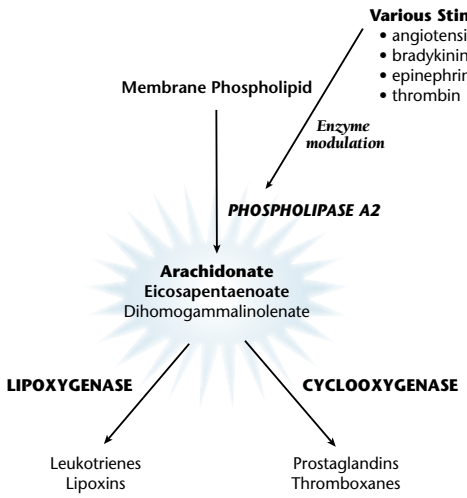

FIGURE 5.10 — Eicosanoids from Cell Membrane Fatty Acids

Polyunsaturated fatty acids normally occupy the second glycerol atom of membrane phospholipids. The fatty acid abundances vary with dietary supply. When phospholipase A2 (PLA2) is stimulated, it cleaves at the two-positions, releasing the fatty acids to be acted upon by lipoxygenase (LOX) and cyclooxygenase (COX) enzymes. The ultimate products control responses of immune, smooth muscle, epithelial, and other tissues.

The parent PUFAs, DGLA, AA, and EPA are processed by the same enzyme for all three series of products. The simple concentrations of the three fatty acids present in the cell membrane thus determine which products will predominate. Membrane composition, in turn, is determined by dietary intake and the desaturation and elongation reactions that we have described.

The lipoxygenase enzyme initiates the sequence of reactions leading to leukotriene formation (Figure 5.11). The 2-series that is derived from AA is by far the most pro-inflammatory. Antagonism of AA by DGLA and EPA for active sites of lipoxygenases moderates inflammatory responses because the 1-series and 3-series leukotrienes are much less inflammatory than those of the 2-series. The anti-inflammatory effects of DGLA and EPA on rheumatoid arthritis and other diseases are mediated by means of this mechanism. They bind to the lipoxygenase in place of AA and stimulate the production of the series 1 (DGLA) and series 3 (EPA) eicosanoids. Dietary fatty acid intake is a primary determinant of flow through this

pathway. This mechanism helps to explain many of the links between the balance of specific fats in the diet and long-term health maintenance.[81] Eating a diet rich in fish oils shifts the dynamic equilibrium toward less inflammatory responses. Routine laboratory profiles of fatty acids in plasma or erythrocytes allow specific intervention in cases in which the imbalance is most prevalent, so that pro-inflammatory and anti-inflammatory responses are kept in balance and other local control processes respond properly to cellular stress.

Endocannabinoids — Endocannabinoids are endogenous metabolites capable of activating cannabinoid receptors. The CB1 and CB2 cannabinoid receptor subtypes are involved in nervous and immune disorders, cardiovascular disease, pain, inflammation, and cancer. Both types of receptors are G-protein-coupled membrane-bound functional proteins. CB1 receptors are found in the central nervous system and in a variety of other organs, including the heart, vascular endothelium, uterus, vas deferens, testis, and small intestine.[82] Within the first few minutes of high-frequency stimulation of glutamatergic corticostriatal synapses, CB1 activation by endocannabinoids is necessary for long-term synaptic depression and recovery.[83] CB2 receptors appear to be associated exclusively with the immune system. The endocannabinoids interact with known regulators of appetite and weight gain. The interactions occur in the limbic system, hypothalamus, intestinal system, and adipose tissue. Various pharmacological and dietary approaches are under study in the pursuit of improved treatments for cachexia and malnutrition associated with cancer, acquired immunodeficiency syndrome, and anorexia nervosa.[84]

The most active endocannabinoids have the arachidonoyl chain attached to either ethanolamine or glycerol. Arachidonylethanolamide (Anandamide or AEA) and 2-arachidonoylglycerol (2-AG) have been reported to have neuromodulator or neurotransmitter properties.[85] Hypothalamic regulation of visceral processes (e.g., food intake, thermoregulation, and control of anterior pituitary secretion) is modulated by endocan-

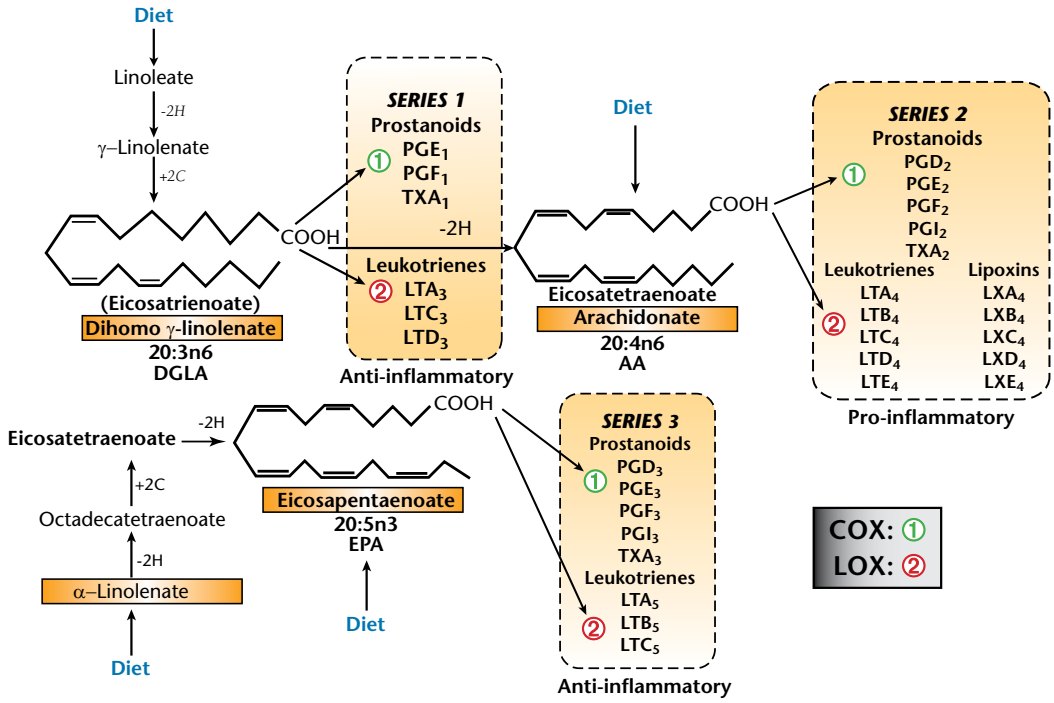

FIGURE 5.11 — **Lipoxygenase and Cyclooxygenase Enzyme Products**

Eicosanoid products of COX and LOX enzymes help maintain the balance of tissue responses to stress signals. The series-1, -2, and -3 products are defined by the number of double bonds they contain. Prostaglandin formation causes loss of two double bonds, so the products of DGLA, AA, and EPA contain 1, 2, or 3 double bonds, respectively. These numbers become the subscripts of the product names (e.g., $PGE_{1, 2, or 3}$).

nabinoids by means of CB1 receptors. Administration of anandamide to rats decreases hypothalamic release of leutenizing hormone and prolactin, causing prolongation of pregnancy and delayed lactation.[86]

Phospholipids containing AA at the 2-position undergo sequential hydrolysis of the 1-fatty acid and the 3-phosphate group to yield 2-AG. In mice, n-3 fatty acid deficiency elevates, and n-3 fatty acid enrichment reduces 2-AG levels in brain.[87] These results suggest that the physiological and pathological effects of 2-AG on the CNS could be modified by manipulation of dietary n-3 fatty acid status. Other evidence indicates that this class of endocannabinoid also includes the analogous compounds produced from the 22-carbon PUFA, DHA.[88] Both arachidonoyl and docosahexacosanoyl forms have been detected in the retina.[89] These finding are significant because of the very high concentrations of DHA in the healthy brain and especially retinal tissue. Thus low n-3 fatty acid levels detected in plasma or erythrocytes may have significant effects in the CNS.

The pharmaceutical industry is keenly interested in endocannabinoid-mimicking drugs because of the potential for weight reduction. Since both cannabis and 2-AG stimulate appetite and increase visceral adiposity, the possibility of causing weight loss with a drug that inhibits such stimulation was explored. The drug Rimonabant was discovered to have powerful CB1 inhibitory properties. It not only induced weight loss, but simultaneously caused a lowering of nicotine effects, helping smokers to stop the habit.[90] The drug, also known as SR 141716, suppresses the reinforcing/rewarding properties of cocaine, heroin, nicotine, and alcohol in laboratory rodents.[91] As might be expected with such pharmacologic-induced inhibitions of fundamental cell regulatory systems, such drugs were found to cause significant side effects. After the first year of use, 1 out of 8 patients reported significant depression, anxiety, and nausea.[92] Such side effects are not surprising when a system that helps to regulate pleasure, relaxation, and pain tolerance is suppressed. Even though tolerance for potential side effects might be justified in view of the rapidly rising incidence of obesity, CB1-inhibiting drugs may be no more effective than the counteracting of appetite and fat cell proliferation by endocannabinoids derived from EPA and DHA. Data regarding the much lower incidence of obesity in Iceland compared with the United States provide evidence in support of such an argument. The obesity rates in the two countries are inversely correlated with fish intake.[93] The latest data on Rimonabant efficacy indicate that the drug-induced weight loss is quickly regained if the drug is discontinued after a year, indicating that it would need to be used chronically to maintain weight loss.[92] Furthermore, onset of multiple sclerosis (MS), a disease of the central nervous system in which the nerves undergo progressive demyelination, has been observed within several months of starting the cannabinoid receptor antagonist.[94] These data suggest that the cannabinoid system might indeed be relevant to disease pathogenesis in MS. Finally, any conclusion as to the impact of drugs that might be used by large numbers of women before and during pregnancy must take perinatal effects into consideration, since endocannabinoids regulate embryonal implantation and neonate feeding, appetite, and neuroprotection.[95]

The Fat-Sensing System: Peroxisome Plasticity

In order to accommodate the heavy baseline flux and large fluctuations in the supply of fatty acids, powerful regulatory mechanisms must act to adjust their use and storage. A primary method of responding to changes in fatty acid intake consists of changing the number of peroxisomes available for metabolic processing. Peroxisomes are eukaryotic organelles within the cell that function in the metabolism of fatty acids, to help rid the cell of toxic peroxides, and provide other essential metabolic functions. Peroxisomes proliferate or decrease in response to dietary lipids, hormones, toxicants, and drugs that bind to nuclear regulatory proteins called peroxisome proliferator-activated receptor (PPAR) proteins. Similar to other nuclear hormone receptors, PPARs acts as ligand-activated transcription factors. The PPAR response provides a mechanism whereby lipogenic enzyme gene expression is strongly suppressed by small additions of dietary PUFAs.[96]

Three varieties of PPAR, α, δ (previously β), and γ, are distributed among various tissues. Unsaturated fatty acids bind to PPAR-α primarily in the liver, heart, muscle, and kidney, activating genes involved in fatty acid uptake, mitochondrial and peroxisomal transport, beta-oxidation, and cytochrome P450 omega-oxidation. During long-term fasting, the free fatty acids that are mobilized from adipose tissue bind to PPAR-α, enhancing hepatic fatty acid oxidation and the production of ketone bodies, averting hypoglycemia.[97] This difference between fatty acids released from adipose tissue

and fatty acids flowing from the gut or from hepatic synthesis is the key to understanding PPAR effects. The explanation is that free fatty acids are released from adipocytes, whereas lipoproteins are generated in enterocytes and hepatocytes. Lipoproteins contain fatty acids primarily as triglycerides, phospholipids, and cholesterol esters. The amounts of free fatty acids that they transport, although small, can contribute to the PPAR effects, but the magnitude of response is much lower than that elicited from adipose mobilization. High-fat diets result in PPAR-α-induced fatty acid catabolism to decrease hypertriglyceridemia and lower the tendency to adiposity.

The δ and γ forms of PPAR have different ligand responses, and induce different sets of genes involved in central energy metabolism. Fibrate drugs like gemfibrozil are PPAR-α agonists.[98] Insulin sensitizers such as thiazolidinediones are PPAR-γ agonists.[99] Although unsaturated fatty acids activate all three classes, most studies have dealt with their effects mediated through PPAR-α.

Responses to PPAR activation are further modified by a mechanism involving coactivation of similar receptors by different ligands. Since a pair of receptor-ligand receptors is required, the functional unit of regulation is called a heterodimer. The vitamin A derivative, 9-*cis*-retinoic acid is the ligand for the second heterodimer receptor (RXR) in PPAR activation. The shift from gene repression to activation occurs as the RXR goes from the state of both ligands unbound or bound, respectively. These two states are illustrated in Figure 5.12. Activation of peroxisome proliferation produces increased cellular peroxisome content, increased rates of fatty acid and glucose metabolism, decreased rates of fatty acid and cholesterol synthesis, increased insulin sensitivity,

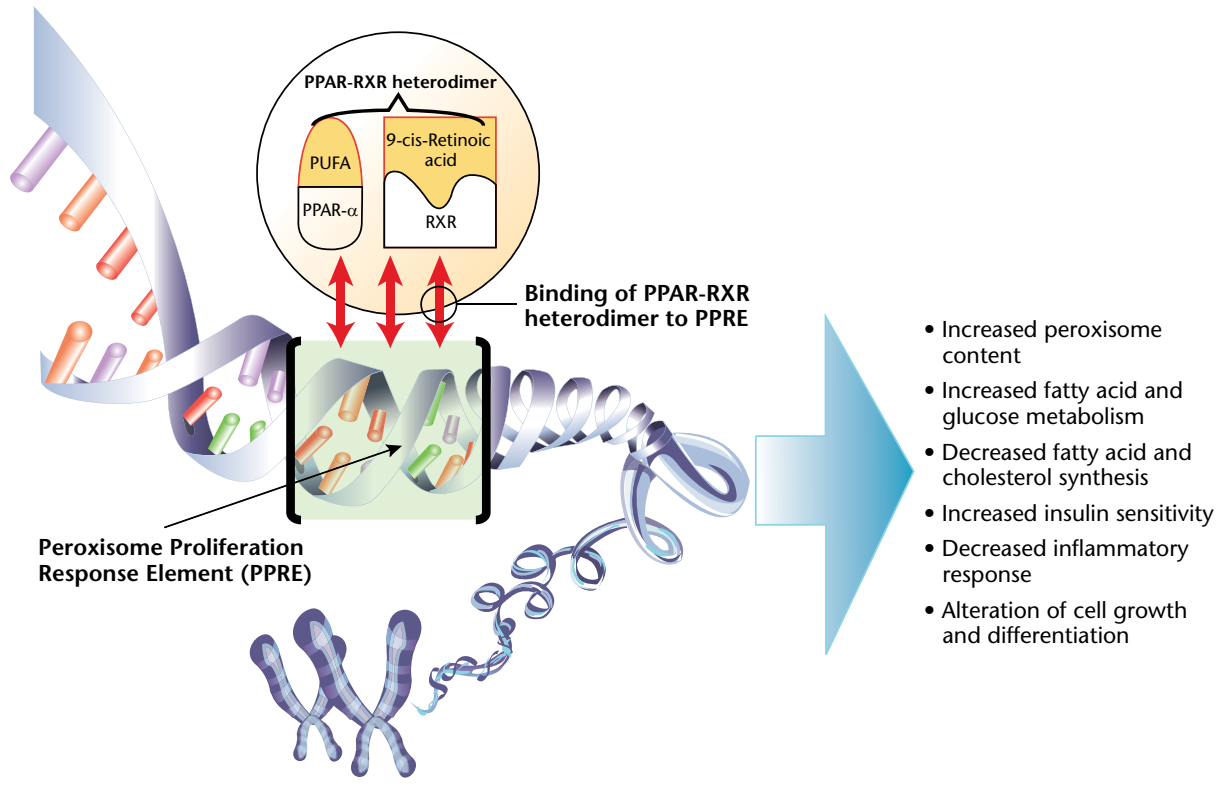

FIGURE 5.12 — PPARα Heterodimer Nuclear Regulation

The peroxisome proliferator-activated receptor (PPAR) acts as a ligand-activated transcription factor similar to other nuclear hormone receptors. When both PUFA and 9-*cis*-retinoic acid ligands enter the nucleus and bind to their respective receptor sites, the PPARα/RXR heterodimer changes conformation. The shift causes binding of co-activator or co-repressor complexes to peroxisome proliferation response element genes, changing nearby histone structures. The full response involves activation of multiple gene expression, leading to the assembly of peroxisomes and, thereby, to multiple metabolic consequences.

decrease of inflammatory responses, and altered cell growth and differentiation.[97, 100-103]

Activation of the PPAR-α/RXR heterodimer increases the rate of fatty acid oxidation in the liver and skeletal muscle, increases oxidized LDL uptake in cardiac tissue, and increases reverse cholesterol transport,[104] lowering total circulating LDL.[105] Failure of PPAR activation leads to the rising levels of serum triglyceride and cholesterol characteristic of metabolic syndrome.[70] The more severe genetic peroxisomal biogenesis defects result in accumulation of VLCFA that affect nervous system structure and function. To gain a greater understand these relationships, knowledge of phospholipid membrane formation and maintenance are recommended.

Blood Plasma and Erythrocyte Specimens

Since the majority of fatty acids in plasma are present in lipoproteins in the esterified form, as glycerol or cholesterol esters, hypertriglyceridemic patients are also hyperlipoproteinemic. These patients will also show elevated plasma fatty acid patterns. Erythrocyte membrane fatty acid profiles do not similarly reflect hypertriglyceridemia. One way to overcome this problem with plasma data is to use the percentage of total fatty acids as the basis for calculation. However, this calculation frequently produces false abnormalities in the majority of non-hypertriglyceridemic patients because of the influence of the variable levels of the dominant fatty acids on the total. For example, a falsely low ALA level (minor component) is produced when oleic and stearic acids (dominant components) are high. The reverse situation results in a falsely elevated ALA. The same is true for all of the minor components. Many researchers also prefer to express fatty acid amounts as a percentage of a specific fraction of fatty acids contained in a tissue or body fluid, such as measurements made on plasma total phospholipid or plasma choline phosphoglycerides, triglycerides, and cholesterol esters.[106]

Fatty acids are present in serum or plasma primarily in the form of lipoprotein particles, of which the LDL species is the most abundant in normal fasting plasma. The small fraction of free fatty acids in blood is bound to transport proteins, principally serum albumin. Profiles report individual concentrations of each fatty acid instead of measuring only the total of all fatty acids present, as is common in a serum triglyceride assay.

Patients with high serum triglycerides have higher levels of some fatty acids in plasma. Conditions, such as insulin insensitivity, that lead to elevated serum triglycerides do so by stimulating endogenous synthesis and transport. When glucose or amino acids are used to build fatty acids in liver or adipose tissue, the principal product is the 16-carbon-long palmitic acid. Thus, an elevated palmitic acid in a plasma profile of fatty acids is evidence of elevated serum triglycerides, and the degree of palmitate elevation is directly proportional to the degree of triglyceride elevation.

The majority of fatty acids do not have such linear relationships with serum triglycerides. Variations of total triglycerides within the normal range have little effect on concentrations of the EFA, ALA, GLA, and EPA, in plasma. Concentrations of these fatty acids in fasting plasma reflect their composition in adipose tissue. Adipose tissue fatty acid composition is primarily affected by dietary intake and rates of endogenous synthesis. Thus levels of fatty acids will depend on the total effects of endogenous synthesis and recent dietary intake. Plasma fatty acids are therefore frequently used in studies of fatty acid status and dietary intake.[107-110] Some researchers also report simultaneous plasma and erythrocyte data from which additional insight is gained

Notes:

regarding fatty acid metabolism. For example, the higher levels of LA metabolites in erythrocyte phospholipids versus plasma lipoproteins was given as evidence of the importance of assaying the very-long-chain members.[111] In addition, a finding of simultaneous decreased plasma and erythrocyte levels of DHA helped support the conclusion that premature infants may have higher n-3 fatty acid requirements than term infants.[112]

Erythrocyte membranes consist of 45% fatty acids in the form of various phosphatides and glycolipids. The most common procedure for fatty acid profiling measures concentrations of individual fatty acids in packed erythrocytes. Fatty acid levels in erythrocytes differ from plasma profiles in that, although not as representative of recent dietary intake and adipose composition, they do provide information about metabolic functions within the cell. Dietary intake factors are seen on a longer time scale, since they influence newly formed red cell populations. Because of the high binding affinities of desaturation enzymes for LA and ALA and GLA, the concentrations of these compounds are lower in cell membranes relative to their levels in plasma lipoproteins that originate in hepatic lipid biosynthetic and export pathways.

Drugs that alter fatty acid metabolism will enhance the differences between plasma and erythrocyte fatty acids levels. The triglyceride-lowering drug gemfibrozil causes a profound suppression of erythrocyte unsaturated fatty acids, even in the presence of high concentrations of the same compounds in plasma. The effect is apparently due to stimulation of the peroxisomal beta-oxidation system.[113] Because of the metabolic effect of the drug, a patient consuming a diet rich in olive oil (largely oleic acid) may show low levels of oleic acid in erythrocyte membranes while maintaining high oleic acid in plasma. Thus, plasma profiles show the dietary changes, whereas erythrocyte fatty acids show the metabolic impact of the drug.

Erythrocyte levels of the long-chain metabolic products of elongation and desaturation are representative of the fatty acid content of other tissues. The DHA composition of brain tissue, for example, is directly correlated with that in red blood cells in rat and primate studies.[114] Thus, although dietary intake is not as closely reflected and metabolic influences of drugs that alter fatty acid metabolism must be accounted for, the richer information content induces many clinicians to prefer data from erythrocytes over those from plasma.[115]

In addition to plasma and erythrocytes, blood spot specimens have been successfully used in assessing fatty acids. The specimen may be useful in nutritional evaluations and epidemiological studies.[116] Applications include the determination of ratios such as AA/EPA and Mead/AA (Triene-Tetraene, or T/T).

Interpretation of Fatty Acid Profiles

It has become common for laboratory profiles of fatty acids to include more than 40 analytes that may be measured accurately from a specimen of plasma or erythrocytes from whole blood. In practice, clinicians generally do not evaluate each fatty acid individually but look for patterns. Topics for discussion, thus, are reduced from the 42 analytes and ratios listed in Table 5.1 to several patterns with a few subcategories.

The Clinically Relevant Patterns

Fatty acid analysis provides useful information about the need for dietary modifications and/or fatty acid supplementation, as well as the need for certain vitamins and minerals. The proliferation of studies reporting favorable neurological effects of the n-3 fatty acids, especially EPA from fish oil, may induce routine recommendation of supplemental fish oils to all patients. However, individual variability of intake, digestion, absorption and degradation can produce very significant differences in fatty acid status. Patients with plasma n-3 fatty acid concentrations in the upper range of normal or higher are unlikely to show significant benefit from added fish oils. Those with low levels, on the other hand, may need aggressive supplementation to assure rapid repletion for positive physiological effects. Thus supplementation can be targeted to appropriately benefit each individual patient. Clinical interventions for fatty acid deficiencies and imbalances often involve modifying diets to include greater quantities of nuts, seeds, or marine oils, along with necessary micronutrients. Various useful ratios and indices may be displayed on laboratory reports to assist interpretation.

Interpretation of laboratory results is simplified by considering clinical conditions rather than individual analytes, since most of the information lies in assessing patterns within families rather than any one member. Thus, one may concentrate on the abnormalities or

TABLE 5.3 — FATTY ACID IMBALANCES AND MICRONUTRIENT DEFICIENCIES COMMONLY EVALUATED BY FATTY ACID PROFILES

	Condition	Fatty Acid Pattern	Corrective Actions
General Fatty Acid Disorder	EFA deficiency	Multiple low n-3 and n-6; high mead; high T/T ratio, high palmitoleic	Add EFA-rich oils
	Hypertriglyceridemia	General elevation of most members of all families	Add fish oil Decrease dietary carbohydrate Antilipidemic medications
Omega 3 Deficiency or Excess	n-3 class deficiency	Low ALA, EPA, and DHA with normal or elevated AA	Add flax and fish oils
	Specific ALA deficiency	Low ALA with normal EPA & DHA	Add flax oil
	Specific EPA,- DHA deficiency	Normal ALA with low EPA & DHA	Add fish oils
	ALA or EPA excess.	High ALA or EPA	Decrease flax oil or fish oil and increase antioxidant intake
Omega 6 Deficiency or Excess	n-6 class deficiency (esp. with n-3 class excess)	All n-6s low with low DGLA/EPA and AA/EPA ratios	Decrease all n-3 supplementation and add corn, evening primrose, or other n-6 oils
	Specific GLA or DGLA deficiency	Low GLA or DGLA with normal LA & AA	Add primrose oil
	Specific LA excess	High LA (see desaturase deficiency)	Reduce LA-rich dietary sources
	Specific AA excess	High AA with normal levels of other n-6s	Check AA/EPA ratio, and decrease red meat intake accordingly
Hydrogenated Oil Toxicity	Toxic interferences from hydrogenated oil use	Elevated palmitelaidic or C18-*trans*-fatty acids (elaidic, *trans*-vaccenic, *trans*-petroselinic)	Reduce hydrogenated oil intake
Micronutrient Deficiencies	Zinc deficiency	Elevated LA/DGLA or ALA/EPA ratios	Zinc supplementation
	Copper deficiency	Elevated stearic and docosadienoic with low oleic and linoleic acids	Copper supplementation
	Vitamin B_{12}, biotin deficiency	Elevated odd numbered fatty acids and vaccenic acid	Vitamin B_{12} and biotin supplementation

patterns as shown in Tables 5.3 and 5.4. This set of conditions may be further classified under the following seven steps of interpretation for clinical assessment:

1. General Fatty Acid Deficiency
2. Omega-3 Deficiency or Excess
3. Omega-6 Deficiency or Excess
4. Hydrogenated Oil Toxicity
5. Micronutrient Deficiencies
6. Metabolic and Genetic Disorders
7. Fatty Acid Ratios and Indices

The assessments require the detection of patterns of abnormalities found within the families of structurally similar members. Thus, patients who have been on diets low in essential fatty acids are likely to have generally low levels of all n-3 fatty acids. Those with metabolic syndrome typically display high levels of the long-chain saturated fatty acids, and so forth.

General Fatty Acid Deficiency or Excess

Disorders in this category have effects across two structural classes of fatty acids. In the extreme case of starvation, tissue content of EFAs is used for basic caloric needs. Much more common are patients who present with a history of chronic, moderate dietary fat

Table 5.4 — Metabolic and Genetic Disturbances Evaluated by Fatty Acid Profiles

	Condition		Fatty Acid Pattern		Corrective Actions
Metabolic and Genetic Disturbances	The Metabolic Syndrome		Elevated C16, C18 and C20 fatty acids with low levels of other members in each class		Steps to increase insulin sensitivity and restrict dietary carbohydrate
	Multiple Acyl-coenzyme A Dehydrogenation disorders (MAD)		High capric, lauric and myristic acids		Riboflavin; 50 mg TID
	MCAT def		Pattern of high to low as chain length increases		Carnitine
	LCAT def or Metabolic Syndrome		Pattern of increase, then decrease with chain length		Carnitine, check insulin and insulin sensitization factors
	VLCAT def		Pattern of low to high as chain length increases		Add carnitine
	Peroxisomal insufficiency	Type 1. Single enzyme defects	X-linked adrenoleuko-dystrophy	Accumulation of fatty acids longer than 22 carbon atoms in length	Mixtures of glycerol trioleate and trierucate may correct the fatty acid status
			β-Oxidation disorders	Accumulation of fatty acids longer than 22 carbon atoms in length	
		Peroxisome Biogenesis Disorders	Zellweger spectrum	Pattern of VLCFA elevation (docosatetraenoioc, nervonic, erucic, behenic, lignoceric and hexacosanoic)	Fish oils or CLA can activate PPAR and natural vitamin A containing the 9-*cis* isomer co-activates PPAR response

restriction. Though research has continued to show that it is the type of fat in the diet rather than the amount that is important for weight loss, long-term intake of low-fat diets is still prevalent.[117-121] Such diets can result in deficiencies of EFAs. Chronic EFA deficiency is indicated by low levels of most n-3 and n-6 fatty acids, as well as palmitoleic acid, mead acid and triene-tetraene ratio elevations. Abnormalities in these markers are first reflected in plasma fatty acid profiles.

EFA deficiency induces compensatory cellular mechanisms to preserve critical functions. Intercellular numbers of microsomes and peroxisomes fall to reduce the flux of fatty acid oxidation pathways. Hepatic fatty acid synthesis increases and endogenously produced fatty acids begin to occupy desaturase enzymes of the ER. Greater levels of mead and palmitoleic, and other unsaturated members are used as substitutes for dietary PUFA at the 2-position of membrane phosphatides. None of the products of de novo endogenous fatty acid biosynthesis, however, can maintain normal eicosanoid responses. Clinical signs and symptoms and their fatty acid association are listed in Table 5.5.

The monounsaturated fatty acids, myristoleic and palmitoleic, are normally found in very small amounts in human tissues, because the desaturase enzymes have weak affinity for their saturated fatty acid precursors, myristic and palmitic acids. In long-term essential fatty acid deficiency, the low concentrations of ALA and LA free up desaturase enzymes to act on saturated fatty acids, forming myristoleic and palmitoleic acids. Thus, elevated levels of these two fatty acids also indicate long-term EFA deficiency.[122]

Individuals with good fatty acid status have low levels of the n-9 PUFA, mead acid, in plasma. Mead acid can be produced in human tissues by repeated desaturation of non-essential fatty acids. Under conditions of EFA sufficiency, the desaturase enzymes stay primarily occupied by the much more strongly binding n-3 and n-6 fatty acid members. As EFA intake falls, mead-acid production rises, making this n-9 fatty acid a marker for EFA deficiency.[123, 124] Although mead acid cannot participate in eicosanoid formation, it does serve to mimic the membrane fluidity contributions of PUFAs derived from essential precursors. Prior to plasma fatty acid profile abnormalities there is a depletion of PUFA in adipose tissues. When their concentrations reach levels low enough to free up binding sites on the desaturase enzyme, oleic acid will be the first non-EFA to take their place, because of its higher binding affinity relative to the more abundant palmitic acid. Formation of mead acid is the result of this phase. Since decreasing AA is a necessary precursor to mead formation, the ratio of triene (mead acid) to tetraene (AA), also known as the triene-tetraene or T/T ratio, provides a sensitive marker of EFA deficiency. Mead acid may be high with normal AA if there has been a recent intake from AA sources. In long-term PUFA deficiency, palmitic desaturation to palmitoleic increases, yielding abnormally high palmitoleic acid. The

TABLE 5.5 — SIGNS AND SYMPTOMS ASSOCIATED WITH FATTY ACID ABNORMALITIES

Signs & Symptoms	Fatty Acid Association	Intervention
Emaciation, weakness, disorientation	Caloric deprivation	Balanced of fat, protein, and CHO
Reduced growth, renal dysplasia, reproductive deficiency, scaly skin	Classic essential fatty acid deficiency	Good quality fats and oils
Eczema-like skin eruptions, loss of hair, liver degeneration, behavioral disturbances, kidney degeneration, increased thirst, frequent infections, poor wound healing, sterility (m) or miscarriage (f), arthralgia, cardiovascular d., growth retardation	Linoleic acid insufficiency	Corn or safflower oils
Growth retardation, weakness, impairment of vision, learning disability, poor coordination, tingling in arms / legs, behavioral changes, mental disturbances, low metabolic rate, high blood pressure, immune dysfunction	Alpha or gamma linolenic acid insufficiency	Flax, primrose or black currant oils
Depression, anxiety, learning behavioral and visual development or cardiovascular disease risk	Long chain PUFA-dependent neuromembrane function Prostanoid balance	Fish oils Avoid hydrogenated oils
Cancer	Low stearic to oleic ratio, Prostanoid imbalance	n3 PUFAs (use n6 PUFAs with caution)
Rheumatoid arthritis	Low GLA & DGLA	Primrose oil
Myelinated nerve degeneration	Increased very-long-chain fatty acids	High-erucate rape or mustard oils
Fatty liver	Saturated and ω-9 accumulation in liver	Restrict alcohol Add lecithin Increase Met
Accelerated aging	High PUFA intake without increased antioxidants	Vitamin E and C and Se, Mn, and Zn

course of appearance in each case will depend on which dietary sources are most severely restricted.

The clinical significance of these interactions may be seen in the reported increase in infant plasma DHA when the LA/ALA ratio of diet liquid formulas was lowered from 10.1 to 5.1.[125] The higher ratio might be found in people consuming diets high in corn oil. In this study, when the ratio was lowered so that LA (18:2) was no longer so abundant, the ALA (18:3) more easily passed into the pathway for conversion to DHA. This phenomenon has important implications for pregnant women. See the section on "Specific EPA and DHA Deficiency" below for discussion of the effects on childhood development.

Sustained high intake of EFAs can produce elevated mead acid because of peroxisomal stimulation and increased rates of fatty acid desaturation. Since the dominant EFA is normally AA, calculating the ratio of mead to AA provides a correction for the false mead elevation. The T/T ratio has been widely used as a marker of EFA deficiency.[126] Thus, an observation of elevated mead should be linked with a check for concurrently elevated T/T ratio for confirmation of deficiency status.

Of course, when the entire profile of data is available, it is easy to see the pattern of low levels for ALA, EPA, DHA, LA, DGLA, and AA in general EFA deficiency. Figure 5.13 shows a generalized time course for appearance of fatty acid deficiency markers.

All patients with intestinal disorders should be suspected of developing EFA deficiency. Even after being placed on standard treatment protocols, up to 25% of such patients showed signs of deficiency in research studies.[127] Supplementation with n-3 fatty acids have been used in the treatment in cystic fibrosis patients, since malabsorption of PUFAs is a clinical

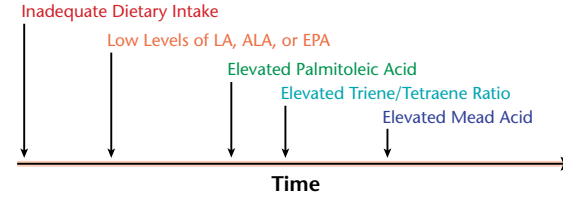

FIGURE 5.13 — Relative Time Course for Appearance of Essential Fatty Acid Deficiency Markers

manifestation.[12, 128] Fish oil supplementation was also found to improve liver and pancreas function, shortened ICU stay and eliminated weight loss in patients undergoing major abdominal surgery.[38] Whether the origin is simple dietary restriction or diseases that reduce intestinal fat absorption, patients may be managed by initial supplementation of dietary oils with high EFA content and by instruction concerning the appropriate intake of foods with high EFA content. Both n-3 and n-6 fatty acids are needed to supply balanced replacement of all PUFAs. In cases of malabsorption due to loss of pancreatic enzyme or hepatic/biliary bile acid secretion, oral therapy with appropriate digestive replacement products may be required.[129]

Omega-3 Deficiency or Excess

Disorders that are specific to the n-3 class of fatty acids are detected by inspecting the levels of ALA, EPA, docosapentaenoic (DPA) and DHA. Deficiency of n-3 fatty acids is the most common area of clinical concern regarding fatty acids. Within this class, however, a patient may show signs of overall n-3 fatty acid deficiency, specific ALA deficiency, or specific EPA and DHA deficiency. In large epidemiologic studies n-3 fatty acids were associated with significant reductions in total mortality, CHD mortality, cardiovascular mortality, and sudden death, even in those patients with no history of cardiac disease.[130-135]

Omega-3 Class Deficiencies — Omega-3 intake in the U. S. is low. Dietary intake of fresh green vegetables or seafood sources of n-3 fatty acids has been low in many countries for multiple generations, resulting in maternally amplified generational effects. The current U.S. total n-3 fatty acid intake is 1.6 g/d, the majority of which is ALA, with only 0.1 to 0.2 g/d coming from EPA and DHA.[136, 137]

The cell membrane content of n-3 fatty acids strongly influences fluidity, as well as the availability to form eicosanoids which influence tissue responses to stimuli. As n-3 fatty acids enter the bloodstream, their presence on lipoproteins sends signals to PPAR that initiate increased production of the entire cellular machinery for fatty acid oxidation. Thus, low intake of n-3 fatty acids has large effects on fatty acid metabolism.

Recognizing the patient who is deficient in the entire class of n-3 fatty acids is a simple matter of finding simultaneous low levels for ALA, EPA, DHA, and the intermediate DPA in results from profiles of fatty acids in plasma, erythrocytes, or whole blood. A plasma fatty acid profile from a typical case is shown in Case Illustration 5.1. This instance also shows the typical pro-inflammatory pattern of elevated AA. The clinical manifestation of this n-3 fatty acid deficiency is one of a chronic inflammatory state rather than one of essential fatty acid deficiency that is associated with the same n-3 fatty acid pattern and low AA. Various combinations of supplemental oils may be used to increase tissue status of these fatty acids. The compositions of most oils used for this purpose are shown in Table 5.2. Repletion of a severely depleted patient may require supplemental oil use over a period of several months. The freshness of food sources is a critical concern because of the great ease of oxidative degradation for these polyunsaturated fatty acids. When dietary supplements are used, they must be kept from air and light, both of which enhance oxidation rates. Plasma levels should show increasing n-3 fatty acid concentrations within 60 days and erythrocyte or whole-blood follow-up testing is best done only after 90 days.

Refer to Case Illustration 5.1

Specific ALA Deficiency — ALA is found in plant sources, but, because of the small amounts of vegetables consumed by most people, it is one of the least abundant essential fatty acids in the average diet. It is found in relatively high amounts in flax, hemp, rape seed (canola), soybean, and walnut oils, and in dark green leaves. It must be supplied by foods, since human tissues lack the enzymes needed for its formation. Dietary insufficiencies and imbalances of ALA and its counterpart, GLA, play a central role in many disease processes. For example, supplementation of n-3 fatty acids was found to increase seizure thresholds and lower inflammatory mediators in patients with epilepsy.[138, 139] The wide range of symptoms and disease associations shown in Table 5.5 is due to the function of this fatty acid in critical cell processes of membrane integrity and eicosanoid local hormone production. The hormone function utilizes EPA, produced from ALA by elongation and desaturation. The various cofactors required for this conversion are dependent on zinc status. Inadequate conversion of ALA into EPA has been used as a marker of zinc insufficiency in animal studies, and is indicated by low EPA and DHA in the presence of normal ALA.[140]

An important fatty acid impact on immune responses is the modulation of adhesion molecules that govern T-cell-B-cell interactions. Levels of soluble vascular adhesion molecule (sVCAM) in dyslipidemic patients were reduced after 12 weeks of supplementation with 15 mL of ALA-rich flaxseed oil per day.[141] Such results provide a basis for the prevention of coronary artery disease by use of PUFA.[141] ALA rich flax may provide an alternative to fish oils to achieve similar long-term cardioprotection.[34] The impact of ALA on heart disease is emphasized by finding that changing the dietary fat fed to hamsters from butter to flaxseed oil resulted in up to 50% lower activities of enzymes that regulate the rate of cholesterol synthesis.[142] This finding, along with epidemiologic evidence, highlights the benefits of a diet rich in n-3 fatty acids, as well as identifying the detrimental effects of a diet high in animal foods that are low in n-3 fatty acids and rich in saturated fats, such as dairy products, beef and pork.[143]

Correction of low body pools of ALA requires dietary changes or the use of dietary supplements containing ALA. When food sources of fatty acids are examined (Table 5.2), it becomes apparent that the n-6 rich fatty acid oils that are so predominant in modern diets, such as corn, safflower, and peanut, contribute to the prevalence of ALA deficiency. Fortunately, there are other food sources for the longer-chain n-3 fatty acids, so humans are not completely dependent on ALA intake for eicosanoid production.

Specific EPA and DHA Deficiency — Insufficiencies of EPA and DHA are likely the most prevalent fatty acid abnormality affecting the health of individuals in Western societies. Low levels in plasma or erythrocytes are indicative of insufficiency. EPA deficiencies have been associated with neurological conditions, arthritis, heart disease, cancer, accelerated aging and autoimmune disorders, presumably as a result of direct or indirect effects of inflammatory responses that may be modulated by raising EPA levels.[134, 144-149] Significant improvements were noted in ADHD, autism, developmental coordination disorder (DCD), learning disabilities and poor cognitive abilities when they were supplemented with fatty acids, primarily EPA-DHA.[150-160]

Research has also provided evidence of the importance of EPA intake in depression or bipolar disorder.[161-163] Since severe depression is associated with general immunoactivation, the general immunosuppressive effect of EPA may be involved. In addition, the ability of EPA to suppress the HPA axis, reversing multidrug resistance, may allow more effective action of standard therapy with dexamethasone.[164] Furthermore, EPA inhibits the synthesis of PGE_2, decreasing this inducer of endogenous and synthetic steroids.[163] When 1 g/d ethyl-EPA was given to inpatients who remained depressed despite adequate standard therapy, 88% had significantly better outcome than the placebo group on three rating scales.[165]

EPA is the parent of the 3-series eicosanoids that moderate the pro-inflammatory effects of the 2-series derived from AA. An entire generation of anti-inflammatory drugs, the COX-inhibitors, is based on compounds that block the cyclooxygenase system. Side effects are generated by the overriding of normal cellular controls for regulating the pathways either up or down in specific tissues to generate appropriate local responses. Optimizing body composition of PUFAs is a way of dealing with excessive inflammatory signals at the level of precursor supply. Fatty acid derivatives with immunosuppressive activities continue to be discovered in natural oils. Oleocanthal from newly pressed extra-virgin olive oil has ibuprofen-like activity for inhibition of cyclooxygenase enzymes.[166] The endocannibinoids add another powerful layer of control exerted by balanced tissue composition of EPA and DHA (see "Fatty Acid Metabolism" above). Favorable effects of DHA supplementation on cancer is proposed to be related to changes in levels of inducible nitric oxide synthase (iNOS).[167] Intake of EPA is generally poor, and although it can be produced from the essential fatty acid ALA, its supply can be limited by concurrent low ALA intake.[136, 137, 168, 169] The conversion of ALA to EPA requires the action of the Δ^6d enzyme, which is critically dependent on adequate zinc and inflenced by low status of magnesium, or vitamins B_3, B_6, and C.[59, 60, 140, 170] Such enzyme impairment would be suspected if plasma or erythrocyte EPA is low and ALA is normal or high. High levels of saturated, monounsaturated or *trans*-fatty acids and defects in desaturase enzymes also slow the conversion of ALA to EPA (as well as GLA to DGLA).[171] Conjugated linoleic acid (CLA) depresses Δ^6 and Δ^9d activity without changes in gene expression.[172, 173] Patients under treatment with CLA may show low conversion of ALA to EPA.

Since both EPA and DHA are critical for vascular system function, a single value that represents the sum of both fatty acids has been proposed as a useful marker

for coronary heart disease, the Omega-3 Index.[133, 174] In meta-analysis, when results were expressed as percentage of the total fatty acids, subjects with a sum of EPA and DHA above 8% had a 90% risk reduction in sudden cardiac death compared to lower values of the sum.[131, 174, 175] Healthy controls were found to have 20% lower values for the sum of EPA and DHA in blood cell membranes than patients with acute coronary syndromes (ACS). In this study, the odds ratio for an ACS event was 0.31 (p for trend <0.0001) in the highest EPA+DHA group compared with the group with the lowest sum.[176] In patients with type 2 diabetes, fish oil supplementation was found to decrease plasma triglycerides, improve n-3 fatty acid status and in some studies reduces insulin response to oral glucose.[177, 178]

DHA may protect against emotional disorders as well as against heart disease. Increased hostility is associated with folate deficiencies and heart disease. Insight about a possible connection between these associations and fatty acids was provided by a study of blood concentrations of folate and DHA in hostile and aggressive subjects. The finding of a strong, direct relationship between folate and DHA was explained as being linked through phosphatidyl choline (PC) formation. Membrane levels of PC are closely related to the ability of hepatocytes to export DHA, and the methylation of phosphatidyl ethanolamine to form PC is strongly dependent on folate adequacy.[179, 180] Fish oil sources of EPA, are shown in Table 5.6.

The growth and development of the central nervous system are particularly dependent on the presence of an adequate amount of the very-long-chain, highly unsaturated fatty acids DPA and DHA.[112, 181] ADHD and failures in development of the visual system in essential fatty acid deficiencies are two examples of this dependency.[182] Adult ADHD has been found to be associated with lower DHA levels,[183] but not all studies show positive effects of supplementation on symptoms once the disorder is present.[184, 185] DHA is an important member of the very-long-chain fatty acids (C22–C26) that characteristically occur in glycosphingolipids, particularly those in the brain. Since this fatty acid is so important in early development, it is worth noting that the levels in breast milk are correlated with the mother's intake of fish oils, which are rich sources of DHA and DPA.[186] Plasma DHA levels should be checked as early as possible in pregnancy. In a functional shortage of DHA, the body synthesizes the most comparable long-chain PUFA in the n-6 family, osbond acid (22:5n-6). The ratio between DHA and osbond acid may therefore be a reliable indicator of functional DHA status.[187] Babies born to mothers supplemented with fish oil during pregnancy had higher DHA, which correlated with lower levels of osbond acid at birth.[188]Animal studies have also shown a deficicney of ALA can reduce DHA and increase osbond acid.[189] The finding of low DHA in plasma or erythrocytes also justifies the use of supplemental DHA for treating depression.[190] Patients with autosomal dominant retinitis pigmentosa (RP) were found to have much lower RBC levels of DHA.[191] The visual process involves rapid cycling of DHA within the membrane to accommodate the binding of DHA to retinoid-binding protein. Studies

Table 5.6 — EPA and DHA Content of Seafood

Food	EPA	DHA	EPA/ DHA	Total n-3
Salmon, atlantic, farmed	0.62	1.29	0.48	2.01
Salmon, atlantic, wild	0.32	1.12	0.29	1.73
Herring, pacific	0.97	0.69	1.41	1.72
Anchovy, european	0.54	0.91	0.59	1.45
Salmon, chinook	0.79	0.57	1.39	1.44
Bluefish	0.25	0.52	0.48	0.77
Shrimps, mixed species	0.26	0.22	1.18	0.49
Halibut	0.07	0.29	0.24	0.43
Tuna, yellowfish	0.04	0.18	0.22	0.23
Cod, atlantic	0.06	0.12	0.53	0.19

Notes:

have found supplementation with DHA and vitamin A slowed the decline of RP.[192, 193] Thus, long-chain PUFAs modulate metabolic processes and attenuate effects of environmental exposures, leading to the pathogenesis of vasoproliferative and neurodegenerative retinal diseases.[194]

Supplementation of DHA also produced significant increases in all phospholipid fractions of plasma, RBC, and mucosal specimens in cystic fibrosis (CF) patients.[128] The product of plasma linoleic times DHA concentrations has been proposed as an alternative to the sweat chloride test for distinguishing CF patients from other disorders.[195] This product is consistently low in CF patients in both non-blinded and blinded trials as shown in Figure 5.14.

The sluggishness of DHA production from ALA, as a result of the transfer from the endoplasmic reticulum to peroxisomes, explains why the desaturation of both n-3 and n-6 fatty acids in humans is quite slow beyond the Δ^5d step that produces EPA.[196] Thus, dietary ALA added during and after pregnancy is ineffective in improving neonatal DHA.[64, 197] Intake of hydrogenated oils contributes to the problem, because *trans*-fatty acid intake is associated with lower essential fatty acid intake, especially linoleic acid, and these factors are thought to interfere with DHA synthesis.[190]

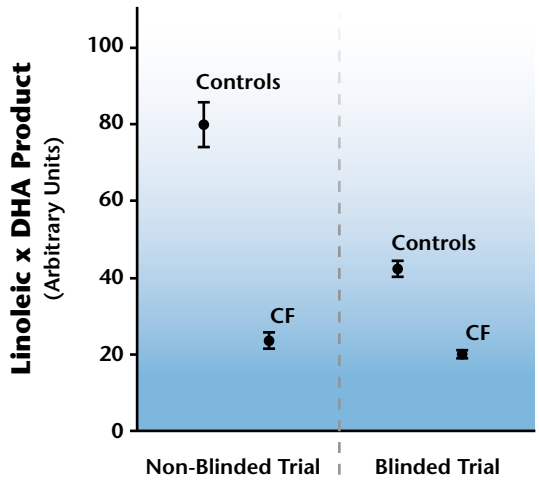

FIGURE 5.14 — **The Product of Linoleic x DHA for Diagnosis of Cystic Fibrosis**

Plasma fatty acid profilng was used to measure concentrations of fatty acids in patients with confirmed CF and controls, and the product of linoleic (18:2 n-6) times DHA (22:6 n-3) was calculated. Mean values for 9 controls and 10 CF patients are shown with standard error bars. Both non-blinded and blinded trials produced marked, consistently significant lower values for the linoleic x DHA product in patients.[195]

Improving neonatal DHA status presents a critical challenge, since this fatty acid is required for brain development,[198] and overall maternal essential fatty acid status tends to decline steadily during pregnancy. Pregnant women have lower levels of EPA and DHA and higher levels of palmitate in both plasma and erythrocytes.[199] The transfer from red cell membrane to fetus may be a special mechanism for ensuring fetal supply of EFA. Under the prevailing dietary conditions, mothers may be unable to meet the high fetal requirement for EFA.[200] Meanwhile, the mother is more likely to experience postpartum depression related to degradation of EFA status.[201]

ALA or EPA Excess — The risk of excessive n-3 fatty acid supplementation has risen along with the awareness of its health benefits. It is important for clinicians to realize the possibility of excessive use of flax or fish (or other n-3) oils can produce a condition described as n-3 dominance. It is detected as high absolute concentrations of the n-3 fatty acid class members with lowered AA. The laboratory report will show elevated EPA/ DGLA and depressed AA/EPA ratios. This imbalance can produce a lowering of peak levels of class 2 eicosanoid signals such as leukotriene A2 in response to distress. Flax oil can produce high plasma ALA without high EPA if the Δ^6d enzyme is inhibited. If fish oil has been used in excess, ALA may be in the normal range, whereas EPA and DHA are greatly elevated.

Several concerns have been raised in regards to n-3 fatty acid supplementation. Though it has not been systematically studied, there have been anecdotal reports of patients with excessive supplementation having difficulty overcoming infection. In placebo-controlled studies of prescription omega-3 ethyl ester supplements (4 grams/day) the incidence of flu symptoms, infection and rashes were 2 to 4 times the rate in control subjects, who had been given corn oil, as placebo.[202, 203] These results are consistent with a blunting of the immune response due to reduced production of series 2 eicosanoids. It is suspected that the incidence of effects would be even greater in the subset of individuals who had higher initial levels of n-3 fatty acids. Researchers have questioned the increased risk of bleeding with supplementation,

though clinical evidence has not supported this.[204, 205] There is also a debate over the potential for toxic mercury accumulation from fish extracts, as well as the risk from carcinogenic contaminants stored in adipose tissue of fish,[206,207-209] though current evaluations indicate that their health benefits outweigh the potential harm.

Another effect of the n-3 fatty acid dominant condition is increased risk of oxidative damage and elevated serum lipid peroxides. Serum lipid peroxides have been shown to be elevated in patients with heart disease,[210] and the association of higher lipid peroxides with rising triglyceride level has been proposed as a mechanism for the origin of atherosclerosis.[211] In a general outpatient population, including those heavily supplementing with fish oils, the levels of serum lipid peroxides tend to rise in proportion to serum PUFA concentrations.[212] Though it is generally important to increase the intake of antioxidants to confer protection from increased lipid peroxides,[213] oxidative stress should be also be monitored by assessing fatty acid and antioxidant nuttrients.[214] Combinations of dietary antioxidants and PUFA treatment has been proposed in the treatment of multiple sclerosis.[14] Depletion of vitamin E may contribute to the EFA deficiencies reported in CF.[215]

Possible increases in serum lipid peroxides provide reason to exercise caution regarding the possibility of exceeding antioxidant protection when PUFAs are supplemented, even though increased hydrogen peroxide production within hepatocytes has been proposed as a mechanistic explanation of the LDL-lowering effects of fish oil supplements.[216] It should not be assumed that there are beneficial effects of elevated serum lipid peroxides, because a specific organelle exhibits beneficial effects from locally produced oxygen radicals. A report that in vivo oxidant stress is reduced without changing markers of inflammation when hypertensive, type-2 diabetic subjects are treated with fish oils may be taken to indicate that other types of patients will respond similarly.[217] The patients in this study were not evaluated for PUFAs, and their history would predict an initially depleted n-3 fatty acid status. It should be noted that, at some point, increasing intake of n-3 fatty acids is unadvisable. Laboratory results may be used to discern such a point by demonstrating the n-3 dominant scenario.

When the n-3 fatty acid dominant pattern is found, the patient should be advised to decrease or discontinue the use of n-3 fatty acid enriched oils. Plasma levels of ALA and EPA may be monitored to determine the return to normality that usually occurs within 3 months of restricted intake. Case Illustrations 5.2 to 5.4 show relevant data from plasma fatty acid laboratory reports where such a tendency was found in three cases with different clinical presentations.

Refer to Case Illustrations 5.2–5.4

Omega-6 (n-6) Class Deficiency or Excess

The great weight of clinical significance of the n-6 fatty acid family arises from a high intake of LA and AA because of the heavy use of LA-rich oils in food preparation and the practice of fattening cows on LA-rich grains before slaughter. The beef from such cows tends to be high in AA and low in ALA and EPA, because the cows were not allowed to mature by grazing on grasses.[218] For patients with an n-6 fatty acid deficiency, the use of n-3 fatty acid rich oils or foods can exacerbate clinical outcomes by competing for the desaturase enzymes. The condition of such patients may be improved by adding n-6 fatty acid rich oils, such as evening primrose oil, instead. Alternatively, a low LA can be improved with the commonly available LA-rich oils or by correcting fat malabsorption.

Omega-6 Class Deficiency — LA is by far the most abundant polyunsaturated fatty acid in most human tissues. It is one of the EFAs because it contains a double bond at the n-6 position, which lies beyond the reach of the human desaturase enzyme. Low levels indicate dietary insufficiency, which can lead to a variety of symptoms (Table 5.3). Some of these symptoms result from a lack of linoleic acid in membranes, where it has a role in structural integrity. Most, however, result from failure to produce the 1-series and 3-series local hormones known as prostanoids. The EPA/DGLA ratio is used to assess the

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overall balance of n-3 fatty acids (series 3 eicosanoids) to n-6 fatty acids (series 1 eicosanoids). Eicosanoids are a group of oxygenated fatty acids that are produced by different cell types and regulate many physiological and pathological functions. DGLA undergoes oxidative metabolism by cyclooxygenases and lipoxygenases to produce anti-inflammatory eicosanoids. Since LA serves as the starting point for this pathway low LA intake almost universally is accompanied by low DGLA. The 1-series prostanoids and leukotrienes are derived from DGLA, so an insufficiency of this fatty acid impairs a wide range of cellular functions and tissue responses. The 1-series compounds act like the 3-series derived from ALA to moderate the proinflammatory 2-series. In tumor response, however, they uniquely serve as promoters of growth, whereas the 3-series are inhibitors. When testing reveals low levels of DGLA, supplementation with borage or evening primrose oils should be considered, but, if there is a history of tumor formation, consideration should be given to ALA sources (black currant oil) as well.

GLA is the precursor of DGLA.GLA is found in hemp, borage, black currant and evening primrose oils. It can be produced in human tissues by the action of desaturase enzymes on LA. GLA has been found to be beneficial in immune disorders.[35] In controlled trials, treatment with GLA produced significant improvement in swelling, tenderness and morning stiffness of rheumatoid arthritis.[219,220]

Although the n-6 fatty acids, and especially LA, are overabundant in modern diets, it is important to recognize that the clinical imperative is to identify PUFA imbalance. Except for analysis of a quantitative diet history, the best way to know whether a patient suffers either n-6 fatty acid deficit or excess is to perform a laboratory evaluation of fatty acid status. The data can then guide interventions that sometimes call for adding, rather than restricting, dietary LA sources.

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In cases of cancer, it is important to evaluate status before supplementing with GLA because high levels of n-6 fatty acids may enhance tumor formation and growth.[221] LA has also been shown to inhibit the growth of *Helicobacter pylori*, the bacterium thought to cause gastric ulcer.[222] Another time of concern for assuring adequate fatty acid status is pregnancy. Research has found normal neonatal status of LA to be marginal, if not insufficient.[123] Fetal LA is correlated with maternal RBC levels.[223]

Omega-6 Class Excesses — Since its dietary sources (especially corn oil) are abundant, LA may be found at above-normal levels in some adults. Excessive LA can contribute to an overproduction of the pro-inflammatory 2-series local hormones derived from AA. The AA/EPA ratio is used to evaluate the level of n-6 fatty acid (AA) in relation to the amount of n-3 fatty acid (EPA). A high ratio has been negatively associated with several medical conditions.[224] Because of the prevalence of corn and corn oil products in feed for cattle and hogs, these meats are also high in AA. Diets high in these red meats are rich in AA, especially in older animals with higher fat content.[225, 226] Very lean beef contains higher EPA content, so it does not have such a large effect on increasing the dietary AA/EPA ratio.[227] AA is a 20-carbon or eicosanoate fatty acid that serves as substrate for the cyclooxygenase and lipoxygenase enzymes, leading to the production of the 2-series prostanoids and leukotrienes. Several of these products have potent pro-inflammatory and thrombogenic activity. Extensive research has identified the neurological effects of arachidonic acid,[228-231] which may be due to arachidonic acid's production of PGD2 from cyclooxygenase. In the brain, the outcome can be alterations in function on multiple levels. For example, the sleep-disrupting effects of mental stress are normally related to higher levels of epinephrine, cortisol, energy expenditure and plasma-free fatty acid concentrations. Research studies have noted the connection between adrenal activation and fatty acids. When men were supplemented with 7.2 g/d fish oil for 3 weeks, their adrenal activation elicited by a mental stress was dimished, presumably through effects exerted at the level of the central nervous system.[232] When children with ADHD were supplemented with graded doses of EPA-DHA concentrates so that their plasma AA:EPA ratios were sustained below 1.0, both psychiatrist-reported assessment of behavior and global

severity of illness scores improved significantly.[233] Decreases in clinical symptoms of depression were also associated with lower values of the EPA/AA ratio in both erythrocytes and plasma.[234] Thus some of the benefits of increasing n-3 fatty acid levels may also come from the concurrent decrease in the AA/EPA ratio. A low AA/EPA ratio is often seen in n-3 fatty acid dominance and may give guidance in decreasing fish oil supplementation.

High AA has also been found to promote gallstone formation by stimulating mucin production in the gall bladder mucosa.[235] Elevated AA is a frequent finding in profiles of fatty acids in plasma or erythrocytes as exemplified in Case Illustration 5.1. Such a finding should trigger counseling of the patient regarding dietary AA reduction and appropriate supplementation to restore n-3/n-6 fatty acid balance.

When n-6 dietary fatty acids are constantly supplied in overabundance, the intermediate products of further desaturation and elongation are not utilized as fast as they are produced. Under these conditions, the process of modification can continue through docosadienoic acid (22:2n6) to the 22-carbon-atom, 4 double-bonded docosatetraenoic acid (22:4n6). These fatty acids can then accumulate in adipose tissue.

Hydrogenated Oil Toxicity

Trans-fatty acids are prevalent forms of *trans*-fatty acids in the diet because of the widespread use of hydrogenated oils in the food manufacturing process. These fatty acids contain one double bond, and thus are included in the unsaturated category. Because of the geometry of the *trans* bond, however, they behave like saturated fats on the one hand, leading to higher cholesterol levels.[236] On the other hand, they mimic unsaturated fats in binding to desaturase enzymes and interfering with the normal production of critical products. The net effect is to raise plasma LDL and lower HDL.The growing consensus among experts is that foods containing hydrogenated oils are to be avoided. The toxic effects of *trans*-fatty acids have been shown to significantly contribute to the development of atherosclerotic disease, as well as having negative effects on cancer risk.[237-243]

The bacteria in the gut of ruminant animals can produce *trans*-fatty acids, which is the reason that beef and milk contain small amounts (1 to 3%) of elaidic acid. Dietary *trans*-fatty acid contributed by milk products is of little concern. A person whose plasma or erythrocyte levels of *trans*-fatty acids are elevated should avoid foods containing hydrogenated oils. Foods that generally contribute the greatest amounts of *trans*-fatty acids include stick margarine, hydrogenated peanut butter, and bakery products such as breads, rolls, cookies, crackers, pies, and cakes. Product ingredient labels that list hydrogenated or partially hydrogenated oil should be avoided.

The principal *trans*-fatty acid in most foods containing hydrogenated oils is elaidic acid (18:1t-n7).There are two other 18-carbon positional isomers of elaidic acid, which are called petroselenic and *trans*-vaccenic acids. Since the clinical impact of *trans*-fatty acids is governed by their total-body status, it is expedient for laboratories to report the total C18 *trans*-fatty acid concentration rather than to attempt to isolate each isomer. After these three isomers, the next most abundant *trans*-fatty acid is palmitelaidic acid (16:1t-n7). The exact food composition of the *trans* isomers depends on the source of seed oil involved. However, overall status of *trans*-fatty acids is reliably assessed by measuring the predominant 18- and 16-carbon members.

Micronutrient Deficiencies

Patterns of fatty acid abnormalities can indicate specific vitamin or mineral deficiencies because of the cofactor requirements of enzymes for fatty acid metabolism. In many of the fatty acid metabolic pathways, the fluxes are so large that abnormalities in fatty acid levels can be highly sensitive markers of early-stage nutrient insufficiency. Small changes in functional levels of nutrients can quickly produce large abnormalities in fatty acids, because the rate of accumulation is relatively great.

Zinc Deficiency — Since desaturase enzymes have specific requirements for zinc and the binding constant is not extremely high, their activity falls dramatically in early stages of zinc deficiency. The result is an abnormal disparity between the concentrations of their substrates and products. The Δ^6d step required for conversion of LA to DGLA is usually the highest flux pathway, so an elevation of the LA/DGLA ratio is a sensitive marker for zinc deficiency. The intermediate fatty acid in this pathway, GLA corrects most of the biological effects of zinc deficiency, indicating that the requirement of the Δ^5d enzyme for zinc is a first-order essential function of zinc.[58, 244] The sign of elevated LA/DGLA ratio is illustrated in Case Illustration 5.5, where a concurrent

erythrocyte elemental analysis confirms the low total-body status of zinc. In cases where LA-rich foods are restricted and extra flax oil is used, the ALA/EPA ratio may also be elevated because that conversion has become a dominant pathway requiring zinc.

REFER TO CASE ILLUSTRATION 5.5

Copper Deficiency — A single report has appeared noting significant shifts in erythrocyte fatty acid patterns in copper-deficient rats.[245] Levels of stearic and docasadienoic acids were higher, whereas oleic and linoleic acids were depressed.

Vitamin B_{12} or Biotin Deficiency — The ratio of vaccenic acid to palmitoleic acid, found to be significantly lower in biotin- deficient rats compared with controls, has been reported to be a possible important indicator of biotin deficiency.[246] Vaccenic acid also seems to have large effects on membrane fluidity, possibly due to the fact that the n-7 double bond does not align with the much more abundant n-9 positions of the majority of fatty acids present in the membrane. Inhibition of tumor growth in cell culture has been reported for vaccenic acid.[247]

Fatty acids with odd numbers of carbon atoms are produced primarily by initiating the synthetic pathway with the 3-carbon compound, propionic acid. Vitamin B_{12} is required for the conversion of propionate into succinate.[248] A deficiency of vitamin B_{12} results in the accumulation of propionate and a subsequent buildup of the odd-numbered fatty acids. The association between vitamin B_{12} and abnormal fatty acid synthesis provides a rationale for the neuropathy of cobalamin deficiency. Odd-chain fatty acids would build up in membrane lipids of nervous tissue, resulting in altered myelin integrity and demyelination, leading to impaired nervous system functioning.[249]

Normal fatty acid biosynthesis also depends on biotin cofactor availability. The initiation of fatty acid synthesis through methylmalonyl-CoA is a biotin-dependent step. In biotin deficiency, the rate at which acetyl-CoA is converted into malonyl-CoA is reduced, allowing propionate to enter in greater amounts. Elevated levels of odd-chain fatty acids have been shown to occur in human plasma phospholipids and in plasma, heart, and liver in experimental animals with biotin deficiency.[250, 251]

The bacteria in the gut of ruminants produce large amounts of propionate, which is absorbed and enters the metabolism. Consequently, the intake of animal and dairy products favors higher levels of odd-numbered fatty acids. Alternatively, it is possible that the bacteria in the human gut could produce sufficient amounts of propionate to lead to an elevation in the odd-carbon fatty acids,[252] a situation that would occur only under conditions of significant gut dysbiosis.

Metabolic and Genetic Disorders

Any of the numerous enzymes and organelle systems involved in fatty acid metabolism are susceptible to gene alterations. Specific patterns of fatty acids may be used to detect their presence, and, in some cases, to direct effective interventions to offset the proteomic alteration.

Hypertriglyceridemia — A plasma fatty acid profile is an amplification of the standard serum triglyceride analysis. In both cases, the fatty acids are present in lipoprotein particles. Instead of measuring only the total of all fatty acids present, the profile test reports the individual concentrations of each fatty acid. An individual with high serum triglycerides will have higher levels of many individual fatty acids in plasma. Of course, disorders associated with elevated serum triglycerides will likewise be associated with elevated plasma fatty acid concentrations. Patterns of the high concentrations in plasma will depend on average dietary intake for the past several weeks, rates of metabolic conversion from precursor to product fatty acids, and the type of fatty acids in long-term adipocyte storage. Plasma is the preferred specimen for routine evaluation of the balance of fatty acids being delivered to the tissues for energy and maintenance. Interpretation of plasma profile data is less complicated by metabolic issues and is useful for determining dietary changes needed to restore balance.

When plasma fatty acids are reported in absolute concentration units (micromole/liter) the presence of hypertriglyceridemia is generally obvious because multiple members of all fatty acid classes are elevated. Another commonly used unit, percent of total fatty acids, does not show these elevations, because the individual fatty acids rise in proportion to the total. This situation illustrates the debate over the preferred unit for reporting. The loss of sensitivity in detecting multiple important fatty acid patterns in the non-hyperlipidemic population

is a significant disadvantage to using the percentage units. Further information on the diagnosis and management of hypertriglyceridemias can be found in standard medical textbooks.

Relatively small amounts of fish oil can have beneficial effects on plasma triglyceride levels in hypertriglyceridemic patients.[253] Oleic acid is the principal lipid that makes LDLs resistant to oxidation, and thus a diet rich in this fatty acid reduces foam cell accumulation rates, and thereby lowers the chances of atherosclerosis.[254] Although the principal concern with dietary fatty acids is adequate levels of the PUFAs, dietary saturated fat may be monitored for another index of risk of disease, especially cardiovascular disease. The Baltimore Longitudinal Study of Aging has reported that the combination of high fruit and vegetable and low saturated fat intakes is protective against mortality in aging men.[255]

The Metabolic Syndrome — Increase in serum triglycerides and cholesterol is one of the earliest manifestations of elevated insulin that is characteristic of the metabolic syndrome.[256] Carbon atoms from glucose are being forced to enter the lipid biosynthetic pathway, increasing the relative abundance of endogenously produced fatty acids.[257] The fatty acid synthase system releases palmitic acid that is easily elongated to stearic and arachidic acids.[258] Since fatty acids of shorter chain length are not produced efficiently, and the very-long-chain members are likewise not proportionately increased due to the lower affinity of the elongase enzymes, a characteristic pattern of saturated fatty acids on plasma profiles tends to develop. On a plasma fatty acid profile it can be seen as a pattern of high long chain fatty acids (palmitic, stearic and arachidic) while medium chain (capric, lauric, mystic) and very long chain saturated fatty acids (behenic, lignoceric, and hexacosanoic) are low. When viewing the bar graphs in the saturated fats section from afar, one can see this pattern, similar to a 'greater than sign' (see case study 5.6). The same chain-length pattern may be found in the monounsaturated and odd-chain classes, since they respond to similar endogenous synthesis factors. Because of diabetic-induced effects on fatty acid transport and metabolism, different patterns may be found in erythrocyte profiles.[259] The proportion of saturated fatty acids to the total of all fatty acids in plasma has been found to be positively associated with diabetes incidence.[260] The liver can convert fatty acids into cholesterol. Although any fatty acid can enter this pathway, palmitic acid is the most stimulatory one known, and high levels lead to increased serum cholesterol, and thus to increased risk of atherosclerosis, cardiovascular disease, and stroke. Most other fatty acids are either cholesterol neutral or have reverse effects, as in the case of EPA. Palm kernel and coconut oils are rich sources of palmitic acid. Palmitic acid synthesis is stimulated by hyperinsulinemia and by diets high in simple carbohydrates.

Refer to Case Illustration 5.6

Multiple Acyl-Coenzyme A Dehydrogenation Disorders — The medium-chain saturated fatty acids, capric, lauric, and myristic, accumulate in the fatty acid catabolic disorders known as medium-chain acyl-coenzyme A dehydrogenation (MCAD) disorders.[261] A common scenario is a previously healthy child who presents with hypoketotic hypoglycemia, vomiting, and lethargy triggered by a common illness. In addition to the abnormalities found in plasma fatty acid profiles, the C6 to C10 carnitine acyl fatty acid esters accumulate in plasma. An additional confirmatory test is demonstration of adipic (C6) and suberic (C8) elevations in urinary organic acid profiles.[262]

Metabolic beta-oxidation disorders where fatty acids of all chain lengths are affected are called multiple acyl dehydrogenase deficiency (MAD). Because of a common clinical sign, these disorders have also been called glutaric aciduria type II. Late-onset cases may present with profound muscle weakness and signs of ethylmalonic and adipic aciduria and low muscle carnitine. Because massive excretion of multiple organic acids is frequently found, organic acid profiles of urine may be the most sensitive way of detecting these disorders.[263] Patients may function normally prior to stressful infections or other life events. Dietary fat restriction and supplementation with riboflavin and carnitine are effective therapies.[264] Some of these enzyme defects are responsive to riboflavin at levels far above normal intakes.[265] Thorough investigation of biochemical effects in one case showed that riboflavin therapy produced coordinated and reversible improvements in multiple riboflavin-dependent enzymes, concurrent with reversal of symptoms.[266] With the widespread availability of biochemical assessment, the prevalence of late onset MAD is increasing because of more accurate diagnoses.[267, 268]

Adrenoleukodystrophy (ALD) — Lack of peroxisomal activity resulting in the accumulation of very-long-chain fatty acids is associated with degenerative diseases of the central nervous system such as adrenal leukodystrophy. Specifically, X-linked adrenoleukodystrophy (X-ALD) is an inherited disorder of peroxisomal fatty acid oxidation in which the erythrocyte hexacosanoic acid level becomes greatly elevated. Treatment with a mixture of olive and canola oil containing predominantly glycerol esters of oleate and erucate were reported to have beneficial effects.[269] Subsequent studies have failed to substantiate the benefit on disease outcomes[270, 271] except in asymptomatic boys.[272]

There are a large number of known genetic disorders involving the accumulation of sphingolipids, which is usually due to the lack of enzymes needed to maintain the turnover of membrane components. Behenic, lignoceric, and hexacosanoic acids, as well as the unsaturated members of the C22 to C24 classes, especially nervonic, are found elevated in such cases. A laboratory report showing specific elevation of VLCFA is shown in Case Illustration 5.7.

REFER TO CASE ILLUSTRATION 5.7

Erucic acid is apparently one of the components responsible for the favorable response of individuals with adrenal leukodystrophy to preparations containing canola and mustard seed oils.[273] Other studies using Lorenzo's oil containing glycerol trierucate revealed no appreciable changes in brain-lipid content.[271] In Zellweger syndrome, in which peroxisomes are absent, erucic and adrenic (docosatetraenoic) acids accumulate. It is speculated that either the anabolic enzymes are inhibited from producing sphingolipids or the catabolic enzymes are stimulated to faster clearance of the offending products.[274]

Nervonic acid contains the longest chain of carbon atoms of all monoenoic fatty acids present at appreciable levels in plasma and erythrocytes. The unique structure of nervonic acid confers special properties to nerve membranes, where it is found in highest concentrations. The myelin sheath is especially rich in nervonic acid. Nervonic acid accumulation in red blood cells yields information on cerebrum maturation in premature infants.[275]

Fatty Acid Ratios and Indices

Red-Cell Stearic/Oleic Index

Oleic acid constitutes 15% of the fatty acids in erythrocyte membranes. In tumor cells, stearic-acid falls as oleic-acid rises, causing a profound shift in the ratio of stearic to oleic acids.[276] The stearic-oleic ratio is used as a monitor of the effectiveness of cancer therapy in certain types of cancer.[277] The shift to higher unsaturation of membrane fatty acids produces increased fluidity of the tumor cell membrane, resulting in more rapid movement of nutrients and waste products and allowing for faster metabolic rate.

Modulation of endogenous desaturation rates is involved in controlling cell proliferation and apoptosis.[278] Increased Δ^9d activity in tumor cells shifts the relative abundance of saturated and unsaturated fatty acids, causing lowered saturated and elevated monounsaturated members that is most highly significant when expressed as the stearic/oleic ratio.[278-280] More specifically, the ratio of stearic to oleic acid in red-cell membranes has been found to be a strong indicator of the presence of malignant tissue, as it reflects the lowered ratio found in malignant tissue-cell membranes.[281, 282] Stearic/oleic values below 1.1 are associated with the presence of malignancy. The ratio was found to respond to hormonal therapy for prostatic cancer, and, in studies following surgical cures, individuals who maintained this ratio above 1.1 had no tumor recurrences. The stearic-oleic ratio, sometimes referred to as a saturation index, is also lowered in carcinoma of the gallbladder.[283] Colonic adenocarcinoma cells display increased stearic acid synthesis with elevated stearic-oleic ratios.[284] The ratio is not a general cancer marker, since some cancers display opposite effects. Movement of a low ratio to higher levels provides an indicator for efficacy of a cancer therapy.

The Polyunsaturated to Saturated Fatty Acid Ratio (P/S)

Originating primarily as a measure of the quality of dietary fats, the PUFA to saturated fatty acid ratio is sometimes used also as a general measure of physiological fatty acid balance. This ratio increases markedly during exercise, as the saturated fatty acids are preferentially utilized as energy sources, causing plasma levels to fall relative to the unsaturated fatty acids.[285] The availability of individual fatty acid data allows the inclusion of *trans*-fatty acids in the saturated group, since their

physiological behavior is the same. This has not normally been done in the food industry to date, resulting in the posting of misleadingly favorable ratios on food labels. The overall effect of higher levels of the polyenoic fatty acids is the suppression of hepatic lipogenesis.[286]

Total Fatty Acid Concentration (Calculated)

The correlation of serum trigyceride levels with plasma saturated fatty acid concentration has been discussed. A tighter correlation may be found by calculating the sum of measured concentrations in a comprehensive fatty acid profile. Widely used treatments for elevated triglycerides involve dietary restriction of fats, the use of pharmacological doses of niacin, and lipid-lowering drugs, depending on the class of hyperlipidemia found by studies of lipoprotein subclasses and cholesterol levels.

When total fatty acids are low, chronic stimulation of peroxisomal oxidation of fatty acids may be present. A number of structurally diverse chemicals have been found to cause proliferation of peroxisomes in liver and other tissues.[287] Paradoxically, high-fat diets also cause such proliferation. The increased oxidative activity leads to higher levels of hydrogen peroxide and development of liver tumors in laboratory animals.[288] In humans, the related disorder, hypobetalipoproteinemia, is associated with increased risk of a variety of cancers and pulmonary and gastrointestinal diseases.[289]

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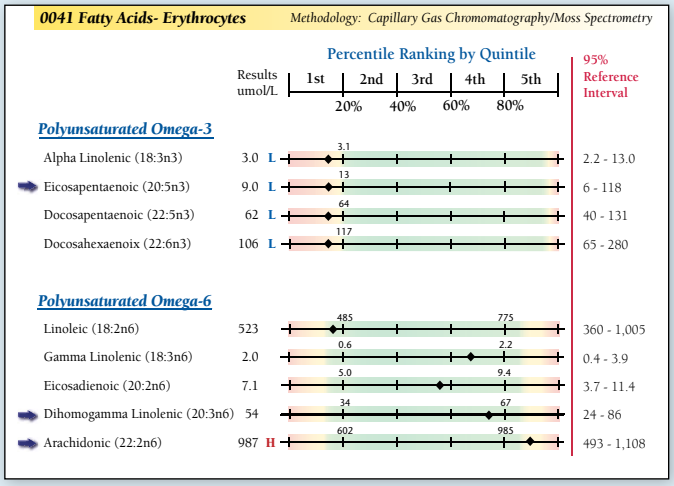
Case Illustrations

Case Illustration 5.1 — A Pro-Inflammatory Pattern

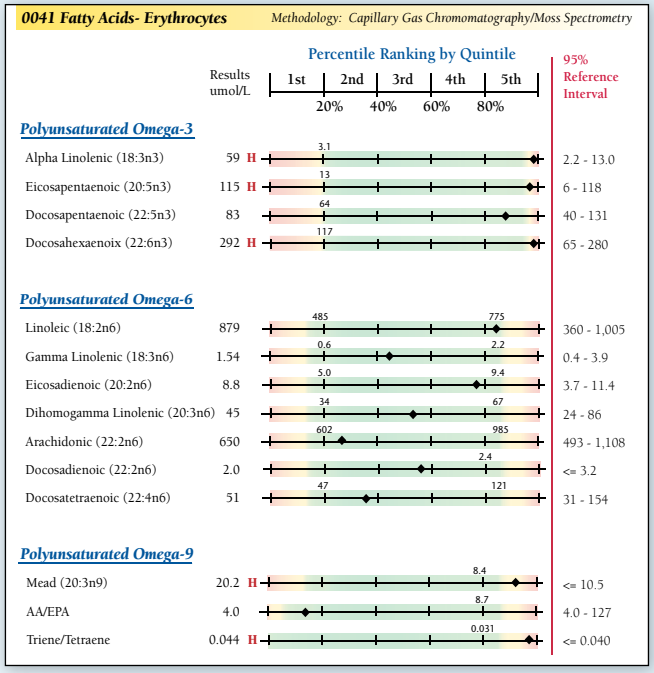
The only information received from the clinician in this case is that the patient is a 78-year-old male. However, the pattern of generally low n-3 fatty acids, including 1st quintile EPA with elevated AA suggests that this patient will have trouble controlling inflammatory response signals.

The abundance of AA and the relative paucity of EPA to supply substrates for the cyclooxygenase and lipoxygenase enzymes cause over-production of the more aggressive series 2 eicosanoids. ❖



Case Illustration 5.2 — Omega-3 Dominant Pattern 1

This pattern was found in a 54-year-old male who, prior to specimen collection, had been eating salmon 4 to 5 times per week for 1.5 months plus supplementing with 800 mg of fish oil and 600 mg of flax oil per day. All four members of n-3 fatty acids are found in the fifth quintile.



The presence of fifth quintile LA with lower second quintile AA shows a significant suppression of n-6 desaturation. The AA/EPA ratio is in the first quintile, but not yet below the 2.5% cutoff for abnormality. However, continuing this level of n-3 oil supplementation can further suppress AA formation to generate a very low AA/EPA ratio.

The concurrent finding of fifth quintile mead acid and very elevated T/T ratio indicates an additional stimulation of peroxisome oxidation by EPA. There are no clear adverse clinical consequences at this stage, but the data provides a warning of the potential for crossing into clinically suppressed AA status and various consequences of sustained high peroxisome status, so supplementation of n-3 enriched oils should be moderated. ❖

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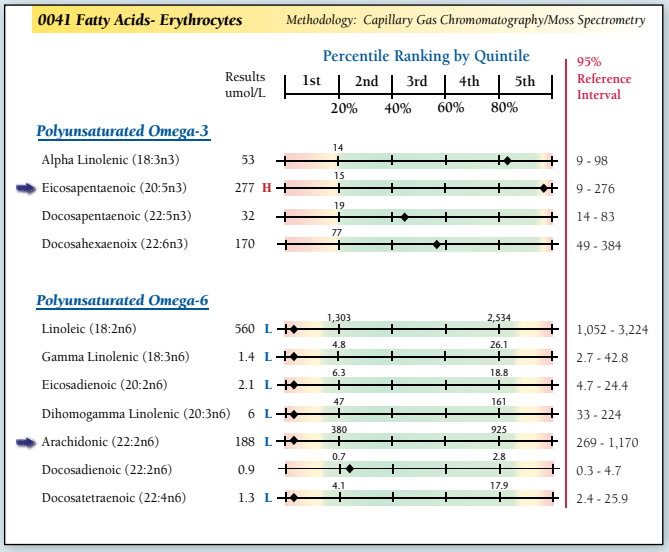

Case Illustration 5.3 — Omega-3 Dominant Pattern 2

For eight years this 21-year-old male with a rare connective tissue disorder had been fed MCFA and fish oils through a gastrostomy tube as part of the medicinal food therapy. He had developed difficulty sleeping along with weight gain and edema. These symptoms improved markedly with alteration of dietary oils to lower n-3 and increase n-6 fatty acid intake, allowing AA to rise. This may be a case where total body AA levels were low enough to produce sleep disturbance from insufficient brain levels for prostaglandin D2 (PGD2) formation. ❖

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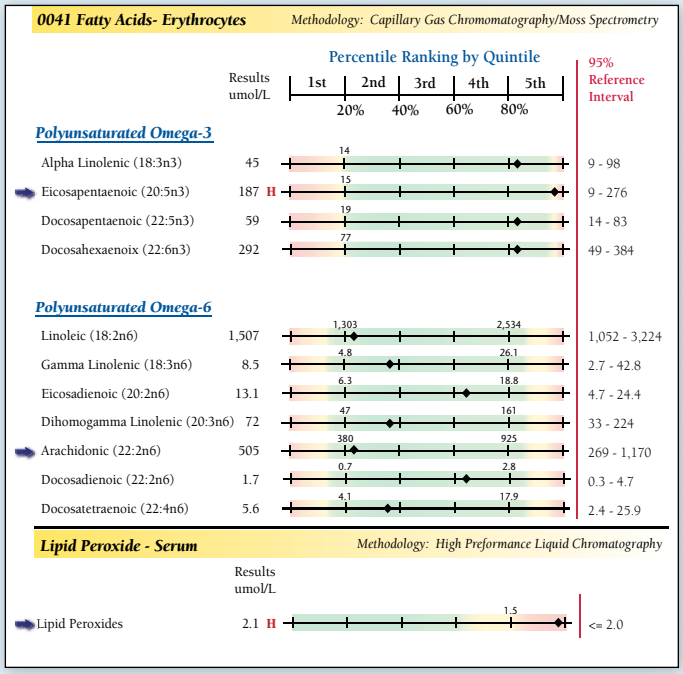
Case Illustration 5.4 — Omega-3 Dominant Pattern 3

Here the n-3 levels are not high enough to cause concern except that the greatly elevated serum lipid peroxide value gives evidence of inadequate antioxidant protection against polyunsaturated fatty acid oxidation. The 55-year-old female patient had been using supplemental fish oil with no antioxidant supplementation, producing the pattern of elevated EPA and low second quintile AA. Improving fat-soluble antioxidant status, confirmed by normalizing of lipid peroxide results may be all that is needed. ❖



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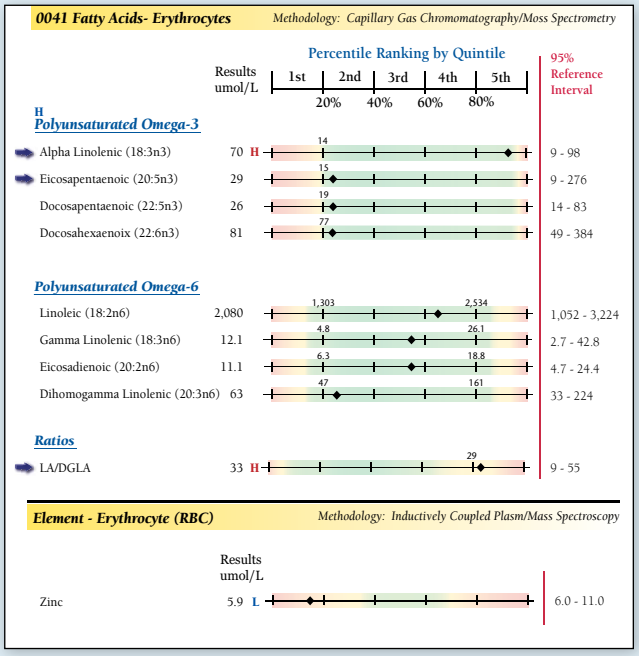
Case Illustration 5.5 — Zinc Insufficiency Sign

This pattern was found in an 82-year-old male with long history of cardiovascular disease. He had undergone a quadruple cardiac bypass surgery and mitral valve replacement. Although the LA result is only in the 4th quintile, with 2nd quintile DGLA, the calculated LA/DGLA ratio is somewhat elevated, a sign of functional zinc insufficiency impairment of desaturase enzyme activity. In this case the pattern is strengthened by examining for desaturase activity failure in the n-3 family. There we find elevated ALA with lower 2nd quintile EPA, confirming the effect. Confirmatory evidence is found in the concurrently run RBC element profile where the zinc concentration is below the low reference limit. ❖

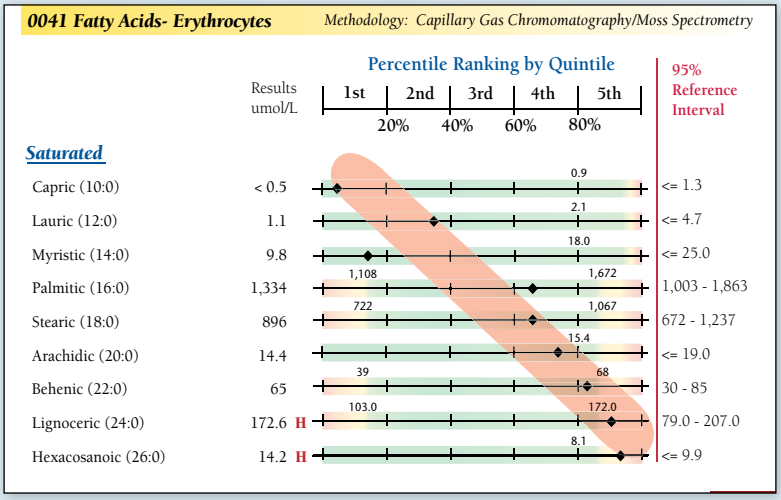


Case Illustration 5.6 — Metabolic Syndrome

This plasma fatty acid profile reveals the effects of chronic insulin stimulation on endogenous fatty acid synthesis. The report was generated for a 48-year-old female with high waist to hip ratio, elevated triglycerides and cholesterol, and high fasting insulin with normal glucose. The fatty acid synthase enzyme complex retains the growing chain until the length reaches 16 carbon atoms (palmitic acid). Elongase enzymes add two-carbon units efficiently to produce the 18- (stearic) and 20-carbon (arachidic) products. By connecting the quintile chart points, a pattern is seen that has the shape of a greater than sign (>). The relative abundances of saturated fatty acids are reflected in their desaturase products, so the same pattern is found in the family of monounsaturated fatty acids. Further confirmation of metabolic syndrome effects on fatty acid metabolism may be found in elevated levels of the LA/DGLA ratio. Elevated insulin suppresses desaturase enzyme activity, slowing the rate of LA conversion to DGLA. ❖

Case Illustration 5.7 —
Very Long Chain Fatty Acyl CoA Dehydrogenase Deficiency

This saturated fatty acid pattern was found in a 70-year-old male. The relative positions progress to higher levels as chain length goes above 20 in the saturated fatty acid family. This type of very long chain fatty acid accumulation is typical of the metabolic disorder in adreno-leukodystrophy (ALD). The levels can be much higher than those shown in this illustration when full symptoms of ALD are present, including (for the childhood form) visual loss, learning disabilities, siezures, dysarthria, dysphagia, deafness, gait disturbances, vomiting, melanoderma, progressive dementia and aggression. ❖



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Chapter 6

Organic Acids

Richard S. Lord and J. Alexander Bralley

CONTENTS

Notes:

TABLE 6.1 — SUMMARY OF ABNORMALITIES FOR ORGANIC ACIDS IN URINE

Name		Potential Intervention	Metabolic Pathway
Fatty Acid Oxidation			
Adipate	H	L-Carnitine, 500–1000 mg TID; L-Lysine (if low), 500 mg TID; B2, 100mg BID See text for other interventions in genetic disorders	Fatty acid oxidation
Suberate	H		
Ethylmalonate	H		
Carbohydrate Metabolism			
Pyruvate	H	B_1, up to 100mg TID with B complex support; For concurrent H Lactate: lipoic acid, 500mg TID	Aerobic/anaerobic energy production
Lactate	H	Coenzyme Q_{10}, 50 mg TID	
β-Hydroxybutyrate	H	Chromium picolinate, 200 µg BID	Balance of fat and CHO metabolism
Energy Production (Citric Acid Cycle)			
Citrate	H	Arginine, 1–3 gm/day	Citric Acid Cycle Intermediates: Renal ammonia clearance
	L	Aspartic acid, 500 mg; magnesium citrate, 500 mg	
Cis-aconitate	H	Cysteine, 1000 mg BID; Check for iron deficiency	
Isocitrate	H	Lipoic acid, 25 mg/kg/day Magnesium, 400 mg; manganese, 20 mg	
α-Ketoglutarate	H	B-complex, 1 TID; lipoic acid 100 mg	
Succinate	H	CoQ_{10}, 50 mg TID, magnesium, 500 mg	
Fumarate	H	CoQ_{10}, 50 mg TID, magnesium, 500 mg	
Malate	H	CoQ_{10}, 50 mg TID, B_3, 100 mg TID	
Hydroxymethylglutarate	L,H	CoQ_{10}, 50 mg TID	(L) Substrate-limited CoQ_{10} synthesis (H) HMG-CoA reductase inhibition
B-Complex Vitamin Markers			
α-Ketoisovalerate	H	B-complex, 1 TID; lipoic acid 100 mg	Valine catabolism
α-Ketoisocaproate	H		Leucine catabolism
α-Keto-β-methylvalerate	H		Isoleucine catabolism
Xanthurenate	H	Vitamin B_6, 100 mg/d	Tryptophan catabolism (hepatic)
β-Hydroxyisovalerate	H	Biotin, 5 mg/day; magnesium, 100 mg BID	Leucine catabolism
Methylmalonate or Propionate	H	B_{12}, 1000 µg TID	Valine or odd-chain fatty acid catabolism
Formiminoglutamate	H	Folic acid, 400 µg/d	Histidine catabolism
Neurotransmitter Metabolism			
Vanilmandelate	L,H	Tyrosine, 1000 mg BID-TID, between meals and phenylalanine hydroxylase cofactors as needed Contraindicated for patients taking MAO inhibitors	(L) Tyrosine-limited or (H) Tyrosine-depleting epinephrine & norepinephrine catabolism
Homovanillate	L,H		(L) Tyrosine-limited or (H) Tyrosine-depleting DOPA catabolism
5-Hydroxyindolacetate	L,H	5-Hydroxytryptophan, 50–100 mg TID; magnesium, 300 mg; vitamin B_6, 100 mg (5-HTP may be contraindicated with SSRI's)	(L) Tryptophan-limited or (H) Tryptophan-depleting Serotonin catabolism
Kynurenate	H	B_6, 100 mg; magnesium, 300 mg	Inflammation-stimulated macrophage and astrocyte kynurenine pathway activity
Quinolinate	H	Magnesium, 300 mg	

Table 6.1 continued on following page...

Table 6.1 continued from previous page...

Name		Potential Intervention	Metabolic Pathway
Oxidative Damage and Antioxidant Markers			
p-Hydroxyphenyllactate	H	Vitamin C to bowel tolerance*	Prooxidant and carcinogen
8-Hydroxy-2'-deoxyguanosine	H	Antioxidants (Vitamins C, E, lipoic acid)	DNA oxidation product
Detecting and Monitoring Inborn Errors of Metabolism			
Homogentisate	H	Low protein diet; vitamin C, 1000 mg TID	Tyrosine catabolism
Detoxification Indicators			
2-Methylhippurate	H	Avoidance of xylene; glycine, 2–5 gm/d; B_5 100 mg TID	Hepatic conjugation
Orotate	H	Arginine, 1–3 gm/day; a-KG, 300 mg TID Aspartic Acid, 500 mg BID; magnesium, 300 mg	Ammonia clearance, Pyrimidine synthesis,
Glucarate	H	Glycine, GSH, NAC, 500–5000 mg/day	Detox. liver enzyme induction
α-Hydroxybutyrate	H	NAC, 1000 mg, glutathione, 300 mg Taurine, 500 mg BID	Hepatic GSH synthesis
Pyroglutamate	H		Renal amino acid recovery
Sulfate	L		Detox & anti-oxidant functions
Dysbiosis Markers (Products of Abnormal Gut Microflora)			
Benzoate	H	Glycine, 2–5 gm/d; vitamin B_5, 100 mg TID	Hepatic Phase II glycine conjugation
Hippurate	H	These compounds may reflect intestinal overgrowth, usually accompanied by microbial hyperpermeability. Take appropriate steps to ensure favorable gut microflora population. Glutamine, 10–20 gm daily, digestive aids (betaine, enzymes, bile) and free-form amino acids help to normalize gut permeability.	Intestinal bacterial overgrowth
Phenylacetate	H		
Phenylpropionate	H		
p-Cresol	H		
p-Hydroxybenzoate	H		
p-Hydroxyphenylacetate	H		
Tricarballylate	H		
D-Lactate			General bacterial or *L. acidophilus* overgrowth
3,4-Dihydroxyphenylpropionate	H		Clostridial overgrowth
D-Arabinitol	H		Intestinal yeast overgrowth

** Bowel tolerance is usually up to 100 mg/kg, as determined by 500 mg dosing repeated every 30 minutes.*

TABLE 6.2 — COMMON PATTERNS SEEN IN RESULTS FROM ORGANIC ACIDS IN URINE

Pattern	Potential Intervention	Explanation
Pyruvate and Lactate High	Lipoic acid, 500 mg	Pyruvate dehydrogenase complex deficiency
Citrate, *cis*-Aconitate and Isocitrate High	Arginine, 500 mg BID	Heavy renal ammonia excretion
- and Orotate High		Hepatic urea cycle capacity exceeded
Glucarate High	Restore orthobiosis	Hepatic Phase I and II up-regulation
- and bacterial markers High		Intestinal bacterial origin of toxin load
β-Hydroxyisovalerate High	Biotin, 5 mg/d and restore orthobiosis	Displacement of biotin-forming species by opportunistic bacteria
- and bacterial markers High		
Hydroxymethylglutarate High	CoQ_{10}	Inhibition of CoQ_{10} synthesis and mitochondrial CoQ_{10} insufficiency (possibly statin drugs)
- and succinate, fumarate, malate High		

INTRODUCTION

Unlike amino acids and fatty acids, the category of compounds called organic acids contains no essential nutrients. Instead of directly measuring nutrient concentrations, abnormal concentrations of organic acids provide functional markers for the metabolic effects of micronutrient inadequacies, toxic exposure, neuroendocrine activity, and intestinal bacterial overgrowth. As such, organic acid testing can indicate the functional need for essential or conditionally essential nutrients, diet modification, antioxidant protection, detoxification and other therapies. Table 6.1 summarizes the relationships between specific organic acid abnormalities and potential nutrient interventions, and Table 6.2 shows some of the common patterns of abnormalities found in urinary organic acid profiles.

All bodily functions are powered by the release of chemical energy. Each day, the energy content of the food for an average person could raise the temperature of about 7 gallons of water to the boiling point. The energy is released through a process of controlled oxidation, where chemical bonds are broken and energy is released. Fats, carbohydrates and amino acids are converted into carboxylic acids before they flow on to the final conversion to carbon dioxide (Figure 6.1). The organic acids that are formed as intermediates in this process are normally absent from urine or present at very low concentrations. When specific reactions are blocked due to insufficient enzymes or cofactors, the organic acids that precede the blocked step accumulate and spill into urine. Genetic polymorphisms in enzyme structure that lead to decreased cofactor binding are another major cause of nutrient deficiencies described by the term, genetotrophic. Individuals with faulty enzyme binding can have increased nutrient needs that will not be revealed by measures of vitamin concentrations in blood. Research has noted mutant enzymes with poor binding affinity account for more than 50 known genetic conditions that can be stabilized with nutrient intakes.[3]

The familial cerebral degenerative disease, maple syrup urine disease (MSUD), provides an example of genetotrophic variations. High urinary levels of the branched-chain keto acids (BCKA) derived from valine, leucine and isoleucine produce an odor similar to that of maple syrup. Elevated BCKA levels are caused by failure of the branched-chain keto dehydrogenase enzyme to oxidize them. This enzyme is highly sensitive to the lack of vitamin B_1-derived thiamin pyrophosphate. Since it is required in a major energy-producing pathway, the appearance of elevated BCKA in urine is a sensitive test for lack of activity due to either simple thiamin deficiency or inborn errors of metabolism. Variants of maple syrup urine disease are due to degrees of impairment of this enzyme (Table 6.3). Genetic variants in which the enzyme is not expressed result in severe clinical consequences in infancy, including failure to thrive and encephalopathy with lethargy, increased or decreased tone and seizures. Similar conditions precipitated by infection or stress may develop in adults with mild levels of functional impairment due to a high requirement for thiamin.[2, 3] Many other such inborn errors of metabolism that produce specific organic acidurias have been reported, and several reviews are available.[4-6]

Urinary organic acid analysis for metabolic profiling has traditionally been used for detection of neonatal

TABLE 6.3 — MAPLE SYRUP URINE DISEASE VARIANTS*

Variant	Enzyme Activity	Organic Aciduria	Clinical Presentation
Classical	< 3%	α-ketoisocaproate, α-ketoisovalerate, α-keto-β-methylvalerate	Symptoms within the first several days of life. Infant death or prominent abnormalities. Developmental delays
Intermediate	3–30%	Similar to classic phenotype, though quantitatively less severe	Developmental delays, irritability, seizures
Intermittent	5–20%	Normal BCAAs when well, similar to classic phenotype when ill	Symptoms appear with illness, stress or high protein intake. Normal early growth and development. Age of onset varies.
Thiamin-responsive (10–1000 mg/day)	2–40%	Profiles improve with thiamine therapy (lower levels in urine)	Large doses of thiamine will increase the enzyme activity and break down leucine, isoleucine and valine. Normal early growth and development. Age of onset varies.

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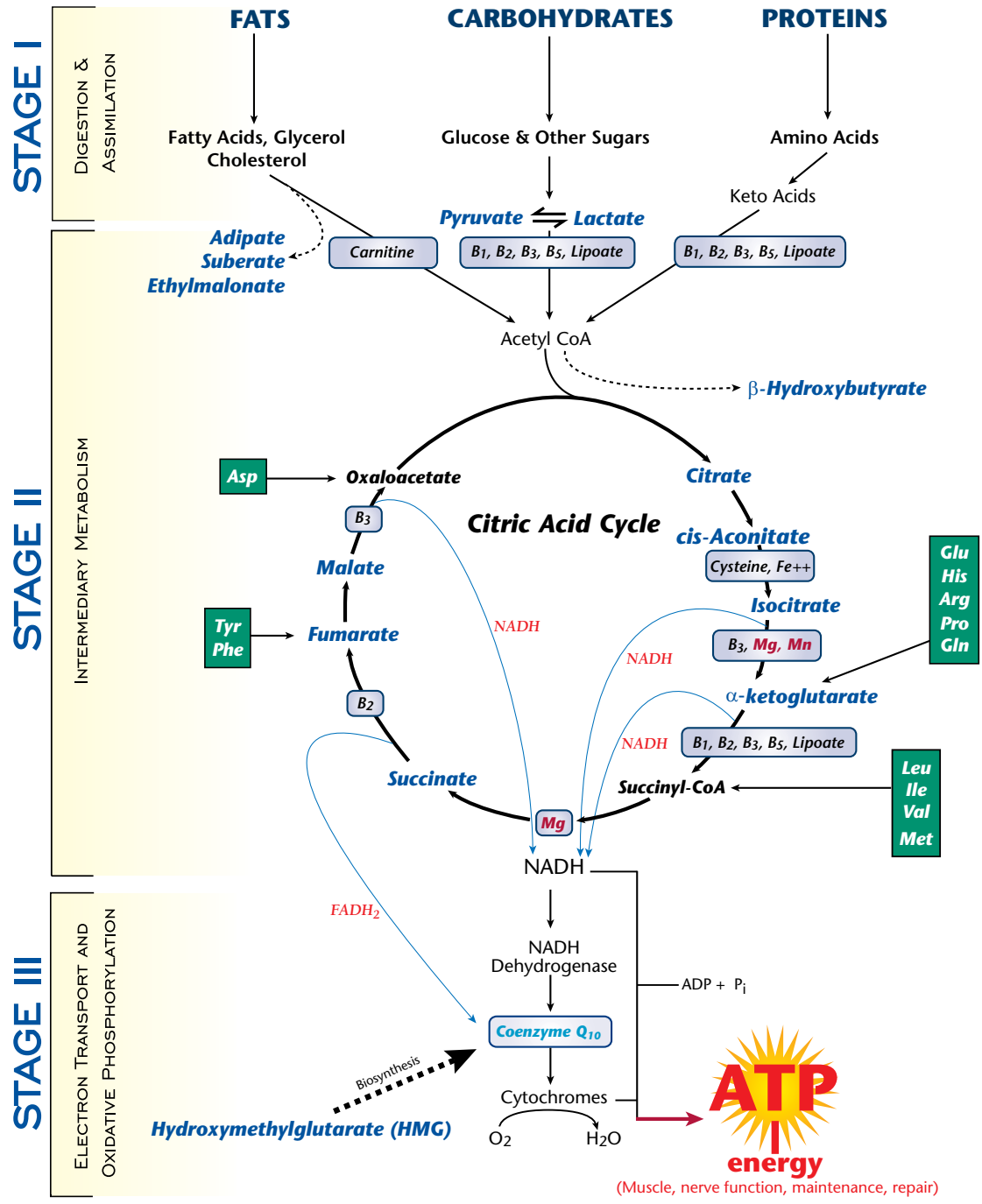


FIGURE 6.1 — **Urinary Markers of Nutrients Involved in Central Energy Pathways**

Intermediates shown in bold purple font on this condensed version of central energy biochemical pathways are compounds normally included on profiles of urinary organic acids. This figure can be useful for patient education regarding the information gained from performing the profile since most people can understand the importance of deriving energy from dietary fat, carbohydrate and protein. Essential nutrient requirements for specific steps are shown in light blue boxes. Elevations of the substrates for those steps can indicate functional insufficiency of the associated nutrient. Amino acid abbreviations shown in green rectangles indicate the points of entry of their catabolic products into the citric acid cycle.

inborn errors of metabolism. Testing organic acids to assess special nutrient requirements of individuals is discussed in a variety of sources.[6, 8-11] Organic acid profiling has also been useful in identification of the source of toxicants from the environment[12] and the gut.[13] Various nutrient-related abnormalities that might appear on a typical quantitative report of organic acids in urine are summarized in Table 6.1. The supplementary nutrient amounts are given as guides for starting points to improve clinical outcomes for adults.

The term "organic acid" refers to a broad class of compounds used in fundamental metabolic processes of the body. Chemically, organic acids share the common features of water solubility, acidity, and ninhydrin negativity (no primary or secondary amines). The term is generally considered to include all carboxylic acids, with or without keto-, hydroxyl-, or other non-amino functional groups, but does not include most amino acids. Some nitrogen-containing compounds are included, such as pyroglutamate, or amino conjugates such as hippurate (benzoylglycine). Short-chain fatty acids are also contained in this group. With adjustments of the method for extraction of the compounds from urine, the analysis may include neutral compounds that possess no acid group, in which case the naming of the entire set as "organic acids" is somewhat inaccurate.

Notes:

Morning urine has become widely accepted for primary study of metabolic disorders. Urination is generally the first post-sleep activity because the cortisol-stimulated, anabolic sleep period requires the metabolic, energy-yielding pathways that produce water. Prior to waking, cortisol peaks and then drops, initiating a shift to greater utilization of catabolic pathways. For these reasons, the first morning urine usually contains a greater array of metabolic by-products than at any other time of the day and is relatively free of the metabolic effects of strenuous muscle activity. The convenience of such collections also leads to high patient compliance. In one laboratory methodology, organic compounds are extracted from the overnight urine sample and analyzed by gas chromatography with mass spectrometric detection (GC-MS). The basic methodologies are well known,[14] but improvements are regularly reported.[15] Most recently, significant advances have come from the use of liquid chromatography with tandem mass spectrometric detection (LC-MS/MS).[16, 17] This technology allows more reliable analyte recovery due to greatly simplified sample preparation. This method does not require organic solvent extraction. It also provides a shorter run time and greater sample throughput.

The information content of an organic acid profile is high, but the interpretation is simplified by keeping in mind that the results supply answers to a few questions of clinical relevance:

1. Are there signs of inborn errors of metabolism?
2. Is mitochondrial energy production adversely affected?
3. Are functional nutrient deficiencies present?
4. Does altered neurotransmitter turnover reveal symptom origins?
5. Are antioxidant nutrients protecting against oxidative stress?
6. Is there a high toxin load and is this adversely affecting detoxification capacity?
7. Are symptoms related to excessive growth of bacteria and fungi in the gut?

Each of several compounds reported in the typical profiling of organic acids in urine will be discussed briefly to indicate why they are related to these clinical questions.

Detecting and Monitoring Inborn Errors of Metabolism

Since the reporting of isovaleric acidemia in 1966, there has been a rapidly growing list of disorders characterized by elevated urinary excretion of organic acid metabolic intermediates.[6,7] Laboratory tests were developed using GC/MS technology to detect inborn errors of metabolism (IEM) that could lead to premature death or retardation in infants. Today qualitative measurements of certain organic acids (and amino acids) are widely performed on neonates using LC/MS/MS technology. These tests screen for extremely high levels of compounds that are not being metabolized due to significant inborn genetic lesions. Quite often, treatments based upon dietary modification and nutrient supplementation are introduced to modulate the effects of the genetic abnormality.

More recently, organic acid methods have been developed to measure lower levels of these compounds for the purpose of identifying more subtle metabolic imbalances related to nutrient deficiencies and toxicities. Metametrix Clinical Laboratory pioneered the clinical application of quantitative analysis of lower levels of organic acids for these purposes in the early 1990's. Other laboratories have since developed the test for clinical use. These tests are increasingly being used to identify biochemical imbalances that underlie chronic diseases and that can be treated by specific nutrient supplementation. Organic acid testing to assess special nutrient requirements of individuals is discussed in a variety of sources.[6, 8–11] This testing has also been useful in identifying the source of toxicants from the environment[12] and the gut.[13] Although the methods used to detect more subtle metabolic imbalances require instrument calibration at much lower concentrations than those used for neonatal screening, extremely high levels can also be identified.

Table 6.4 — Top Frequencies of Clinical Symptoms in Mitochondrial Respiratory Deficiencies[35]

Symptom	Occurrence in Affected Individuals
Truncal hypotonia	36%
Growth failure	31%
Cardiomyopathy	24%
Intrauterine growth restriction	20%
Encephalopathy	20%
Liver failure	20%
Cranial nerve involvement	18%
Myopathy	13%
Spasticity	11%
Gut involvement	8%

Mitochondrial Function Assessment

Because of the centrality of ATP in all life processes, the factors affecting mitochondrial ATP synthesis have diverse implications for human health. The basic sequence of chemical reactions responsible for the transformation of the chemical energy in foodstuff into ATP has been understood for several decades. However, routine clinical assessments of mitochondrial function and interventions to improve it are quite a recent development. Once again, we find much of the early work coming from attempts to discover neonatal diseases that produce profound metabolic consequences. Six chapters in the current edition of the book *Metabolic and Molecular Origins of Disease* are devoted to disorders of mitochondrial function, where neuromuscular symptoms are the most frequent initial presentation.[6, 32] The scope of other symptoms found in mitochondrial deficiencies is shown in Table 6.4. The origins of these symptoms are restricted to diseases found to be genetic polymorphisms in the respiratory chain components. The scope and numbers of individuals expands broadly when insufficiencies of carnitine and coenzyme Q_{10} are added to inherited traits that negatively impact mitochondrial efficiency. Examples of the more subtle effects of mitochondrial inefficiency are obesity, diabetic tendency for intramuscular fat accumulation and the age-associated decline in insulin sensitivity.[33, 34, 605]

Fatty Acid Metabolism Markers

The three organic acids in this category are markers of carnitine sufficiency. Fatty acid metabolism by mitochondrial β-oxidation is the dominant energy-yielding pathway in most tissues. It involves the transfer of fatty acids (as fatty acyl-Coenzyme A esters) across the mitochondrial outer and inner membrane prior to the catabolic action of the fatty acid β-oxidation complex (Figure 6.2). The transfer is dependent on the formation

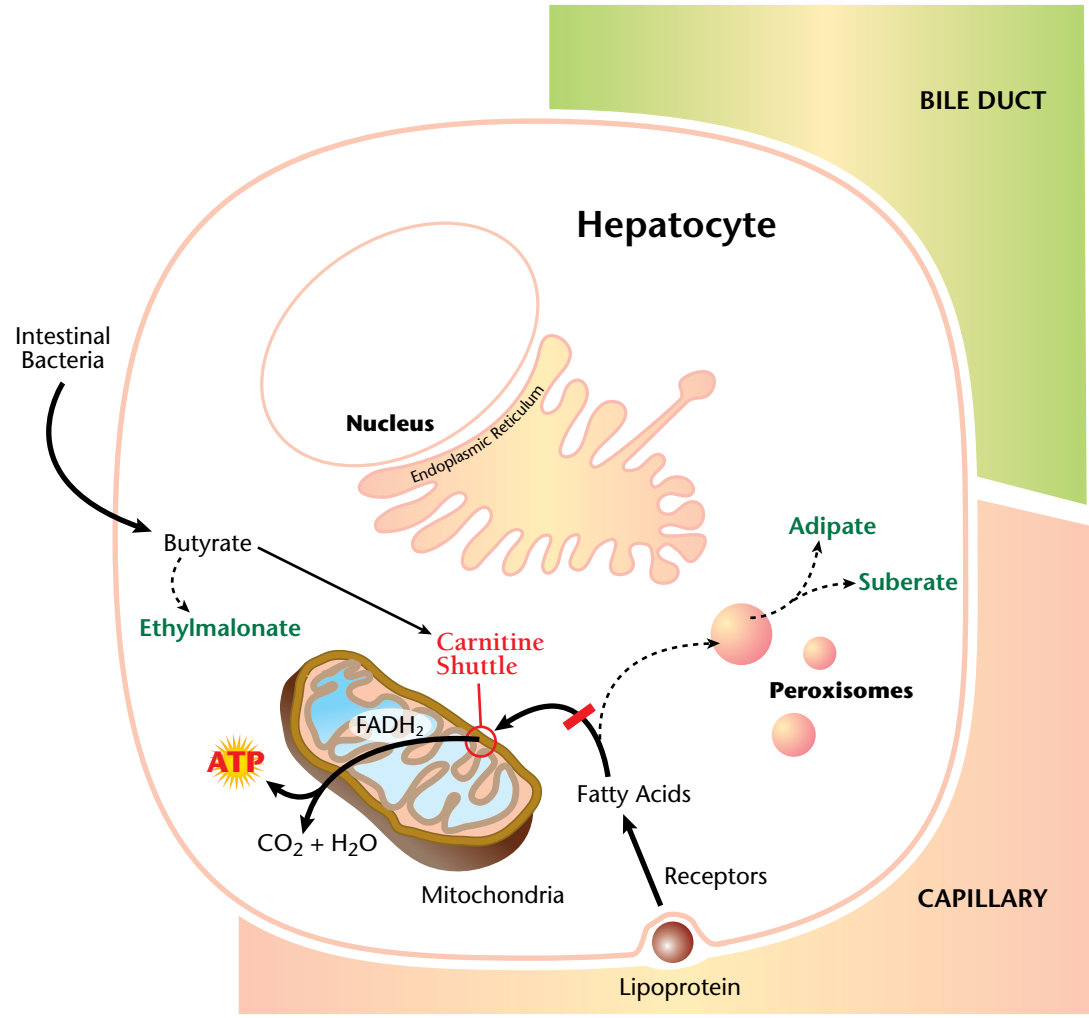


FIGURE 6.2 — **Mitochondrial Fatty Acid Metabolism Markers**

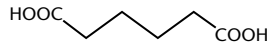
In all tissues except brain, most ATP generation is derived from oxidation of fatty acids. The process is initiated by entry of the fatty acid into the matrix of mitochondria. The rate limiting step for entry is the formation of fatty acyl carnitine by one of three enzyme systems that operate on medium, long or very long chain fatty acids. Even a slight interruption of this dynamic pathway causes increased amounts of fatty acids to be processed via omega oxidation occurring in peroxisomes. The lower efficiency of peroxisomal processing allows intermediates to escape and be lost when the blood is filtered in the kidneys. Adipate and suberate are biochemical markers that reflect the degree to which mitochondrial entry is impaired due to insufficiency of carnitine or other genetotrophic factors. See Figure 5.4 (Fatty Acids) for carnitine shuttle details.

of fatty acyl-carnitine, and this reaction is governed by carnitine concentration. The extreme importance of fatty acid oxidation to provide cellular energy is indicated by the redundancy of systems. Both β- and ω-oxidation systems are contained in peroxisomes. When the mitochondrial system fails to meet demands, peroxisomes can take over to a limited degree, but lack of the double membrane containment of the mitochondrion means that the system is less efficient because substrates may escape and be lost as renal excretory products. Examples of human mitochondrial β-oxidation deficiency are well known to produce infant death, though some individuals exhibit normal development for a few weeks or months.[36] As usual, the severe manifestations of such

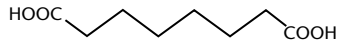
enzyme polymorphism is always accompanied by less severe forms that manifest as milder and, frequently, later syndromes.[37-40]

Carnitine can be synthesized from the essential amino acid, L-lysine. However, limitations of available lysine due to dietary deficiency or digestive impairment or genetic polymorphism of the required enzymes can cause carnitine requirements to exceed biosynthetic capacity. Therefore, carnitine is a conditionally essential dietary component. The most extensive clinical evidence for the conditional essentiality of carnitine comes from management of cardiomyopathy.[41-44] Some treatment regimens have included carnitine to assist mitochondrial fatty acid oxidation along with a peroxisomal proliferation agonist for stimulation of the secondary role of peroxisomal oxidation.[45]

Adipate



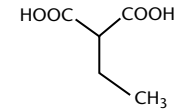
Suberate



Adipate (adipic acid) and suberate (suberic acid) are six- and eight-carbon-long dicarboxylic acids, respectively. Low carnitine availability or impaired enzyme activity can slow fatty acid oxidation by decreasing the transport of fatty acids into the mitochondria as discussed in Chapter 5, "Fatty Acids". Need for extra carnitine may be indicated when urinary levels of adipate and suberate are elevated.[46-49] Adipic and suberic acids are by-products of an alternative fatty acid oxidation pathway that is utilized when mitochondrial oxidation is limited.[50, 51] The principal alternate pathway occurs in peroxisomes where multiple types of oxidizing enzymes are present, in contrast to the constrained fate of fatty acids undergoing β-oxidation in mitochondria (Figure 6.2). Once the dicarboxylic acids are formed, they diffuse away from the peroxisomal sites to be excreted in urine.

A secondary cause of elevated adipate and suberate is riboflavin insufficiency.[52] Once they are inside the mitochondria, fatty acids cannot undergo oxidative metabolism without riboflavin coenzymes. Riboflavin is needed for sustaining levels of flavin adenine dinucleotide (FAD), the critical cofactor at the succinate dehydrogenase step of the citric acid cycle (CAC) (see below). Thus, riboflavin insufficiency is a secondary indication of elevated adipate and suberate. A further effect of riboflavin insufficiency is succinate accumulation that causes inhibition of enzymes required for mitochondrial fatty acid processing. This scenario is indicated when succinate, adipate, and suberate are concurrently elevated.

Ethylmalonate



Ethylmalonate accumulation is traced to different pathways than the long-chain fatty acid oxidation origins of adipate and suberate. Although the exact origin is somewhat unclear, current evidence suggests that ethylmalonate is formed from butyrate. Patients with ethylmalonic aciduria also have elevated levels of butyryl-CoA (and isobutyryl-CoA from isoleucine catabolism).[53, 54] Circulating butyrate, a short-chain fatty acid with four carbons, may be derived from intestinal bacterial metabolism. Butyryl-CoA is normally carried by carnitine into oxidative pathways. Just as for the long-chain fatty acids, carnitine may be insufficient to clear butyrate or riboflavin may be insufficient to sustain FAD, causing slowing of the rate of oxidation. Under these conditions free butyrate is available for other reactions, including carboxylation to form ethylmalonate. Thus, urinary ethylmalonate elevation shares carnitine and riboflavin dependencies with adipate and suberate, but the precursor arises from different metabolic sources. This may explain why in evaluating large numbers of organic acid profiles some individuals have elevated adipate and suberate with normal ethylmalonate, whereas others show the opposite pattern.

Patients with ethylmalonic acidurias should not be treated with medium-chain triglycerides that produce metabolic stress from the accumulation of medium-chain fatty acyl-carnitines.[55] Glycine (250 mg/kg/d) has

Notes:

been found to be a useful adjunct in addition to carnitine and vitamin B_2 in the treatment of these patients.[49] An interesting association of ethylmalonate and obesity is found in a report on lean and fatty Zucker rats. The "fatty" rats excrete high levels of ethylmalonate and adipate, indicating impaired oxidation of short chain fatty acids. When the lean rats were fed diets enriched with medium-chain triglycerides or sodium butyrate, their excretion of ethylmalonate rose almost as high as the fatty rats on basal diet. The fatty rats appear to have impaired ability to clear butyrate that is an optimal primer for synthesis of long-chain fatty acids, thus offering an explanation for their tendency for obesity.[56] No similar studies have been reported in humans.

The enzyme that controls the butyrate oxidative pathway is short-chain acyl-CoA dehydrogenase (SCAD). Its activity may be lowered by genetic mutations resulting in increased urinary ethylmalonate excretion.[48, 57] Monitoring for elevation of urinary ethylmalonate can signal the onset of ethylmalonic encephalopathy in affected individuals.[58, 59] A variant SCAD allele (A625) resulting from a guanine to adenine polymorphism occurs in homozygous or heterozygous form in 7 and 34.8%, respectively, of the general population. The A625 allele confers susceptibility to the development of ethylmalonic aciduria.[60] Neonatal SCAD deficiency may present as acidosis with ammonemia and elevated liver enzymes.[53]

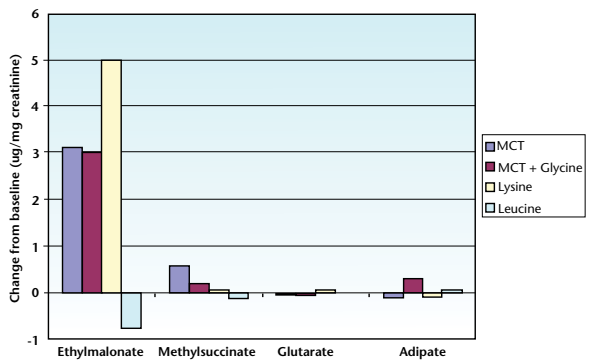


FIGURE 6.3 — Urinary Organic Acids in Short Chain Acyl Dehydrogenase Deficiency[53]

These data are derived from results reported for a single neonate with SCAD deficiency.[53] Compared to control urine, her mean baseline ethylmalonate was very elevated (5.2 vs < 0.3 µg/mg creatinine). The bars show how oral challenge with medium-chain triglycerides (MCT) or lysine produced large increases of ethylmalonate over an already elevated baseline. Addition of glycine to the MCT formula had no additional effect, while leucine showed an apparent stimulation of the metabolism of ethylmalonate, causing lower post-treatment concentrations. Changes for methylsuccinate, glutarate and adipate were much smaller and of low significance.

The related genetic disorder of multiple acyl-CoA dehydrogenase deficiency (MAD), also known as glutaric aciduria type II disorders, is characterized by elevated urinary ethylmalonate. Therapy with vitamin B_2 may partially or totally reverse the associated symptoms, such as profound muscle weakness.[61,62] Ethylmalonate elevation is also found in glutaric aciduria type II disorders that can appear as adult onset with episodic acute pancreatitis.[62] In this case and in a rat model where ethylmalonic aciduria had been induced by experimental riboflavin deficiency, the organic aciduria was reduced by adding L-carnitine, demonstrating the joint action of vitamin B_2 and carnitine in these conditions.[63]

These disorders have multiple clinical presentations due to the varieties of genetic polymorphisms of the enzymes.[64] In one affected family, a child born prematurely had unexplained cholestasis and hepatomegaly in the first year of life, whereas the mother displayed only hemolytic complications of pregnancy and other siblings had no signs of hypotonia, developmental delay, or episodes of ketotic hypoglycemia.[65] Other presentations include ophthalmoplegia and multicore myopathy,[66] vascular lesions of the skin and acrocyanosis,[54] or neonatal neurologic dysfunction with only transient ethylmalonic aciduria.[67] The metabolism of short-chain fatty acids is impaired in these conditions, as shown in Figure 6.3.

Ethylmalonic aciduria can be caused by exposure to toxicants instead of the more commonly encountered nutrient-induced or genetic focal metabolic interferences. Ingestion of the ackee plant results in SCAD inhibition due to the presence of the toxin, hypoglycin. This dietary-related illness has been called Jamaican vomiting illness because of the high frequency due to common use of ackee in that country. It has also been reported in the United States from ingestion of canned ackee.[68] Ethylmalonate excretion may also be stimulated by isoleucine loading, indicating that it may be an intermediate produced in the isoleucine catabolic pathway.[69]

Patients with elevations of adipate, suberate or ethylmalonate generally have variants of the genetic polymorphisms that affect the formation of fatty acyl carnitine formation and metabolism. These patients may have normal serum carnitine, requiring greater concentrations to overcome their enzyme polymorphisms. There is another

form of systemic carnitine deficiency associated with idiopathic dilated cardiomyopathy (IDCM) that may present with normal levels of the organic acids.[41] Since serum carnitine is low in these cases, they may arise from an inability to synthesize carnitine from lysine. Serum carnitine levels increase when patients with both IDCM and celiac disease are placed on gluten-free diets.[41]

The patterns of organic acid elevations in milder carnitine insufficiency vary. Depending on severity, symptoms may include periodic mild weakness, nausea, fatigue, hypoglycemia, "sweaty feet" odor and recurrent infections.[70] Abnormalities have been reported in children with attention deficit disorders. Patients may also exhibit a Reyes-like syndrome in dicarboxylic aciduria, which has been associated with various metabolic toxins from viral infections that affect mitochondrial function. Patients with low carnitine respond well to therapeutic riboflavin.[71] Case Illustration 6.1 shows an instance where carnitine marker abnormalities were found in a patient with schizophrenia.

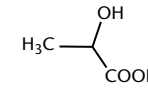
Refer to Case Illustration 6.1

The use of aspirin can change the interpretation of results for the three marker compounds because salicylic acid is an inhibitor of fatty acid β-oxidation and may lead to elevated markers.[72] The β-oxidation enzymes involved also respond to environmental toxin exposure with altered lipid metabolism that can lead to impaired immune responsiveness and mitochondrial DNA damage.[73] Supplementation of carnitine and riboflavin is indicated when adipate, suberate, or ethylmalonate are elevated.[74] However, some inborn errors of metabolism produce ethylmalonic aciduria that is unresponsive to carnitine or riboflavin.[54]

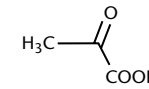
Carbohydrate Metabolism Markers

The operation of the central energy pathway of glycolysis, the catabolism of glucose, generates large, dynamic pools of lactate and pyruvate. Oxidation of fatty acids and ketone bodies similarly generates pools of β-hydroxybutyrate and acetoacetate. When these pathways are perturbed, excretion of their intermediates can shift in response to the metabolic acidosis.[4] Although some patterns of abnormalities for these compounds may be indicative of specific micronutrient insufficiencies, they usually require further evidence to specify the origin of the metabolic impairment. That evidence is frequently supplied by reading the rest of the laboratory report where organic acid profiles are generated.

Lactate



Pyruvate



Abnormalities of urinary excretion of pyruvate and lactate provide useful insight to basic metabolic factors due to their position in the energy production process. Pyruvate and lactate are anaerobic breakdown products of glucose. Under conditions favoring anaerobic metabolism, pyruvate is reduced to lactate by the action of lactate dehydrogenase. Conditions favoring aerobic metabolism increase the clearance of pyruvate by oxidation to acetyl-CoA, avoiding the buildup of lactate. Mitochondrial conversion of pyruvate to acetyl-CoA requires the pyruvate dehydrogenase complex (PDC), the enzymatic gatekeeper for generation of acetate units to drive the citric acid cycle. The three-step operation of the PDC is carried out by enzymes E1, E2 and E3 as described in Figure 6.4.[76] The cycle of reactions requires cofactors derived from thiamin, riboflavin, niacin, lipoic acid and pantothenic acid (the dietary essential precursor of coenzyme A). Pyruvate is decarboxylated at E1, and the acetyl residual group is transferred to coenzyme A at E2. The disulfide must be regenerated by oxidation as the right-hand lipoyl arm swings to the E3 subunit for oxidation by the bound FAD. To complete the sequence, the reduced $FADH_2$ group is oxidized back to FAD by reduction of NAD. Differences in binding affinities alter the impact of individual cofactor status on the overall process of pyruvate conversion to acetyl-CoA to maintain ATP formation via the CAC. Thiamin, which binds on the E1 subunit, is usually the most strongly

Notes:

dependent nutrient for the PDC although genetic polymorphism of one of the three enzymes may generate specific alterations in other cofactor binding. Large doses of thiamin have been shown to increase the function of the PDC.[3]

Lactate (or lactic acid) is a principal product of glucose oxidation in skeletal muscle. There is a chiral center in lactate, so laboratories should designate whether they are reporting the L or D forms or the total concentration of both entantiomers. Because D-lactate is discussed later in this chapter, it is important to understand that the human metabolic product being discussed here is L-lactate. The "L" enantiomeric designation will be dropped for brevity, and the "D" form will always be specified. Although many of the studies cited refer to blood lactate, blood and urinary lactate levels are strongly correlated.[595]

Since lactate is processed through pyruvate for oxidation in the CAC, lactate also can accumulate if any PDC cofactors are insufficient, if a structural polymorphism is present for any enzyme of the PDC or if CoQ_{10} deficiency creates a block in the final oxidative phosphorylation stage of energy production.[77] Lack of mitochondrial ATP formation can induce a strong elevation of lactate with concurrent severe depression of pyruvate. This metabolic consequence is because of the necessity of cycling NAD^+ to allow alternative energy yield from glycolysis (see Figure 6.6). The reduced cofactor (NADH) produced in oxidation of glucose is reconverted to the oxidized form (NAD^+) as pyruvate is reduced to lactate. The regenerated NAD^+ allows another molecule of glucose to enter the pathway. Because of these metabolic relationships, when simultaneous elevation of both lactate and pyruvate is found, normal mitochondrial ATP output is indicated with blockage of the PDC, causing poor clearance of pyruvate with increased conversion to lactate.

Because of the multiplicity of causes, lactic aciduria is the most common organic aciduria associated with inborn errors of metabolism. In addition to PDC cofactor deficiencies and mitochondrial function factors, lack of oxygen can slow lactate clearance. Experimental anoxia in sheep produces immediate elevation of urinary lactate due to down-regulation of the oxidative pathways.[78] Lack of oxygen or coenzymes results in inactivation of the citric acid cycle and a decrease in mitochondrial activity. The effectiveness of CoQ_{10} supplementation for normalization of high lactate[77–79] demonstrates that a blockage at the electron transport level of mitochondrial

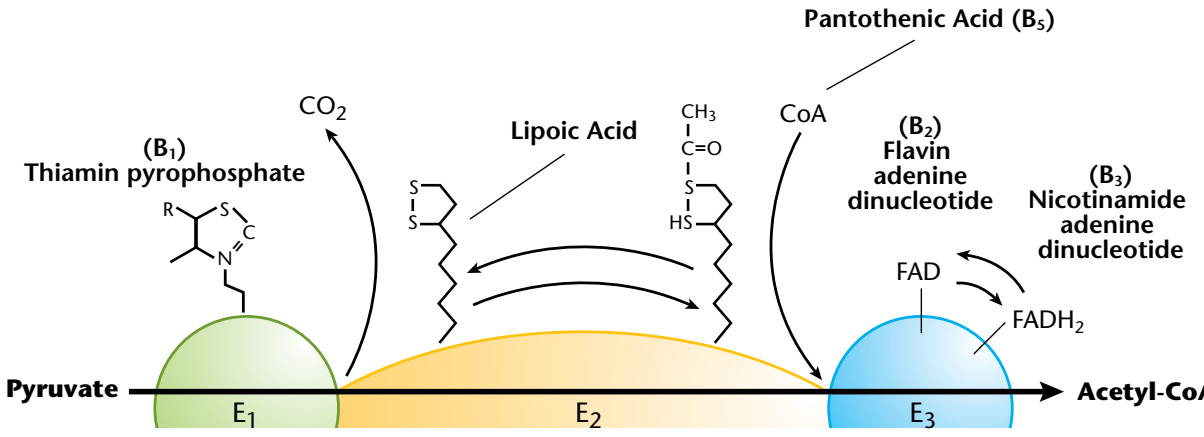


FIGURE 6.4 — **The Pyruvate Dehydrogenase Complex**

The large enzyme complex contains multiple copies of three subunits, E_1, E_2 and E_3. This figure emphasizes the bound cofactors, thamin pyrophosphate on E_1, two lipoyl groups in peptide linkage to lysine side chains of E_2 and flavin adenine dinucleotide on E_3. An isoenzyme that carries out similar oxidative decarboxylation of branched chain amino acids is illustrated in Chapter 4, "Amino Acids," Figure 4.13. A third isoenzyme carries out the oxidative decarboxylation of α-ketoglutarate. The three forms differ in amino acid sequence for E_1 and E_2, while maintaining the same gene for E_3. That means that genetic polymorphisms of E_3 will manifest metabolic interferences in all of the keto acid pathways similar to that found in simple B-vitamin deficiency. Generic alterations of pathway-specific E_1 or E_2 can produce specific abnormalities in pyruvate or α-ketoglutarate levels.

function is common. As noted, elevated lactate, therefore, is a sign of potential CoQ_{10} insufficiency. Detecting deficiencies of the other vitamin-derived cofactors of the PDC that can contribute to lactate elevation will be covered in later sections

Clinical Associations: Elevated levels of pyruvate have been traced to insufficiency of thiamin.[80] Animal studies have also identified increased levels with insufficient pantothenic acid.[81] Pyruvate elevation in the brain accompanies the encephalopathy associated with alcohol use in the thiamin-responsive Wernicke-Korsakoff syndrome.[82] Infants with thiamine-responsive congenital lactic acidosis often respond to thiamin stimulation (see Figure 6.5). Clinical features are variable, but detection through laboratory assessments of lactate and pyruvate can allow early initiation of thiamin therapy that can prevent permanent brain damage.[83] Dietary supplementation with pyruvate has been found to prevent fatty liver caused by chronic alcohol ingestion in laboratory animals.[84] The beneficial effect of pyruvate is explained as a stimulation of glucogenesis, which results in inhibition of fatty acid synthesis.

Lactate accumulation has been reported in asthma,[85] mitochondrial encephalomyopathy,[86] HIV-infected patients[87] and diabetics using biguanide medications.[88, 89] The lactic acidosis produced when HIV-infected patients were treated with nucleoside-analogue reverse transcriptase inhibitors, was found to be reduced by treatment with two or more cofactors (thiamin, riboflavin, CoQ_{10}, and L-carnitine), which lead to a lower overall mortality.[90] After glucose loading, lactate and pyruvate levels are significantly increased in patients with type 2 diabetes. Short-term treatment with 600 mg of lipoic acid orally prevented the hyperglycemia-induced increments of lactate and pyruvate.[91] This effect was mediated through increased activity of the pyruvate dehydrogenase enzyme, indicating that the coenzyme role of lipoate may be inadequate without the supplemental lipoic acid.

A case of lactic acidosis and marked hepatic steatosis was normalized with administration of 50 mg of riboflavin per day.[92] Both lactate and pyruvate may be elevated by alcohol consumption prior to specimen collection.[93] This effect may be due to the niacin-depleting effect of alcohol. The conversion of lactate to pyruvate (Figure 6.6) and the further oxidation of pyruvate are NAD-dependent reactions.

Lactic acidosis with increased excretion of hydroxybutyrate and fumarate were biochemical signs of the metabolic disturbance found in a 42-year-old woman who presented with a 10-year history of ophthalmoplegia, malabsorption resulting in chronic malnutrition, muscle atrophy, and polyneuropathy.[94,95] The underlying biochemical lesion in this mitochondrial, multisystem disorder was found to be genetically defective cytochrome C oxidase (complex IV of the respiratory chain). This case illustrates the potential for graded metabolic effects from genetic polymorphic expressions. Interestingly, this defect did not manifest until adulthood. Compensatory mechanisms maintained nominal function until the genetic weakness became manifest, which was most likely due to accumulation of environmental insults and dietary inadequacy. As we age, metabolic systems function less efficiently. How many of our "degenerative diseases" are genetic weaknesses manifesting as our systems become less efficient?

Investigators studying the metabolic profile of cancer proposed that apoptotic resistance, thought to promote tumor growth, is produced when mitochondrial glucose oxidation is inhibited. This inhibition is thought to be due to stimulation of pyruvate's reduction

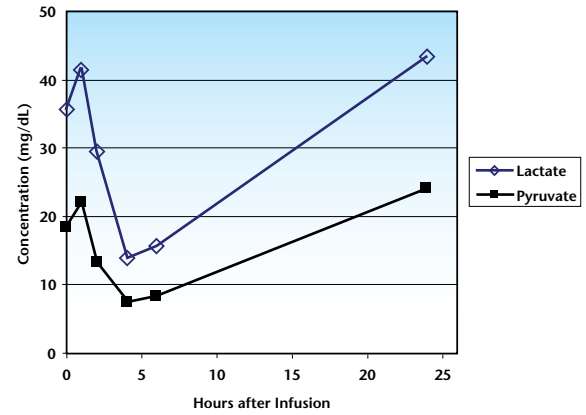


FIGURE 6.5 — **Thiamin Effects on Blood Lactate and Pyruvate[83]**

Changes of blood lactate and pyruvate after intravenous administration of thiamine hydrochloride (125 mg/kg) in a 2-month-old female with congenital lactic acidosis. The sudden rise of vitamin B_1 to high concentrations in blood causes rapid intracellular uptake and conversion to the thiamin pyrophosphate (TPP) form with subsequent enzyme saturation and metabolic correction of abnormal levels of metabolites. Within four hours the metabolites fall to near-normal levels even though genetic polymorphism has produced defective TPP binding. The effect is short-lived, however, showing the need for regular vitamin administration to sustain the metabolic effect.

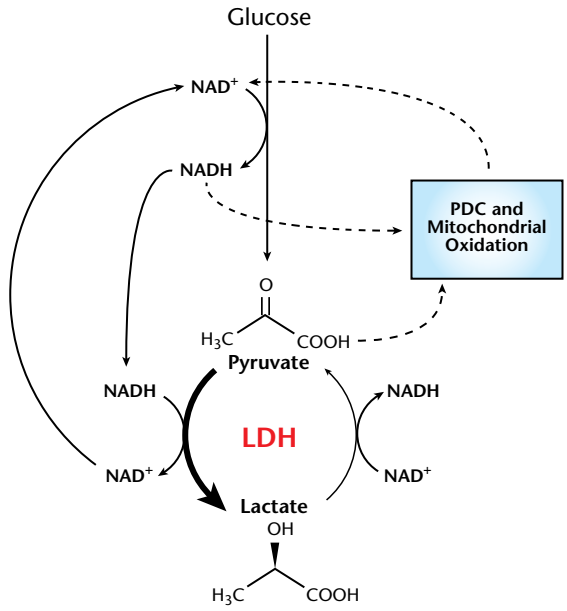


FIGURE 6.6 — **Conversion of Pyruvate to Lactate Regenerates NAD+**

The kinetics for lactate dehydrogenase catalysis of pyruvate reduction to lactate allow for the reaction to be easily reversed with net synthesis of pyruvate from lactate. However, when the PDC is inhibited, the normal transfer of electrons that regenerates NAD+ falls behind. In order to allow cellular energy flow to continue, the reduction of pyruvate to lactate becomes strongly favored over the oxidation of lactate to pyruvate. The outcome is lowering of urinary pyruvate and elevation of lactate.

to lactate in preference to its oxidation to acetyl-CoA.[96] The inactivity of the mitochondria produces a need for NADH oxidation that is reflected as high lactate production and depletion of pyruvate as discussed above and illustrated in Figure 6.6. The study also found that the suppressed oxidative metabolism of cancer cells could be reversed by a medication (dichloroacetic acid) used to increase the function of the PDC enzyme. A urinary profile showing high lactate and low pyruvate in a patient with cancer, is evidence of the effect in that patient. A number of chronic nutrient insufficiencies will be discussed that can contribute to low-level mitochondrial activity restrictions.

Studies in rats have shown an increase in lactate excretion when dietary carbohydrate is switched from fructo-oligosaccharides to maltodextrin, indicating that lactate production by intestinal bacteria may have shifted (see below in this chapter for discussion of D-lactate).[97] Although such variations in humans may be encompassed by the normal range limits set by clinical laboratories, definitive studies of such dietary effects in humans have not been done. Patients with moderate lactic aciduria may carry mild enzyme defects that manifest as symptoms only when metabolic stressors exceed the ability to maintain normal function. The age of onset and the degree and duration of functional loss depend on an individual's ability to maintain organ reserve. The incidence of such latent defects can be high relative to more severe forms of genetic disorders. Since there are a very large number of amino acid substitutions possible for every enzyme, it is logical to assume that for every serious enzyme polymorphism that produces profound organic acidurias there can be less severe forms in larger numbers of individuals that produce moderate abnormalities. The less severe forms may become clinically significant only when triggering events occur. The example of multiple forms of PKU known to be caused by over 400 single nucleotide polymorphisms was described in Chapter 4, Amino Acids, and the organic acidurias involved in multiple forms of maple syrup urine disease described above further illustrate this point.

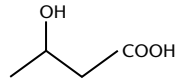
Notes:

OF FURTHER INTEREST...

Metabolic Acidosis:

- Increased levels of organic acids due to their increased production or failure to be removed
- Narrowly defined as either diabetic or lactic acidosis, but more broadly includes elevated levels of any organic acids in body fluids
- High anion gap due to organic acids

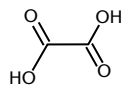
β-Hydroxybutyrate



β-Hydroxybutyrate is a ketone body. Ketone bodies are a feature of metabolic acidosis due to failure of glucose utilization as with diabetes. Ketone body production increases in diabetes because the oxidation of free fatty acids is stimulated, and excess acetyl-CoA is converted to the four-carbon organic acid, β-hydroxybutyrate. This acetyl-CoA spillover phenomenon occurs because the control of ATP production from fatty acids cannot be regulated as well as from carbohydrate oxidation. Individuals with normal blood glucose who respond to insulin do not produce high concentrations of ketone bodies because their production of energy from glucose is well controlled. Elevations of β-hydroxybutyrate in an overnight urine collection may indicate inefficient utilization or mobilization of glucose.[95] Chromium and vanadium supplementation have been found to support carbohydrate utilization by improving the action of insulin.[98–99] Defects in cytochrome oxidase enzymes of the electron transport system are another reason for elevated hydroxybutyrate.[100] These enzymes are heme dependent, thus iron deficiency could be the origin of their lowered activity.

Restriction of dietary carbohydrate, a common dietary approach to weight loss, produces elevated β-hydroxybutyrate. As in the diabetic association, the diet induces dependency on fat oxidation, leading to acetyl-CoA spillover.[101] Any severe caloric restrictions such as fasting produce this same effect.[102]

Oxalate



Oxalic acid is primarily known in medicine for its role in the formation of calcium oxalate kidney stones. At neutral pH, oxalate, the smallest possible dicarboxylic acid, forms complexes with calcium that have limited solubility. Because individuals who have a tendency to form renal oxalate stones frequently experience intense pain on passage of a large stone, the issue of management of oxalate stone formation has been extensively investigated.

Dietary Contributions to Urinary Oxalate: Oxalic acid is a product of the glyoxylate pathway that is present in human tissues and in oxalate-accumulating plants and some microorganisms. Both diet and intestinal flora can contribute to human oxaluria. Many leafy vegetables and some fruits contain appreciable oxalate, but may not produce significant urinary oxalate increases because the calcium and other divalent elements in the foods have already complexed with the oxalate, rendering it unavailable for absorption. Therefore urinary oxalate levels do not directly correlate with dietary oxalate content.[103] Peanuts and almonds contain sufficient free oxalate to raise urinary levels.[104] Spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries can cause a significant increase in urinary oxalate excretion, though, again, the increases are not in proportion to oxalate content, due to calcium content.[103] Soy products contain up to 50 mg of oxalate per serving, but their even higher content of phytate prevents oxalate absorption and makes them of low risk for contributing to urinary oxalate in most individuals.[105]

Oxalate Status Modulation by Intestinal Bacteria: Some intestinal bacteria can produce oxalate, whereas others degrade it.[106] The total oxalate degrading activity in the stool is inversely related to urinary oxalate excretion, and calcium oxalate stone-forming individuals frequently are lacking oxalate-degrading bacterial activity.[107] Daily administration of a mixture containing 8×10^{11} freeze-dried lactic acid bacteria *(Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Streptococcus thermophilus, Bifidobacterium infantis)* for 4 weeks produced a reduction of urinary oxalate from average values of 55 to 28 mg/24 h 1 month after the treatment period in 6 patients with idiopathic calcium-oxalate urolithiasis and mild hyperoxaluria (> 40 mg/24 h).[108] However, at 1- and 2-year post-treatment follow-up visits, lack of intestinal colonization was found with such lactic acid bacteria. *Oxalobacter formigenes* appears to have therapeutic potential for long-term lowering of urinary oxalate by oral application as a probiotic agent.[109, 110] Studies in vitro have shown that *O. formigenes*, detected in human feces by 16S rDNA amplification, actively metabolizes oxalate, helping to lower intestinal assimilation of oxalate.[111]

Production and Deposition of Oxalate in Human Tissues: The rate of oxalate production in human tissues is low in most individuals, but deficiency in the liver-specific peroxisomal enzyme, alanine-glyoxylate

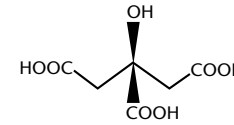
aminotransferase results in the congenital defect in glyoxylate metabolism known as primary hyperoxaluria type I that is associated with over 50 disease-producing mutations.[112] Whether oxalate accumulation is due to absorption of intestinal oxalate or endogenous production, the effects can be more general than kidney stones. In addition to the crystallization of calcium oxalate in the kidney, musculoskeletal and systemic manifestations can be produced from oxalate crystals deposited in bone, skin, vessels, and inside the joints.[113] Maintaining high urinary magnesium and citrate can be a helpful way to avoid renal calculi and oxalate crystal deposition disease, as discussed further under "Citrate. Isocitrate, and cis-Aconitate" below.[114] Calcium supplementation can reduce renal stone disease,[115] and the enhancement of calcium absorption by vitamin D is well known. Polymorphism in the vitamin D receptor gene has been proposed as a marker for calcium oxalate stone disease.[116] In addition, oxalate-induced free radical oxidative stress may be reduced by antioxidant therapy.[117] Hyperoxaluric rat kidney shows high levels of superoxide and H_2O_2-generating enzymes such as glycolic acid oxidase and xanthine oxidase, and accumulation of transition metal ions, iron, and copper that increases formation of hydroxyl radicals.[117]

Analytical Issues: Urinary oxalate may be measured by enzymatic assay, but there are several interfering compounds that can produce false-positives. Application of GC/MS requires derivitization of the two carboxyl groups, leading to extreme difficulty with controlling losses of the product because of the small molecular weight of the simple two-carbon compound. Attempts to develop such methods have shown variability far outside of acceptable limits.[596] These limitations may be overcome by using LC/MS-MS techniques, where urine is simply diluted with a solution of internal standard and injected onto the liquid chromatography column. Columns must be chosen to allow elution of the highly polar oxalate ion without excessive spreading of the peak.

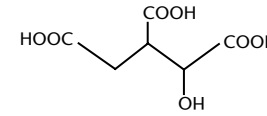
Central Energy Pathway Markers

Dietary carbohydrate, fat, and protein (amino acids) are catabolized to the common metabolic intermediate acetyl-CoA. The subsequent metabolic conversions responsible for extracting energy from the bonds between carbon and hydrogen with ultimate consumption of oxygen and release of carbon dioxide and water are known as central energy pathways.

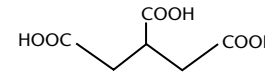
Citrate



Isocitrate



cis-Aconitate



The CAC is not only the final common pathway of energy release from food components, but is also the source of basic structural or anabolic molecules that are drawn away from the cycle to support organ maintenance and neurological function. Therefore, the CAC serves both anabolic and catabolic functions of the body, representing the "crossroads" of food conversion and utilization (see Figure 6.1).

Conversions of the CAC intermediates are under the control of enzymes, many of which require vitamin-derived cofactors and essential elements for their function. This fundamental pathway of energy flow is critical for all organ systems. Abnormal spilling of CAC intermediates in urine can indicate mitochondrial inefficiencies in energy production. Detection of such abnormalities can explain the biochemical basis of excessive fatigue and weakness and guide ways to improve energy production by supplying specific B-complex vitamins. Since the compounds that make up the pathway are related in a cyclic manner, a block at any step can cause accumulation of multiple compounds that precede that step. In cytochrome-c oxidase deficiency, inefficient utilization of NADH, the primary product of the CAC, has been shown to cause citrate, malate, fumarate, and α-ketoglutarate elevations.[118] In such conditions of genetic origin, interventions must focus on optimizing other ways of managing energy demands if approaches to enhancing formation of

the heme cofactor for the oxidase enzyme (including iron supply) are ineffective. Coenzyme Q_{10} deficiency can also result in elevated CAC intermediates (see "Hydroxymethylglutarate" below). Toxic effects of drugs like gentamicin are, in part, due to loss of mitochondrial integrity. Such drugs inhibit isocitrate dehydrogenase, the enzyme that converts isocitrate into α-ketoglutarate. Lipoic acid, used at 25 mg/kg/d, protects against such cytotoxic effects.[119] Isocitrate dehydrogenase activity also requires the presence of magnesium, manganese, and NAD from vitamin B_3.

Citrate is frequently the most abundant organic acid in urine. The range of normal values is broad because citrate excretion varies with changes in renal metabolism and acid-base disturbances.[120] Citrate elevation can be utilized as a marker of ammonia accumulation due to arginine insufficiency.[121,122]

Ammonia, a common metabolic byproduct, is toxic and must be neutralized via the urea cycle or excreted. Whenever the ammonia clearance capacity of the urea cycle is exceeded, the rise of blood ammonia triggers increased ammonia excretion by the kidneys. The anionic properties of citrate, *cis*-aconitate and isocitrate are used to counterbalance the cationic ammonium ion excretion. Abnormally high levels of these three organic acids in urine can indicate that this renal ammonia removal mechanism is being used. A caveat about such a conclusion, however, is that elevation of the entire CAC intermediate group is a sign of functional coenzyme Q_{10} insufficiency. This scenario is discussed further under "Succinate." Orotate elevation is an additional, sensitive and specific marker for ammonia build-up due to levels that exceed the capacity of the hepatic urea cycle (see "Orotate" later in this chapter).

Low levels of citrate, isocitrate, and *cis*-aconitate may occur due to the multiple pathways that draw CAC intermediates away for biosynthesis, such as the initiation of heme synthesis by succinate. When this happens, enzymes that serve to refill the cycle should be activated. Aspartic acid is a preferred substrate for the anaplerotic (refilling) pathway that restores CAC levels. By a single vitamin B_6-dependent transamination step, aspartate is converted into oxaloacetate, the immediate precursor of citrate. The reaction yields a net gain of cycle intermediates, a result that cannot be achieved from the flow of acetyl-CoA. Catabolism of phenylalanine and tyrosine produces fumarate, and several amino acids are converted into α-ketoglutarate (see Figure 6.1).

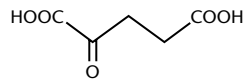
Low urinary citrate is related to urolithiasis. Both cystine and calcium calculi are increased when citrate excretion is low. Hypocitraturia is the most common metabolic abnormality (found in 52% of patients) associated with the nearly 5-fold increase of urolithiasis in children from 1994 to 2005 in one hospital.[123] A similar conclusion was drawn from measurement of urinary citrate in patients with cystic fibrosis.[124] Urinary citrate was highly inversely correlated with cystine excretion when 18 children with cystine stones were compared with 24 healthy children. Cystine excretion decreased by 43%, whereas urinary citrate increased almost 3-fold when the patients were treated with1 mEq/kg potassium citrate and 15 mg/kg α-mercaptopropionylglycine daily.[125] This mercapto compound reacts with renal free cysteine in a mechanism independent of citrate concentration. High-protein, low-carbohydrate diets decrease the excretion of citrate, raising the risk of kidney stones.[126, 127]

A pattern of high *cis*-aconitate with low isocitrate is consistent with a defective aconitase enzyme. Aconitase, which requires cysteine and iron as cofactors, is one of the mitochondrial matrix enzymes most sensitive to oxidative damage.[128] In rats, normal rates of oxidative damage produce an age-related decline of aconitase activity.[129] Significant evidence exists for iron regulation of aconitase,[130-136] so iron deficiency would also tend to produce an effect like that seen on these reports. The aconitase responses are tied to iron and nitric oxide effects through cellular regulation involving mitochondrial ATP production.[137] Signs of oxidative damage that might produce elevated cis-aconitate may be investigated by inspecting for high xanthurenate and 8-hydroxy-2'-deoxyguanosine. As discussed below under "Xanthurenate," accumulation

Notes:

of this tryptophan catabolite has capacity for generating reactive oxygen species.[138] Elevated 8-hydroxy-2'-deoxyguanosine provides evidence of increased rates of DNA oxidative damage as discussed below under "8-hydroxy-2'-deoxyguanosine."

α-Ketoglutarate



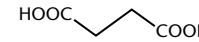
The oxidation of isocitrate produces α-ketoglutarate (α-KG), also known as 2-ketoglutarate or 2-oxoglutarate. In the next reaction of the CAC, α-KG is oxidized in an energy-releasing step that requires a dehydrogenase enzyme complex, called α-ketoglutarate dehydrogenase complex (KGDC), which is similar to the pyruvate dehydrogenase complex described above. Low activity of this enzyme has been shown to produce elevated excretion of α-KG.[139] The same five B vitamins are necessary for the action of KGDC, so elevations of α-KG can signal B-complex deficiencies. α-Ketoglutarate dehydrogenase deficiency can range from near absence to marginal reduction in activity, with outcomes ranging from severe neonatal acidosis to interruptions of normal development by infections.[139] Clinical signs, neuroimaging results, and biochemical correlations associated with movement disorders in childhood organic acidurias have been the subject of review.[140]

REFER TO CASE ILLUSTRATION 6.2

The compounds that make up the CAC can be derived from amino acids as discussed above. This may explain the energy-boosting effect people often report when they take free-form amino acid supplements. The effect is due to the conversion of specific amino acids directly into depleted CAC intermediates needed for the energy-producing cycle, such as α-KG, succinyl-CoA, fumarate and oxaloacetate. The fatigue-reducing effect of supplementation of aspartate salts and ketoglutaric acid has been attributed to such a mechanism.[141,142] The importance of α-KG in cell energetic balance is shown by the effectiveness of oral α-KG for reducing toxic consequences of cyanide poisoning where the availability of oxygen is the primary deficit.[143] Administration of a single 2 g/kg oral dose of α-KG protected brain and liver against mitochondrial damage from otherwise lethal doses of cyanide in rats.[144]

Levels of α-KG can also serve to mark an aspect of the carbohydrate and fat metabolic relationship. When metabolic conditions, such as insulin intolerance, act to stimulate fatty acid (especially palmitate) synthesis, the rising levels of palmitoyl-CoA cause an effective inhibition of glutamate dehydrogenase. Since this enzyme is the gatekeeper of the dominant pathway for production of α-KG, mitochondrial α-KG may be depleted when fatty acid synthesis is stimulated.[145] To state the corollary, low urinary α-KG is a marker for up-regulated fatty acid synthesis, increased palmitic acid in plasma and cell membranes, and increased serum triglycerides.

Succinate



Succinate is a CAC intermediate that donates electrons directly to complex II in the electron transport system via succinate dehydrogenase. Succinate dehydrogenase is the part of the electron transport chain that acts to initiate the sequence to transport electrons from succinate to oxygen. Succinate cannot play its role in cellular energy production when CoQ_{10} is inadequate because the electrons must be passed directly to bound CoQ_{10}. Elevated succinate excretion is a marker for increased requirement for both CoQ_{10} and riboflavin.[146] Clinical signs of CoQ_{10} and riboflavin deficiencies include fatigue, lassitude and myocardial and neurological degeneration.[147] CoQ_{10} depletion can be tissue specific. For example, a 4-year-old boy who presented with progressive muscle weakness, seizures, and cerebellar syndrome had greatly reduced muscle CoQ_{10} without corresponding CoQ_{10} deficits in lymphoblasts or skin fibroblasts.[79] His muscle mitochondria had severely restricted ability to utilize succinate. A laboratory report with abnormalities indicative of succinate dehydrogenase deficiency is shown in Case Illustration 6.3. The feature that distinguishes this scenario is the pattern of very low cis-aconitate and isocitrate. CoQ_{10} insufficiency would generally cause high levels of these intermediates, not low levels as seen in this instance.

REFER TO CASE ILLUSTRATION 6.3

The reaction in which succinate is converted into fumarate depends on the presence of flavin adenine dinucleotide (FAD) derived from riboflavin. Riboflavin administration has been shown to produce dramatic regression of neurological impairment in cases of deficits in the complex II respiratory chain succinate dehydrogenase enzyme (Figure 6.7).[71,146]

When succinate levels drop below normal, leucine and isoleucine are effective precursors that are converted into succinate to assure functioning of the CAC. Since this conversion requires adequacy of vitamin B_{12}, functional adequacy of B_{12} should be assured when amino acids are used to raise succinate. Individuals with inherited disorders such as fumarase deficiency, in which the final steps of amino acid conversion to succinate are blocked, exhibit serious neurologic symptoms, especially encephalopathies.[149]

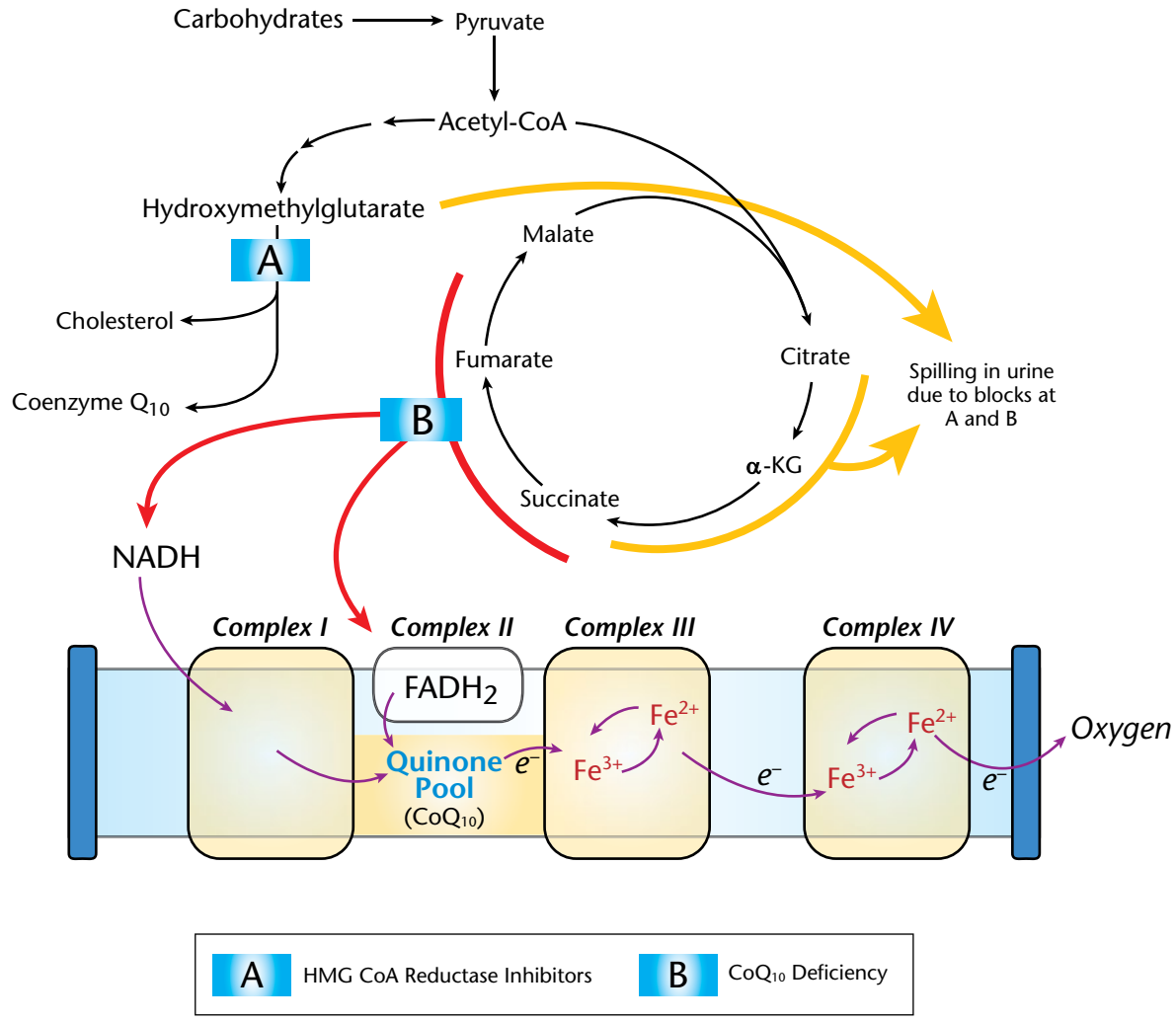
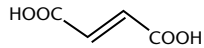


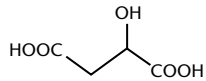
FIGURE 6.7 — **Markers of Coenzyme Q_{10} Synthesis and Function**

Elevation of organic acids in urine can reveal inhibition of the coenzyme Q_{10} biosynthetic pathway (**A**) or interruptions of the electron transport system (**B**) from inability to sustain adequate CoQ_{10} levels in the inner mitochondrial membrane. Both endogenous regulators and statin drugs act at the HMG-CoA reductase enzyme gateway for two-carbon unit entry into the pathway that leads to synthesis of cholesterol and CoQ_{10}. Accumulation of intermediates of the citric acid cycle, on the other hand, signals a functional insufficiency of inner mitochondrial membrane CoQ_{10} to sustain the flow of electrons.

Fumarate



Malate



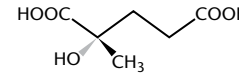
The removal of electrons from succinate in the reaction shown in Figure 6.7 generates fumarate. Fumarate is then converted to malate. Because of the sequential nature of these reactions in the mitochondrial matrix, fumarate and malate elevations serve as additional markers to substantiate the extent of metabolic interference from CoQ_{10} deficiency or cytochrome oxidase defects.[94] Malate is also actively pumped across the mitochondrial membrane as a way of transporting reducing equivalents in or out of the mitochondria. Methylmalonate inhibits the malate transporter, suggesting that elevated malate may also appear as part of the pattern of vitamin B_{12} deficiency. This effect has been suggested as an explanation of the hypoglycemia and ketosis produced in methylmalonic aciduria.[150]

Elevated malate also could result from fatty acid synthesis stimulation. The fatty acid synthesis product palmitoyl-CoA is known to inhibit the malate dehydrogenase enzyme.[145] Urinary malate elevation due to CoQ_{10} insufficiency is difficult to predict, however, because of individual variation in regulation of mitochondrial oxidation. In type 2 diabetic men, for example, lower insulin sensitivity is associated with lower glucose-stimulated rise in cytosolic malate, suggesting an abnormality in fatty acid synthesis regulation by malate.[151]

Low fumarate and malate concentrations in urine are a sign that the refilling reactions for the CAC are not keeping up with losses of intermediates to other pathways. Amino acid catabolism supplies a major portion of compounds that resupply the CAC (see Figure 6.1). The addition of tyrosine or phenylalanine can help supply fumarate when this intermediate is low.

Genetic origins of fumarate accumulation are known. In the citric acid cycle, fumarate is converted to malate by the enzyme fumarate hydratase, also simply called fumarase. Fumarase deficiency produces an autosomal recessive encephalopathy. It is characterized by a clinical history of generalized seizures, psychomotor deterioration, and massive fumaric aciduria.[152-154]

Hydroxymethylglutarate



β-Hydroxy-β-methylglutaryl-CoA, commonly referred to as hydroxymethylglutarate (HMG) is the metabolic precursor of both cholesterol and CoQ_{10} (Figure 6.7). Low levels of HMG may reflect inadequate synthesis and possible deficiency of CoQ_{10}. The conversion of HMG to mevalonic acid is the rate-limiting step in the isoprenoid pathway that yields cholesterol and CoQ_{10}. Statin pharmaceuticals used to lower serum cholesterol do so by inhibiting the HMG-CoA reductase; however, they also simultaneously inhibit the endogenous synthesis of CoQ_{10} and cause accumulation of HMG.[155]

CoQ_{10} is utilized in the mitochondrial oxidative phosphorylation pathway for ATP synthesis and is a potent antioxidant. CoQ_{10} has been used extensively as a cardiovascular protective agent and has been shown effective in clinical trials of Parkinson's disease.[156] Depletion of CoQ_{10} in skeletal muscles can cause a myopathy characterized by easy fatiguability of muscle and aching muscles.[157] In extreme cases it can cause fatal rhabdomyolysis. Supplementation with CoQ_{10} for patients on statin drugs is widely recommended because the therapy produces decreased muscle CoQ_{10} levels that can lead to drug-related myopathy.[157-159] Testing of urinary HMG, along with functional markers of CoQ_{10} insufficiency and serum $CoQ_{10,}$ can optimize patient management

Notes:

with supplemental CoQ_{10} by helping to differentiate the patient needing 120 mg/d from those for whom 30 mg/d is sufficient.

Although it may seem paradoxical, cases where low serum CoQ_{10} levels are found with low urinary HMG suggest that CoQ_{10} inadequacy may be associated with low as well as high levels of HMG. The rationale for this association is that a block earlier in the pathway inhibits the formation of HMG, producing low levels in urine, whereas a block subsequent to the formation of HMG produces high levels. Either mechanism may lead to reduced formation of CoQ_{10}.

A rare genetic disorder results in elevated HMG due to deficiency of the enyzme HMG-CoA lyase that is required for the catabolism of leucine. Leucine is metabolized to HMG, which can then either be split into acetate groups by HMG-CoA lyase or enter the steroid biosynthesis pathway via HMG-CoA reductase. However, since HMG-CoA reductase is under strong feedback regulation, the HMG tends to accumulate. Infants with this condition present with symptoms resembling Reye's syndrome, neurologic dysfunction (such as obtundation, combativeness, and/or posturing), tachypnea, vomiting, hypoglycemia, hyperammonemia, hepatomegaly, and elevated transaminases in blood, but without ketosis.[2] The behavioral characteristics make this disorder likely to be diagnosed as autism. Such an apparent case is shown in Case Illustration 6.4, where greatly elevated HMG was found with concurrent high CoQ_{10}, indicating that the HMG was passing into the pathway for CoQ_{10} synthesis.

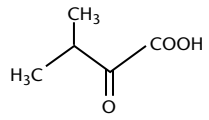
Refer to Case Illustration 6.4

B-Complex Vitamin Markers

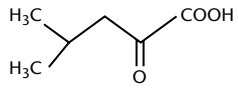
All of the compounds in this category are metabolic intermediates in the degradation of amino acids (Figure 6.8). These pathways are highly active, with large flux of substrates for individuals with normal caloric intake. Once again, vitamin insufficiencies result in slower rates of those reactions where the vitamin-derived cofactor is essential for enzyme activity. Because of the specificity of individual enzymes utilizing the vitamin-derived coenzymes, discrete patterns of elevated excretion of specific intermediates signal functional deficiencies of individual vitamins.

Vitamins $B_{1, 2, 3,}$ and $_5$

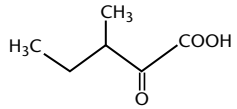
α-Ketoisovalerate



α-Ketoisocaproate



α-Keto-β-Methylvalerate



When hepatic or small intestinal enzymes remove the amino groups from the branched-chain amino acids (BCAA) valine, leucine, and isoleucine, they form the branched-chain keto acids (BCKA), α-ketoisovalerate, α-ketoisocaproate, and α-keto-β-methylvalerate, respectively. In most current biochemical literature, these compounds are named 2-oxo acids, rather than α-keto acids. In medical discussions, the older Greek letter designation still prevails.

These compounds have been discussed in the introduction to this chapter with reference to maple syrup urine disease. Although that discussion noted the specific response to thiamin, the enzymes require cofactors derived from five B-complex vitamins: B_1, B_2, B_3, B_5, and lipoic acid. Elevations of the branched-chain keto acids provide functional assessments of the sufficiency of these vitamins, especially thiamin.[14, 160] The molecular lesion site involving the branched-chain keto acid dehydrogenase (BCKAD) enzyme has been discussed in more detail in Chapter 4, "Amino Acids." The keto acids were also discussed in Chapter 2, "Vitamins," in the "Assessment of Status" section under each of the relevant vitamins. Figure 6.8 provides an overview of the amino acid pathways where the marker compounds are formed. Recall also that regarding the branched-chain keto acids, the enzyme required is similar to the pyruvate dehydrogenase complex discussed above under "Lactate and Pyruvate."

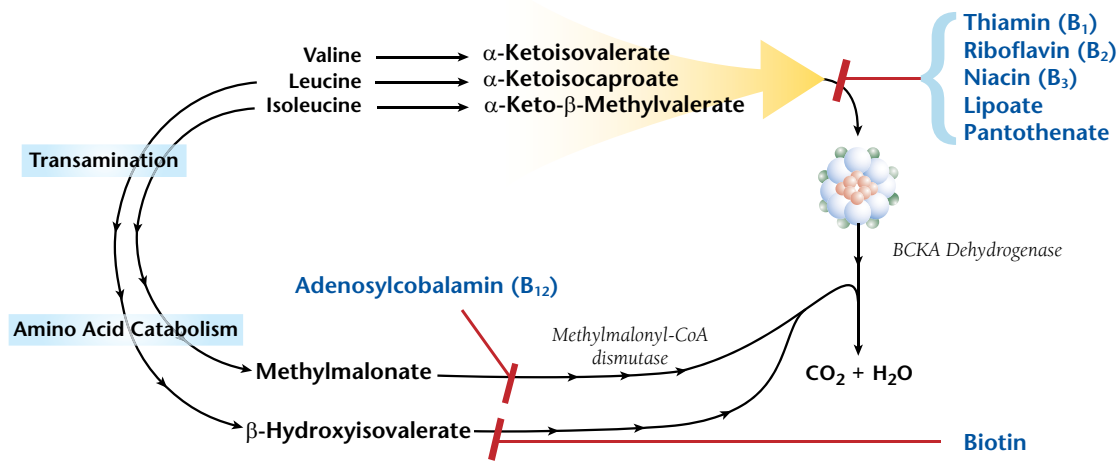


FIGURE 6.8 — Biochemical Markers For Vitamins B_1, B_2, B_3, B_5, Lipoic Acid, Vitamin B_{12} and Biotin

Catabolism of the branched chain amino acids, valine, leucine and isoleucine creates intermediates that can serve as markers of restricted rates of metabolism when they accumulate due to insufficient levels of their cofactor forms to sustain the dependent enzymes. There are three separate steps of isoleucine breakdown that signal insufficiencies of the thiamin group shown, vitamin B_{12} or biotin, depending on which intermediate spills into urine. This pathway schematic shows the relevant enzyme substrates that accumulate in each scenario.

There are six genes that encode the human BCKAD complex, where 100 mutations have been identified.[161] The accumulation of branched-chain keto acids has been shown to be a potential cause of the predominant neurological features of MSUD via their induction of oxidative stress in glioma cells.[162] The oxidative stress is further shown to be due to high rates of nitric oxide production and inhibition of phosphocreatine formation.[163] Additional neuronal damage may occur due to glutamate toxicity because the BCKA inhibit uptake of glutamate by cerebral cortical slices from rats.[164] On the other end of the clinical spectrum of keto acid disorders, patients with renal insufficiency who are placed on low-protein diets frequently have low branched-chain amino acids and may benefit from supplementation.[165] Adding BCKAs improved renal function in such patients along with improving levels of plasma amino acids.[166] Patients taking the hypolipidemic agent, clofibrate, may have diminished levels of urinary BCKA because the drug stimulates their metabolism by the BCKAD complex.[167]

Notes:

A different metabolic lesion has been reported to produce isovaleric acidemia without affecting the other BCKA. The enzyme, isovaleryl-CoA dehydrogenase, that acts subsequent to the BCKAD reaction in the valine catabolic pathway is specific for the isovaleryl group, so only valine is affected. Of the two variants that have been reported, the one responsible for type II isovaleric acidemia produces a mutation that interferes with the anchoring of the enzyme to the inner mitochondrial membrane.[168] This autosomal recessive inborn error was the first organic acidemia recognized in humans. Although it can cause significant morbidity and mortality, early intervention with a protein-restricted diet and supplementation

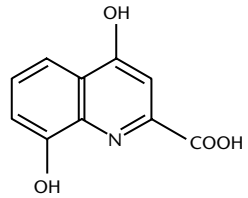
with carnitine and glycine are effective for promoting normal development in affected individuals.[75]

Refer to Case Illustration 6.5

The common pattern seen in simple deficiency of thiamin or other B-complex vitamins is one of increasing levels for all three branched-chain keto acids. When all three are above their 95th reference limit, a more severe thiamin deficiency or BCKAD polymorphism is indicated. In this scenario, it is common to also find elevated pyruvate and α-ketoglutarate because of their similar enzyme cofactors. Milder variants of nutrient insufficiency may show fewer elevations. In such instances, supplementation with branched-chain amino acids challenges the capacity of the pathway and may reveal abnormalities. Very severe abnormalities of specific markers can reflect genetic abnormalities, as illustrated in Case Illustration 6.5.

Vitamin B_6

Xanthurenate



Xanthurenic acid is the first to be discussed of several tryptophan metabolites arising from the pathway that converts tryptophan to nicotinic acid. Vitamin B_6 insufficiency leads to elevated excretion of xanthurenate and kynurenate because of a pyridoxal-5-phosphate-dependent step in this metabolic pathway, called the kynurenin pathway (Figure 6.14).[169]

The liver regulates tryptophan levels in plasma through the kynurenin pathway that is initiated by the heme enzyme, tryptophan-2,3-dioxygenase (TDO) or indoleamine-2,3-dioxygenase (IDO) (see "NMDA Modulators" below). Postprandial tryptophan in excess of what can be utilized for protein synthesis enters this pathway to be converted into nicotinate, offering obvious additional benefit from the high-protein meal. However, the enzyme kynureninase that converts the intermediate 3-hydroxykynurenin into 3-hydroxyanthranilate has a strict requirement for pyridoxal-5-phosphate derived from vitamin B_6.[170] Dietary deficiency of B_6 quickly manifests as slower rates of the B_6-dependent step, with accumulation of 3-hydroxykynurenin, which causes accumulation of kynurenin.[171] These compounds are rapidly converted to xanthurenate and kynurenate, respectively, which appear at elevated concentrations in urine. In most individuals the urinary xanthurenate rises to the higher concentrations, sometimes appearing as the only positive marker.[172, 173] Elevated kynurenate provides biochemical confirmatory evidence of B_6 insufficiency, especially when quinolinate is not high. As discussed below under "Kynurenate and Quinolinate," cells of the immune system and brain express only the first several enzymes of the hepatic kynurenin pathway, resulting in net accumulation of quinolinic acid rather than nicotinic acid.

There are important consequences of the tissue-specific gateways for initiation of kynurenin synthesis. In general, however, the pathway in which kynurenic, xanthurenic and quinolinic acids are formed from L-tryptophan serves the following functions:

1. Clearance of excess L-tryptophan
2. Maintenance of nicotinic acid levels
3. Regulation of glutamatergic neuronal activity
4. Enhancement of macrophage defense functions

Dietary tryptophan is an essential amino acid that often is the rate-limiting amino acid in biosynthetic reactions. Therefore, there is little need for the tryptophan clearance function in most individuals except following high-protein meals. When an L-tryptophan load is received, the kynurenin pathway should become active in the liver. The extra metabolic load is the basis of using a 3 to 5 g L-tryptophan challenge to increase the sensitivity for detecting marginal vitamin B_6 status.[174] Although the excretion of both kynurenate and xanthurenate are increased when vitamin B_6 is insufficient to maintain this function, xanthurenate is the principal product that appears in most cases. This is due to the further metabolism of kynurenate to citric acid cycle intermediates in the liver. Lower rates of renal clearance for kynurenate are indicated by measurements of plasma levels of both compounds in piglets on long-term parenteral vitamin B_6 supplements.[175]

Elevated urinary xanthurenate may be produced from dietary tryptophan even when there is no extra tryptophan challenge. In such cases, vitamin B_6 insufficiency is significant enough to interfere with even the normal conversion of dietary tryptophan. When piglets were used to study vitamin B_6 deficiency, urinary levels of kynurenate and xanthurenate were elevated early in vitamin B_6 deficiency, and continued to rise as the deficiency worsened.[171] Since challenge of the hepatic kynurenin pathway by tryptophan is necessary for xanthurenate production, patients may be advised to eat a large portion of a high protein-content food or take a tryptophan supplement on the evening of the specimen collection. This advice applies to several of the organic acid markers dereived from pathways of amino acid catabolism.

Dietary tryptophan can be used to form nicotinic acid, sparing the need for dietary niacin for making NAD+ and NADP+. If dietary tryptophan is limiting, then dietary niacin is required. However, even if the supply of tryptophan is in excess, niacin cannot be formed in the absence of vitamin B_6. The kynurenine pathway offers an excellent example of the complex interplay of essential nutrients. Eating disorders that restrict vitamin B_6 or protein may manifest signs of the niacin deficiency disease, pellagra, including photosensitivity, dermatitis, diarrhea and dementia.[176] Estrogen hormones inhibit the initial conversion of tryptophan to kynurenine, offering an explanation of the twofold excess of females over males in many outbreaks of pellagra.[177, 178] Other estrogenic effects that produce higher pathway intermediates may be due to increased losses of vitamin B_6 by individuals with high estrogen levels.[104]

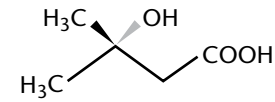
Vitamin B_6 deficiency produces elevated xanthurenate that, in turn, produces increased oxidative stress. The hydroxylated quinone structure of xanthurente has been shown to bind iron, producing a complex that increases DNA oxidative damage that may be shown by increased production of 8-hydroxy-2'-deoxyguanosine.[138] This means that, in addition to indicating a need for therapy with vitamin B_6, elevated xanthurenate leads to suspicion of antioxidant insufficiency.

Interpretation of elevated urinary xanthurenate should take into account kynurenin pathway modulation by steroid hormones[177] and bacterial endotoxins,[179] plus decreased flux of tryptophan through the pathway for individuals on low-protein intake.[180] The low dietary protein effect may be minimized by using an oral challenge of L-tryptophan (adults: 2 g) as long as laboratory reference range adjustments are allowed for such dosing.

Xanthurenic acid forms complexes with insulin, decreasing circulating concentrations and reducing activities of the hormone.[181, 182] Significantly elevated excretion of xanthurenate has been found in diabetic patients and animal models of diabetes.[183] Xanthurenate excretion has been proposed as a marker to both monitor and detect diabetes.[184] In women with chemical diabetes of pregnancy, xanthurenic acid seems to dominate, reducing the concentration of active insulin in the plasma. In pregnant women with gestational diabetes, excessive amounts of urinary xanthurenate were found. Treatment with 100 mg of pyridoxine daily for 14 to 23 days restored the urinary xanthurenic acid to normal in all patients tested.[185] In women who received pyridoxine for 14 days oral glucose tolerance also improved.[186]

Biotin

β-*Hydroxyisovalerate*



Biotin is obtained from food, and is also produced by predominant organisms (especially bifidobacteria) in the healthy human gut. Biotin deficiency was once thought to be rare because such small amounts are required and clinical signs of deficiency are non-specific.[187] It was thought that only certain disorders of infancy, such as Leiner's disease (desquamative erythroderma) and other forms of seborrheic dermatitis, are clinical manifestations of biotin deficiency. Recent findings are shifting the clinical outlook for biotin deficiency.

Biotin is a cofactor in acetyl-CoA carboxylase, pyruvate carboxylase, methylcrotonyl-CoA carboxylase, and propionyl-CoA carboxylase. The biochemical function of biotin-requiring enzymes is the insertion of carboxyl. Methylcrotonyl-CoA carboxylase catalyzes an essential step in the metabolism of leucine. Because leucine turnover amounts to many grams per day, its catabolism is a high-flux process that offers a biochemical marker of biotin deficiency. The product formed after the first three steps of the pathway, β-methylcrotonyl-CoA, requires a biotin-dependent carboxylation to allow the flow to continue. Deficiency of biotin causes accumulation

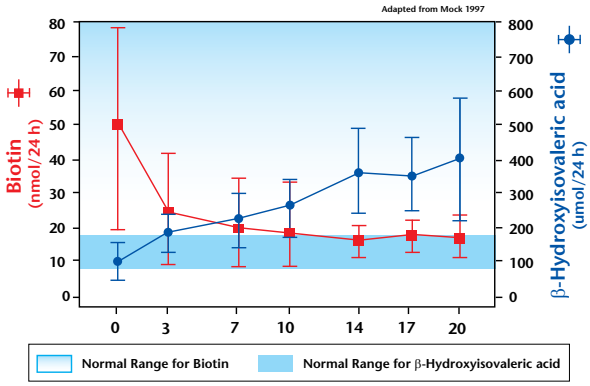


FIGURE 6.9 — **Human Biotin Deficiency and β-hydroxyisovalerate Excretion**

Within about ten days, 24 hour urinary biotin excretion falls below normal levels when the biotin-binding protein avidin is fed to human subjects. The biotin-dependent metabolic intermediate, β-hydroxyisovalerate, however, rises above normal ranges by the third day, showing the functional impact of insufficient biotin on amino acid metabolism far ahead of total body biotin depletion.

of this biotin-dependent intermediate, and its hydrated product, β-hydroxyisovalerate, spills in urine. β-Hydroxyisovaleric aciduria appears early in people who are made biotin deficient by consuming the biotin-binding protein, avidin. After starting avidin administration, elevated β-hydroxyisovalerate appears at the third day, whereas serum biotin concentrations remain in the normal range until the tenth day (Figure 6.9). The effects are completely reversible.

Additional studies have confirmed the high sensitivity and specificity of elevated β-hydroxyisovalerate for detection of biotin insufficiency.[188] Even antibiotic-induced disruptions of gastrointestinal bacterial biotin synthesis can produce biotin insufficiency detectable by elevated β-hydroxyisovalerate.[189] In addition to increased β-hydroxyisovalerate, biotin deficiency may produce elevations of lactate and alanine in urine and accumulations of odd-chain fatty acids (C 15:0 – C29:0) in plasma or red blood cell membranes.[190] The lactate and alanine effect results from decreased rates of gluconeogenesis in biotin deficiency. The fatty acid effect is due to initiation of long-chain fatty acid synthesis with the three-carbon chain of propionate. The sequence of elongations produces abnormally high levels of fatty acids with chain lengths of 13 to 31 carbons in plasma and erythrocyte membrane lipids.[191] See Chapter 5, "Fatty Acids" for further discussion of odd chain fatty acid abnormalities.

Heritable disorders of biotin metabolism lead to the condition called multiple carboxylase deficiency (MCD), in which the activities of enzymes that have absolute requirements for biotin to carry out carboxylation reactions are deficient.[190] β-Hydroxyisovalerate is a compound that is elevated in biotin deficiency and multiple carboxylase insufficiency.[188] Biotin deficiencies of various degrees have been shown to develop in normal pregnancies[192] and in patients on long-term anticonvulsant therapy.[193] When β-hydroxyisovalerate was used to assess biotin in pregnant women, 9 out of 13 women studied were biotin depleted, even in early pregnancy.[194] Symptoms of biotin deficiency include alopecia, skin rash, Candida dermatitis, unusual odor to the urine, immune deficiencies and muscle weakness. Six human missense mutations that produce MCD have been shown to be responsive to biotin therapy because normal circulating biotin levels are 100 times lower than the concentrations needed for half saturation of the enzyme. Dietary biotin supplementation can easily result in saturation of the available mutant enzyme with restoration of activity.[195]

Biotin from food or from intestinal microbial synthesis is absorbed in the upper small intestine and transported to tissues bound to several blood proteins. The enzymes that use biotin as a cofactor are called carboxylases because they use carbon dioxide to insert carboxyl groups into substrates. Cellular biotin must be incorporated into the carboxylase enzymes by the action of other enzymes called synthases (Figure 6.10). If these enzymes are not fully active, higher biotin concentrations can increase enzymatic activity and enhance the reaction rate. The carboxylase enzymes have critical roles in major pathways for the utilization of energy from amino acids (where β-hydroxyisovalerate is formed), the synthesis of fatty acids for cell membrane replacement, and the maintenance of blood glucose via

Notes:

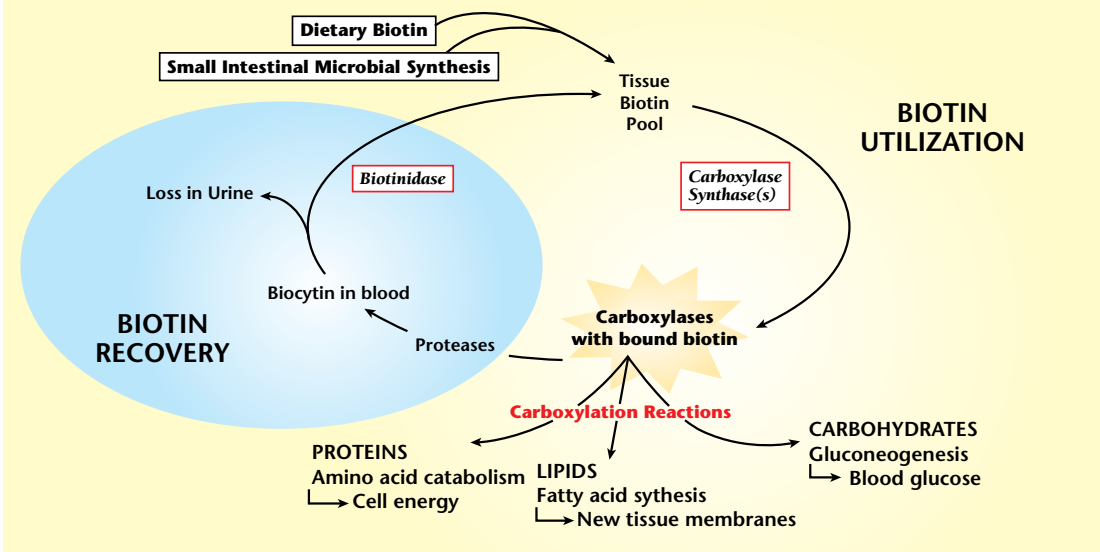


FIGURE 6.10 — **Defects in Biotin Pathways**

The activity of cellular carboxylase enzymes is determined by the total biotin pool size and the rates of formation and degradation. Cellular biotin is covalently bound to carboxylase enzymes by their respective carboxylase synthases. When they are degraded by proteases, the enzymes release biotin as biocytin that may be recovered when biotinidase is active. Thus, dietary, intestinal and genetic factors interact to determine individual biotin status. β-Hydroxyisovalerate is a substrate for one of the biotin-requiring enzymes in leucine catabolism.

gluconeogenesis. At cell death, the biotin may be recovered if there is sufficient activity of the enzyme biotinidase, which acts on the biotin-peptide fragments called "biocytin." The major factors shown as boxed text in Figure 6.10 contribute to the maintenance of carboxylation actions. Biotin insufficiency can be caused by lack of biotin-rich foods or genetic variations in the enzymes. Antibiotic overuse also may contribute to biotin insufficiency by lowering the population of biotin-producing organisms and favoring the overgrowth of non-biotin producing species. Lactobacillus acidophilus inoculation of broiler chickens fed a diet marginally deficient in biotin caused decreased liver biotin content and reduced growth rates, presumably due to competition by the probiotic for dietary biotin.[196]

TABLE 6.5 — BIOTIN DOSES FOR 2-YEAR-OLD MALE WITH BIOTINIDASE DEFICIENCY

Biotin Dose	Clearing Signs/Symptoms
6 mg	Truncal ataxia Convulsions Alertness
20 mg	Organic acids Leukocyte carboxylase
30 mg	Dermatitis (returned at 25 mg)

As the daily biotin dose was increased (shown in the left-hand column), the patient's signs and symptoms cleared in the order shown in the right-hand column.

A case involving biotinidase deficiency illustrates the use of biotin replacement. A 2-year-old male patient presented with ataxia, seizures, and a history of normal development for 19 months, and then experienced loss of language and developmental milestones and progressive gait disturbances.[197] On physical examination, the patient was obtunded with eczematous dermatitis, truncal ataxia, and a fine volitional tremor on the arms. Symptoms of ataxia, tremors, loss of language development, gait disturbances, and dermatitis cleared only after increasing biotin intake to 30 mg/d. The data presented in Table 6.5 are from this report. After 1 year of high-dose biotin, the symptoms did not return and there was no further progression of hearing loss, but there was some attention disorder with hyperactivity.

Over 20 point mutations that cause deficiency of the biotin-dependant enzyme, propionyl-CoA carboxylase (PCCA) have been identified in North America and

Europe. Along with the ensuing propionic acidemia, the accumulation of other toxic metabolites can impair mitochondrial function, especially affecting the basal ganglia and other regions of the brain with high rates of aerobic metabolism. Clinical presentations vary widely because of the variety of enzyme polymorphisms and triggering events that can range from protein overloading to fasting or febrile infections.[198] Table 6.6 shows typical symptoms found in patients with propionic acidemias categorized by age of onset. A case of novel PCCA mutations has been reported in which the patient had unremarkable medical history until 4.5 years old. A simple infection caused her to become comatose, whereupon the diagnosis of two new missense mutations of PCCA was made. In spite of combined therapy with biotin and carnitine, restriction of protein intake, high caloric nutrition, and correction of acidosis, the girl died 2 days after admission due to cardiac arrhythmias.[199]

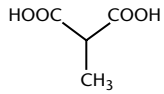
Table 6.6 — Propionic Acidemias

Neonatal	Vomiting, lethargy, neurologic symptoms, and keto-acidotic coma
Late-onset	Abnormal psychomotor development, mental and psychological disabilities
	Severe movement disorders, basal ganglia lesions
29-year-old	Dystonia, choreoathetosis

Methylation Pathway Markers

Vitamin B_{12}

Methylmalonate



Methylmalonyl-CoA is produced in the multistep process of breaking down the amino acids isoleucine, valine, methionine, and threonine, or from catabolism of odd-chain fatty acids.[200] Methylmalonate (MMA) is converted into succinic acid by a vitamin B12-dependent enzyme, methylmalonyl-CoA mutase (see Chapter 2, "Vitamins"). The lack of vitamin B_{12} impairs this conversion as well as the one in which methionine is recovered from homocysteine (tHCYS), leading to accumulation of both tHCYS and MMA. Thus, an increase in MMA or tHCYS, can signal a vitamin B_{12} deficiency.[201] Research studies have looked at both blood and urine levels of MMA, and have found overnight fasting urinary MMA concentrations to have a strong linear relation to serum MMA.[202] Vitamin B_{12} deficiency adversely affects both energy-yielding and methylation pathways. Such biochemical impairment helps explain the fatigue resulting from vitamin B_{12} deficiency and the rapid improvement of symptoms vitamin B_{12}-deficient patients experience with administration of intramuscular vitamin B_{12}. By combining measures of MMA and tHCYS, the various permutations of abnormalities may be used to specify insufficiencies as shown in Table 6.7.

Vitamin B_{12} deficiency has been demonstrated to be present in over 12% of the free-living elderly population in the United States[203] and is especially common in the early stages of HIV infection.[204] About 40% of patients with acquired cobalamin deficiency have neuropsychiatric syndromes characterized by progressive and variable damage to the spinal cord, peripheral nerves and cerebrum.[205] Methylmalonic acidemia is frequently accompanied by propionic acidemia. Acute or chronic vomiting and dehydration were the most common symptoms and physical findings, respectively, in emergency presentations of patients with methylmalonic acidemia, propionic acidemia, or branched-chain amino acidemia (MSUD).[206]

Occasionally a patient is found to have elevated urinary MMA even though vitamin B_{12} supplementation has produced elevated serum cobalamin levels. This situation might be produced by poor conversion of supplemental cobalamin to adenosyl and methylcobalamin. Several genetic defects of these steps, defined as cobalamin complementation groups have been identified.[207] Alternatively, specific polymorphism of the methylmalonyl-CoA mutase enzyme could prevent cofactor or substrate binding that would not respond to increased adenosylcobalamin concentrations. Mild polymorphisms of this type could be relatively common, since it is possible that the specific clinical consequence of methylmalonic aciduria could be benign. One reported case of this type showed psychomotor delay.[208] In the benign polymorphism scenario, all other B_{12} metabolic functions may be working perfectly, and the test returns a false-positive because the pathway step that we have

picked happens to be one that is mildly faulty in some individuals. A third alternative is that the intracellular cofactor concentration has been increased sufficiently to saturate the enzyme, but the turnover is insufficient to process excessive amounts of propionate. Clinical and biochemical benefit in two patients with methylmalonic aciduria following metronidazole therapy suggests that an antibiotic with specific activity against anaerobic bacteria can be effective for removing the propionate precursor of MMA. The virtual elimination of propionic acid from the stool of these patients suggests that propionic acid derived from fecal bacterial metabolism contributes substantially to MMA production. This novel treatment for these disorders of intermediary metabolism indicate the importance of microbial gut flora in normal human metabolism.[209]

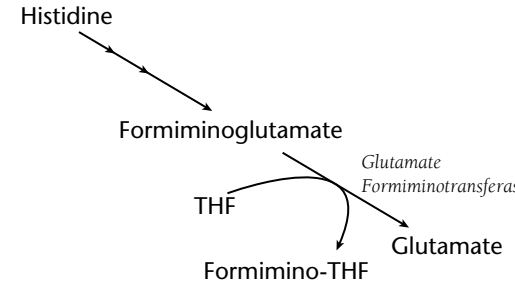
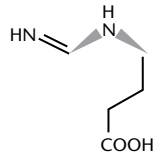


FIGURE 6.11 — The Formiminoglutamate Step in the Catabolism of Histidine

After meals containing protein, lack of adequate tetrahydrofolate (THF) cofactor slows the postprandial catabolic clearance of histidine, causing spilling of formiminoglutamate into urine.

FOLIC ACID

Formiminoglutamate



Formiminoglutamic acid (FIGLU) is an intermediate in the deamination of the amino acid histidine. Folic acid is the cofactor required by the enzyme formiminotransferase that converts FIGLU to glutamic acid (Figure 6.11). Folate accepts the formimino group from FIGLU to yield N5-formimino-THF. Descriptions of the "FIGLU Test" to determine folate sufficiency appeared as early as 1965.[210] FIGLU excretion increases in folate deficiency, especially when histidine is administered as an oral load.[211] Up to 15 g of oral histidine has been used as a FIGLU metabolic challenge for adults. Additionally, FIGLU excretion has been used to measure the influence of drugs or alcohol on functional folate status.[212,213]

Functions of folate include amino acid metabolism, cell division and replication, and nucleic acid synthesis, purine and pyrimidine synthesis in particular. Consequently, deficiency of folate can lead to inhibition of DNA synthesis, impaired cell division, and alterations in protein synthesis. Mild folate deficiency is characterized by low plasma folate and hypersegmentation of polymorphonuclear leukocytes. After about 4 months of low folate intake, red blood cell folate concentrations diminish.[214] High urinary FIGLU appears about 90 days after a period of insufficient folate availability. Urinary excretion of FIGLU in one group of folate-deficient patients was 113 mg/24 h, compared with 16 mg/24 h in healthy controls or vitamin B_{12}-deficient individuals,[215] after 15 g of histidine hydrochloride was administered in three 5 g doses at 4 h intervals (urinary collection started after the first dose). This experiment resulted in the recommendation that folic acid-deficient individuals should be treated with 1 mg of folic acid along with 5 g of histidine for 3 to 5 days to overcome anemia of folic acid deficiency (see Chapter 4, "Amino Acids," under "Histidine").

Since its function is to accept a single carbon group from the substrate in the FIGLU reaction, the simple folic acid form of the cofactor is needed. Another form of folate, 5-methylfolate, functions as a methyl-donor in the methionine-homocysteine pathway. Deficiency of this form of folate results in high levels of tHCYS. Elevated tHCYS increases the risk of ischemic heart disease, stroke, and deep vein thrombosis.[216] Differences in binding affinity and in the forms of folate make it possible to have elevated FIGLU with normal tHCYS. The FIGLU pathway can be thought of as folate loading, whereas the tHCYS pathway is folate unloading. Addition of methyl groups in the tHCYS pathway requires loaded (methyl-) folate, whereas removal of formimino groups in the FIGLU pathway requires only folic acid. Therefore, simple folic acid supplementation (not methyl forms) is needed to normalize FIGLU elevations.

A serum folate concentration of less than 0.3 ng/mL is evidence of deficiency, but false-normal values are

Table 6.7 — Differentiating B_{12}, Folate, and B_6 Deficiency

Scenario	Markers	Potential Deficiency
1	Homocysteine ↑ with no other test result	Either B_{12}, folate or B_6, or methyl donor compounds
2	Methylmalonate ↑ with Homocysteine and FIGLU normal	B_{12}
3	Homocysteine ↑ with normal methylmalonate	B_6 or folate
4	Both Homocysteine ↑ and methylmalonate ↑	B_{12}, folate, and B_6
5	Homocysteine ↑ with MMA and FIGLU normal	B_6 and possible methyl donor compounds
6	HCys, MMA and FIGLU all ↑	B_6, B_{12}, folate and methyl donor compounds

frequently found due to the response of serum levels to transient dietary intake changes that do not correspond to tissue repletion of the nutrient. The period of deficiency that must pass before erythrocyte folate drops below normal levels is longer than that resulting in hyperhomocysteinemia. Changes in leukocyte folate levels occur at the same rate as erythrocyte. Leukocyte folate thus is not a valuable early marker for deficiency. Combination assays of the methylation pathway markers FIGLU, methylmalonate, and homocysteine in urine have been proposed as the most sensitive and cost-effective means for routine diagnosis of megaloblastic anemia.[217]

Elevated tHCYS is a sensitive marker of folate inadequacy at the biochemical level. However, it is not necessarily due to folate deficiency. Serum folate can be used as a confirmatory test. A differential diagnosis of vitamin B_{12}, folate and vitamin B_6 deficiencies can be made with simultaneous assay of MMA, FIGLU and tHCYS (Table 6.7). If only MMA is elevated (Scenario 2), only B_{12} supplementation may be needed. In Scenario 5, folate is indicated, and though B_{12} may not be needed according to the normal MMA, the tHCYS elevation may be signaling a need for methyl donor compounds like betaine, dimethylglycine or subcutaneous injection of methyl-B_{12}. If all of the markers are found to be elevated (Scenario 6), then the full spectrum of cofactors and methyl donating nutrients should be considered.

Folate deficiency can occur for a number of reasons. Since humans cannot synthesize folate and it must be provided by the diet, poor dietary intake of folate-rich foods or excessive intake of processed foods increases the chances of insufficiency. Impaired absorption, inadequate utilization due to a metabolic block, increased demands, increased excretion or increased destruction of folate all contribute to folate insufficiency. Prescription drugs, lack of specific nutrients, genetic enzyme defects, many diseases, and alcoholism are factors that can influence the likelihood of a folate deficiency. Methotrexate (MTX) is a competitive inhibitor of dihydrofolate (FH2) and prevents recycling of folate to its usable THF form. Oral contraceptive use has been shown to impair folate metabolism as measured by serum folate, erythrocyte folate and FIGLU.[218] Vitamin B_{12} deficiency results in reduced folate uptake across gut and other cell walls, and reduced cell retention. Ascorbate protects folate from oxidative destruction, thus low dietary vitamin C influences folate status. Chronic alcohol consumption impairs folate coenzymes and, in folate deficiency, causes possible malabsorption of enterohepatically-circulated folates.[219]

A wide variety of clinical conditions can suggest folate evaluation. Extra folate may be needed to improve endothelial function and lower risk of heart disease.[220,221] Women with inadequate folate have a 53% higher risk of breast cancer, and folate adequacy reduces the increased breast cancer risk from alcoholic beverage consumption.[222,219] Patients with small cell carcinoma of the lung who were losing weight had elevated FIGLU. It was demonstrated that these individuals had high demands for one-carbon units supplied by folate.[223] Cigarette

Notes:

smoking-associated increase in pancreatic cancer is reduced when folate function is assured.[224] Smoking seems also to be associated with elevated homocysteine levels in patients with early atherosclerotic development.[225] Patients with Crohn's disease should be evaluated for folate adequacy to control homocysteine-associated thrombotic events.[226] Demands for folate increase with gestation.[227] Supplementation of folate in women of childbearing years not only prevents neural tube defects, but also prevents megaloblastic anemia, which can complicate pregnancies.

Analytical difficulties have challenged accurate FIGLU determinations, partly because the compound contains both negatively charged organic acid and positively charged amino groups, making it zwitterionic at neutral pH. An improved method for simultaneous determination of multiple zwitterionic metabolic products in urine by ion exchange chromatography coupled with tandem mass spectrometric detection has been developed.[17] This method allows these compounds to be measured accurately over the full range of normal physiological variation as well as for the elevated concentrations found in patients with folate deficiency.

Neurotransmitter Metabolism Markers

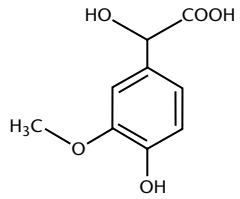
Neurotransmitter disorders have been recently described as "an enigmatic and enlarging group of neurometabolic conditions caused by abnormal neurotransmitter metabolism or transport."[228] Accurately determining the central nervous system (CNS) level of neurotransmitters has proven difficult. This may be in part because neurotransmitters are rapidly formed and degraded in nervous tissue, leaving neurotransmitter concentrations in other tissues largely unrelated to CNS levels. An exception is the reflection of CNS levels of serotonin by platelet or platelet-rich plasma serotonin concentrations and corresponding associations with serotonin-related pathologies.[229-231] Even when 24-hour collection specimens are used, urinary serotonin is found to vary widely among healthy males under clinically controlled conditions.[232] Healthy male smokers who were treated with the selective serotonin reuptake inhibitor Paroxetine had median platelet serotonin decrease by 16% after 24 hours, whereas urinary serotonin increased by 89%.[233]

Regarding dopamine, even in the extreme chronic elevation conditions of neuroblastoma, direct urinary dopamine to creatinine ratios were far less revealing than the dopamine catabolite vanilmandelic acid.[234] Urinary dopamine levels rise significantly and reproducibly only in cases with large, inoperable bilateral carotid body tumors.[235] In a retrospective study of 5,933 adults and 467 children conducted over a 57-month period, high 24-hour urinary dopamine levels were found in less than 3% of adults, and those instances were associated with overcollection, drug effects, and neural crest tumors.[236] Sleep has effects on dopamine excretion, as shown in a study that found roughly equivalent reductions for urinary dopamine and its catabolic product homovanillic acid in healthy adults with periodic leg movements in sleep.[237] Although renal dopamine synthesis rates may be reflected as changes in urinary dopamine,[238, 239] studies specifically seeking evidence of dysregulation of dopaminergic activity in the brain have failed to find any relationship with urinary dopamine levels.[240, 241] Therefore, whatever clinical utility may be claimed for measurement of urinary neurotransmitter concentrations should not be associated with any effects related to actual levels in brain or spinal cord neurons.

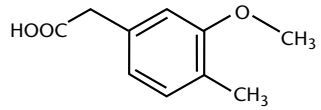
Notes:

CATECHOLAMINES

Vanilmandelate (VMA)



Homovanillate (HVA)



Vanilmandelate, also known as vanilmandelic or vanillyl-mandelic acid (VMA), is the main urinary metabolite of the catecholamines, epinephrine, and norepinephrine. Homovanillate (HVA) is the main metabolite of dopamine that appears in urine (Figure 6.12). In controlled laboratory animal experiments, low urinary levels of VMA and HVA have been associated with low CNS levels of these neurotransmitters.[242] Low levels of these neurotransmitters are associated with symptoms that include depression, sleep disturbances, anxiety and fatigue. Because these neurotransmitters are products of the amino acid tyrosine, treatments aimed at improving protein digestion and supplementation with tyrosine may normalize CNS levels.[243] The relationship of tyrosine to epinephrine also gives rise to the antihypertensive role of tyrosine.[244]

Elevated levels of VMA and HVA signal an increased rate of synthesis and degradation in normal tissue or abnormal production by tumor tissue. Neuroblastic tumors frequently cause a profound elevation in VMA, which may be expressed as the VMA/HVA ratio.[245] The incidence of elevated values appears to increase as a function of tumor size, and small tumors are not likely to result in positive urinary measurements.[246] Elevation of VMA along with specific elevation of cardiac troponin I are, together, precursors to myocardial injury.[247]

In the absence of such disease processes, increased catecholamine synthesis results from the synergism of pituitary adrenocorticotropic hormone (ACTH) and adrenal cortisol. This constitutes the widely recognized chronic stress response that manifests as heightened sympathetic reactions to stress.

The use of specific dopamine reuptake inhibitors like risperidone can cause shunting of dopamine away from uptake into presynaptic vesicles and into the methylation pathway that leads to (nor)epinephrine. Patients on such drugs frequently have low HVA with elevated

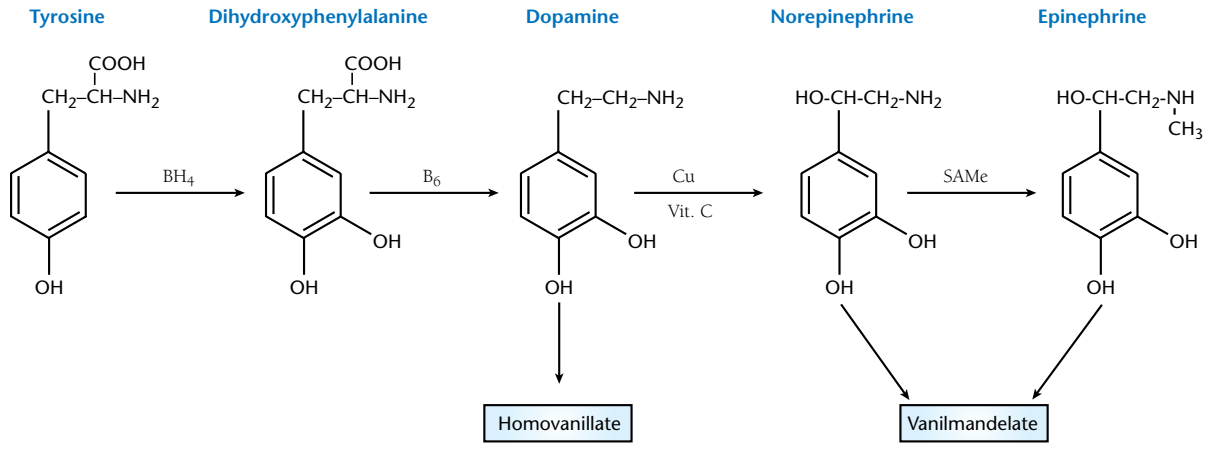


FIGURE 6.12 — Catecholamine Turnover Markers

Adrenergic neurons and adrenal medullary cells utilize tyrosine to produce dopamine, norepinephrine and epinephrine. The urinary products homovanillate and vanilmandelate reflect the overall rates of biosynthesis and breakdown in these pathways.

VMA. A patient who displays increased psychoses when using drugs like risperidone may be displaying a (nor)epinephrine overload.

The dopamine theory of schizophrenia was developed from multiple observations of the effectiveness of neuroleptic drugs that block brain dopamine receptors and are effective in treating psychoses of diverse origins. The brain dynamically regulates dopamine synthesis in response to stimuli, resulting in phasic increases of dopamine and its catabolic product, HVA.[244, 248] Thus, it has been proposed that HVA levels in cerebrospinal fluid (CSF) vary as a function of psychosis rather than being related to the diagnosis of schizophrenia per se.[249] Plasma HVA levels predicted the number of in-patient drug-free days and the duration of illness in schizophrenic patients,[246, 250] as well as pateint response to risperidone treatment.[251]

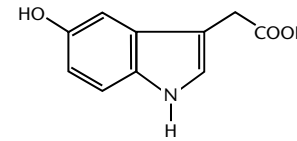
Dietary copper deficiency results in higher dopamine and lower norepinephrine levels in rats due to lower activity of dopamine-β-monooxygenase, even though the copper deficiency leads to greater production levels of the enzyme as well as tyrosine monooxygenase.[252, 253] Similar, though more dramatic changes have been reported in heart and spleen from pigs.[254, 255] These catecholamine changes may be reflected as elevated levels of HVA and suppressed levels of VMA in urine due to copper deficiency. The urinary HVA/VMA ratio test has been found to be a sensitive and specific tool to screen for the copper deficiency disorder of Menkes disease.[256] This ratio may be of similar clinical utility for less severe copper insufficiency states. (See "Copper" in Chapter 3, "Nutrient and Toxic Elements.")

Interpretation of urinary HVA must take into account dietary influences. A large fraction of ingested quercetin is metabolized by intestinal bacteria to homovanillic acid.[257] Significant elevations of serum HVA can result from quercetin ingestion by healthy adults. Serum HVA levels rose 5- to 20-fold during the first 24 h after 1,200 mg doses of quercetin by 4 healthy adults.[258] The estimated average daily dietary consumption of quercetin of about 30 mg is unlikely to produce significant elevations of HVA in urine. However, urinary HVA levels can also be raised by ingestion of bananas, which have high concentrations of dopamine relative to other foods.[236, 259]

Occupational exposure to low levels of aluminum can affect the neurobehavioral function and metabolism of monoamine neurotransmitters. Both urine VMA and HVA levels were higher in the workers exposed to aluminum, although serum aluminum was not significantly different.[260] Compared with controls, the exposed workers in this study had significant differences in neurobehavioral tests.

SEROTONIN

5-Hydroxyindoleacetate (5-HIAA)



Catabolic breakdown of serotonin leads to excretion of 5-hydroxyindoleacetate (5-HIAA) (Figure 6.13). Abnormally high levels of this metabolite result from the use of serotonin-specific reuptake inhibitor (SSRI) drugs, 5-hydroxytryptophan supplementation, or increased release of serotonin from any of three primary sites: central nervous system, intestinal argentaffin cells, or platelets. The effect of an SSRI can cause strong elevations of 5-HIAA. We have found that patients who show no elevation of urinary 5-HIAA in response to SSRIs concurrently often report poor antidepressive responses to the drug. This observation suggests that some people may have an unusual serotonin receptor characteristic that prevents the reuptake inhibition of the drug. Antidepressant drugs show a wide range of relative selectivity for serotonin over noradrenaline and dopamine as shown in Table 6.8. The drugs with greater serotonin selectiveness are more likely to cause urinary 5-HIAA elevation. Conversely, there are individuals who, in the absence of any known drug effect, have greatly increased 5-HIAA excretions. Such individuals provide evidence of a non-pharmacological effect that blocks serotonin reuptake or otherwise leads to serotonin degradation. Xenobiotic compounds are candidates for this action because some, such as acrylamide, are known to interact with serotonin receptors.[261]

A principal reason for difficulty with interpretation of urinary 5-HIAA is the lack of tissue-specific knowledge of its origin. Although serotonergic neurons in the brain have been most intensely studied, they also are abundant in the spinal cord and other tissues, notably the gastrointestinal tract. Spinal cord serotonin may participate in neurotransmission of somatomotor,

autonomic and pain signals.[262] Formation of both serotonin and 5-HTP within the spinal cord have been shown to be stimulated by administration of 5-HTP, and the increase is enhanced in experimentally induced encephalomyelitis (EAE), an experimental model system for human multiple sclerosis.[263] Spinal cord generation of 5-HIAA increases in the initial inflammatory phase of the disease and decreases after destruction of nerve fibers.[264] Decreased serotonin reuptake by damaged neurons and disruption of the blood-brain barrier have been suggested as mechanistic explanations. Such rationale allows understanding of how serotonin may enter the CNS from 5-HTP produced in the periphery.[263] The inflammatory phase in the more caudal regions of the spinal cord also results in marked elevation of quinolinate in rats with induced EAE.[265] Quinolinate is discussed further in the next section. Tryptophan supplementation increases survival in EAE.[266]

Carcinoid tumors composed of chromaffin tissue can release large amounts of serotonin.[268, 269] Urinary 5-HIAA has been recommended for diagnosis of some types of carcinoid tumor cases.[270] Investigations of the pathogenesis of serotonin secretion and high 5-HIAA excretion in carcinoid heart disease showed no association with transforming growth factor beta or fibroblast growth factor.[271] Among patients with carcinoid tumors, only those who show high variability in 5-HIAA excretion in overnight urine experience watery diarrhea.[272]

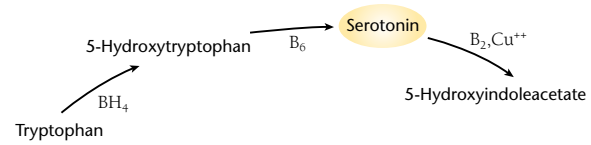


FIGURE 6.13 — **Formation and Clearance of Serotonin**

The availability of tryptophan in serotonergic neurons determines their ability to sustain serotonin levels. Anything that increases serotonin turnover, such as increased neuronal activity or drugs that block synaptic re-uptake cause increased rates of 5-hydroxyindoleacetate formation and higher concentrations of the catabolic product in urine.

Cerebrospinal fluid 5-HIAA and HVA showed highly significant positive correlations with Alzheimer's disease.[273] These monoamine disturbances may be in response to the pathophysiology of this disease. Experimental allergic neuritis also exhibits elevated serotonin precursors, indicating that they may play a role in the clinical course of this disorder.[274] 5-HIAA is also greatly increased in the first attack of chronic relapsing allergic encephalomyelitis, indicating a potential explanation of neurological signs.[264]

TABLE 6.8 — **RELATIVE SELECTIVITY OF NEW ANTIDEPRESSANTS FOR SEROTONIN OVER NORADRENALINE AND DOPAMINE UPTAKE**[230]

Antidepressive Drug	5HT vs. Norepinephrine Selectivity	Likelihood for elevation of 5-HIAA AND VMA
Citalopram	1500	Low
Paroxetine	320	
Sertraline	190	
Fluvoxamine	180	
Fluoxetine	20	
Venlafaxine	3.1	
Nefazodone	1.1	
Clomipramine	13	
Imipramine	0.65	
Amitriptyline	0.91	High

Antidepressive Drug	5HT vs. Dopamine Selectivity	Likelihood for elevation of 5-HIAA AND HVA
Citalopram	3900	Low
Paroxetine	1800	
Fluvoxamine	1600	
Clomipramine	1200	
Fluoxetine	170	
Imipramine	85	
Amitriptyline	54	
Sertraline	32	
Venlafaxine	13	
Nefazodone	-	High

The values show the relative selectivity for serotonin reuptake versus either noradrenaline or dopamine. Citalopram, with a ratio of 1500:1, is highly selective for serotonin receptors, so it produces increased turnover of serotonin with corresponding tendency to deplete tryptophan pools with little effect on tyrosine demand for noradrenaline production. Amitriptyline, on the other hand, has a ratio near 1:1, so it tends to cause increases in both neurotransmitters, thus increasing the urinary concentrations of their catabolic products, 5-HIAA and VMA. For most of the drugs, the effects on dopamine relative to serotonin parallel those for noradrenaline. A notable exception is that a patient using clomipramine is unlikely to show elevated HVA along with the elevating effect on 5-HIAA. The data do not indicate the potencies for serotonin uptake. Thus, while citalopram is more selective than sertraline, it is a less potent serotonin reuptake inhibitor.

Because of the magnitude of total body serotonin synthesis, increased rates of serotonin turnover indicated by elevated urinary 5-HIAA can lead to depletion of the essential amino acid precursor, L-tryptophan. A very high 5-HIAA result calls attention to potential deficiency of tryptophan. The diversion of tryptophan away from the kynurenin pathway also causes an increased risk of niacin deficiency in carcinoid cancer patients.[275] Plasma amino acid analysis provides a useful, direct measure of this amino acid (see Chapter 4, "Amino Acids").

Since several commonly consumed foods contain appreciable concentrations of serotonin, patients should be advised to eliminate those foods for at least 12 hours before gathering specimens for urinary 5-HIAA testing. Table 6.9 shows serotonin content for some foods with especially high levels. A single high serotonin meal made up entirely of the foods shown in bold font in Table 6.9 produced a rise of urinary 5-HIAA to 5.6 µg/mg creatinine from a fasting concentration of 0.85 µg/mg creatinine.[276]

The measurement of urinary 5-HIAA is sufficiently sensitive to allow establishment of a low population normal limit. Individuals with values below this limit have very low total body turnover of serotonin. Serotonin is required for control of gut motility as it activates smooth muscle activity. Inadequate serotonergic activity contributes to constipation.[278] However, increased serotonin output in response to constipation is the dominant effect that has been reported.[279] Ethanol consumption also causes lowered 5-HIAA excretion, due to serotonin metabolism interference.[280] When urinary 5-HIAA levels are low, increased consumption of foods high in tryptophan, including chicken, red meat, dairy products, nuts, seeds, bananas, soybeans and soy products, tuna, shellfish, and turkey can minimize the need for oral tryptophan or 5-hydroxytryptophan.

Acute appendicitis has been shown to produce sharp elevation of 5-HIAA in controlled studies on rats.[281] The potential for serotonin production by enterochromaffin cells is illustrated by the magnitude of urinary 5-HIAA increase in acute appendicitis. Sensitivity and specificity of 98% and 100% were found for discriminating appendicitis in clinically suspected patients, using a cutoff of 20 µmol/L.[282]

Figure 6.13 shows the requirement for tetrahydrobiopterin to catalyze the initial hydroxylation reaction to form 5-hydroxytryptophan. Human tissues may be unable to produce sufficient BH_4 when mutations affect the biosynthetic pathway for conversion of guanosine triphosphate into BH_4 via sepiapterin (see Chapter 2, "Vitamins," Figure 2.9). Mutation of the sepiapterin reductase gene causes a loss of monoamine neurotransmitters due to restriction of the insufficiency of BH_4. These patients have progressive psychomotor retardation, dystonia, and severe dopamine and serotonin deficiencies, indicated by low levels of 5-hydroxyindoleacetic and homovanillic acids.[283]

Table 6.9 — SEROTONIN CONTENT OF SELECTED FOODS

Food	Serotonin mg/100 g
Butternuts	398
Black Walnuts	**304**
English Walnuts	87
Plantain	30
Pecans	29
Pineapple	**17**
Banana	**15**
Kiwi fruit	**5.8**
Plums	4.7
Tomatos	3.2
Haas Avacado	1.6
Dates	1.3
Grapefruit	0.9

Oxidation products of dopamine, epinephrine, norepinephrine, serotonin and melatonin may act as cumulative neruotoxins.[23] The toxic effects of such products may interfere with one-carbon metabolism.[24] Such metabolic toxicant effects add potential significance to findings of elevated VMA, HVA, and 5-HIAA, which are the oxidized products of the neurotransmitters.

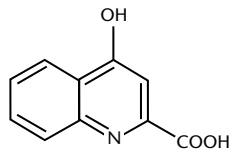
Severe neonatal epileptic encephalopathy has been associated with decreased hepatic activity of the enzyme aromatic-L-amino acid decarboxylase (AADC). An infant with AADC deficiency showed improved clonic contractions with vitamin B_6 therapy, apparently restoring activity to the vitamin B_6-requiring enzyme that catalyzes the conversion of 5-hydroxytryptophan and L-dihydroxyphenylalanine to serotonin and dopamine, respectively. Urinary and CSF concentrations of VMA, HVA, and HIA were decreased in the infants who responded to supplementation of vitamin B_6 with improvement of clonic contractions and lip-smacking automatisms, but died in the third week of life.[284]

Since stress is an important part of depression, studies have measured both urinary HVA and 5-HIAA and found them to have strong positive correlation with depression.[285] Because of the prevalence of enterochromaffin cells in the transitional gut, there is considerable capacity of serotonin output in response to stress.[286, 287]

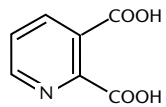
Various lines of evidence from animal studies suggest gastrointestinal microbial origins for elevated 5-HIAA. In animal studies, microbes or microbial toxins increase serotonin and 5-HIAA. When animal feed is contaminated with yeast (*Fusarium*) mycotoxins, serotonin and 5-HIAA are elevateld in the brain, along with lowering of brain concentrations of tryptophan.[288] Intraperitoneal injection of bacterial cell wall lipopolysaccaride increases dopamine catabolites, 5-HIAA, and tryptophan in all brain regions.[289] Cattle grazed on fungal-infected fescue have high pituitary levels of 5-HIAA.[290] Guinea pigs and kittens exposed to *Escherichia coli* endotoxins have elevated levels of 5-HIAA.[291] This evidence might explain why elevated neurotransmitter turnover is frequently found with elevated dysbiosis markers.

NMDA Modulators

Kynurenate



Quinolinate



The hepatic kynurenin pathway (Figure 6.14) has been discussed under "Xanthurenate" with regard to evaluating vitamin B_6 status. There are two other organic acid products of that pathway for which elevated levels in urine can have quite a different meaning. The kynurenin pathway in interferon-gamma-stimulated macrophages, astrocytes, and microglial cells operates as a mechanism of defense against intracellular pathogens and as a mediator of stress response signals to the brain (Figure 6.15).[292] Depending on the timing and juxtaposition of signals, this pathway serves to either activate or inhibit neuronal responses to acute and chronic stress.[293, 294]

Kynurenic acid (KYNA) is an endogenous ligand that antagonizes all types of ionotropic glutamate receptors. It has preferential affinity for the glycine-binding site of the N-methyl-D-aspartate (NMDA) receptor that governs synaptic plasticity, learning and memory. Intrathecal kynurenic acid has antinociceptive effects. It interacts with endomorphin-1 in rats to decrease pain perception.[255] Other studies suggest that elevated KYNA may represent a pathophysiological condition analogous to that seen in schizophrenic patients.[295] Because of the activity of pyridoxal-5-phosphate in this pathway (discussed above), these effects may mediate the clinical uses of vitamin B_6 in treatment of neurological disorders. Even transient deficits of B_6 may cause significant shifts in brain physiology.[296–297]

Quinolinic acid (QUIN) provides a critical link between the immune system and the brain. Stimulation of the inflammatory response causes release of interferon-gamma (IFN-γ) by macrophages. QUIN interacts with NMDA receptors of glutamatergic neurons that respond to pain and other peripheral signals. This biochemical event is the origin of the typical pain symptoms of viral infections. If they are overstimulated, the neurons can degenerate with permanent loss of brain function, known as glutamate excitotoxicity.[298] Their loss is associated with the effects of stroke and with the end stages of HIV infection. Studies have shown that QUIN has a role in the etiology of HIV-related neurologic dysfunction.[299,300] QUIN is selectively elevated in spinal cords of rats with experimental allergic encephalomyelitis, where its neurotoxic effects are suspected to contribute in the etiology of the disorder.[265] Quinolinate toxicity is also suspected as an etiologic factor in Alzheimer's disease. Immunohistochemical studies of brain sections from patients with dementia revealed high quinolinic acid due to up-regulated kynurenin pathway in the hippocampus.[301] Figure 6.16 illustrates the neuronal release, binding and recycling of glutamate.

Toxicants can enhance sensitization of NMDA receptors, decreasing the threshold for QUIN-induced neuronal loss. Rats exposed to methyl mercury at gestational day 8 show significant increases of brain QUIN at day 21, suggesting that kynurenin pathway alterations may mediate mercury effects on brain development.[302] The widespread exposure to phthalates in plastic products has also

been shown to have potential for enhancing quinolinate production by inhibition of an alternative tryptophan pathway in rats.[303]

A useful outcome of the conversion of tryptophan to QUIN is the ultimate production of nicotinic acid to supply its cofactor form, NAD. This step requires the enzyme quinolinic acid phosphoribosyl transferase for the linkage of the adenosyl moiety of NAD. The transferase has low activity in the brain, thus giving rise to increased QUIN as an unprocessed intermediate. In the liver QUIN may be largely processed to NAD.

Vaccination with measles virus induces INF-γ stimulation of the gate keeper enzyme, indoleamine-2,3-dioxygenase (IDO), for viral clearance.[304-306] IDO catalyzes the initial oxidation of tryptophan to start the conversion to QUIN (Figure 6.14). IDO is stimulated by glucocorticoid hormones, whereas estrogens oppose this effect, reducing the rate of kynurenin pathway activity

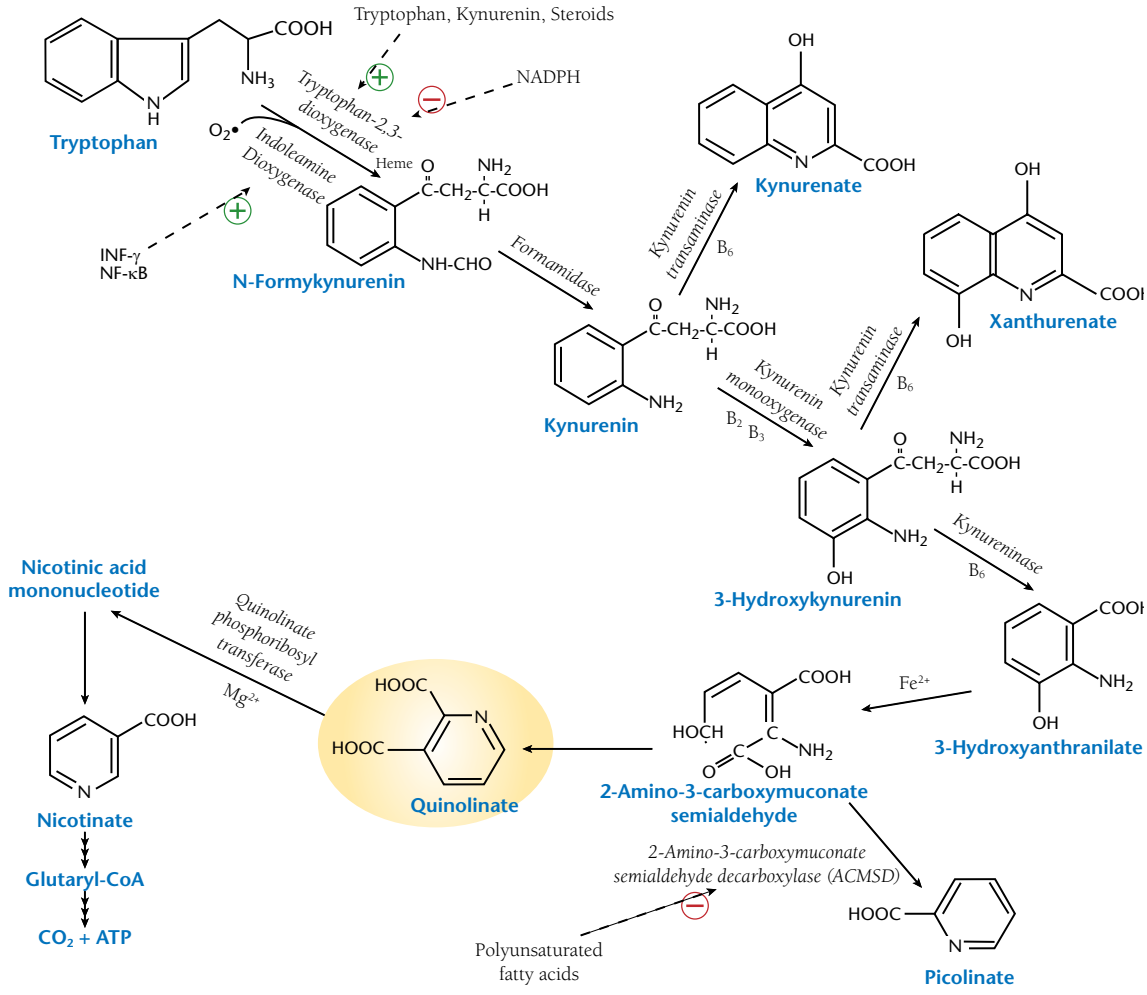


FIGURE 6.14 — The Kynurenin Pathway

Entry of tryptophan into the kynurenin pathway is governed by tryptophan-2,3-dioxygenase that is subject to regulation by tryptophan, NADPH, kynurenin and steroid hormones. The pathway leads to either quinolinate or nicotinate, depending on tissue location as shown in Figure 6.15. The critical point for vitamin B_6 functional assessment is the pyridoxal-5-phosphate requiring kynureninase step. Cofactor insufficiency to sustain this enzyme activity causes production of xanthurenate and kynurenate. Omega-3 polyunsaturated fatty acids suppress transcription of ACMSD, decreasing picolinate production. Primary human neurons do not convert quinolinate to nicotinate because they do not express the enzyme *Quinolinate phosphoribosyl transferase*.

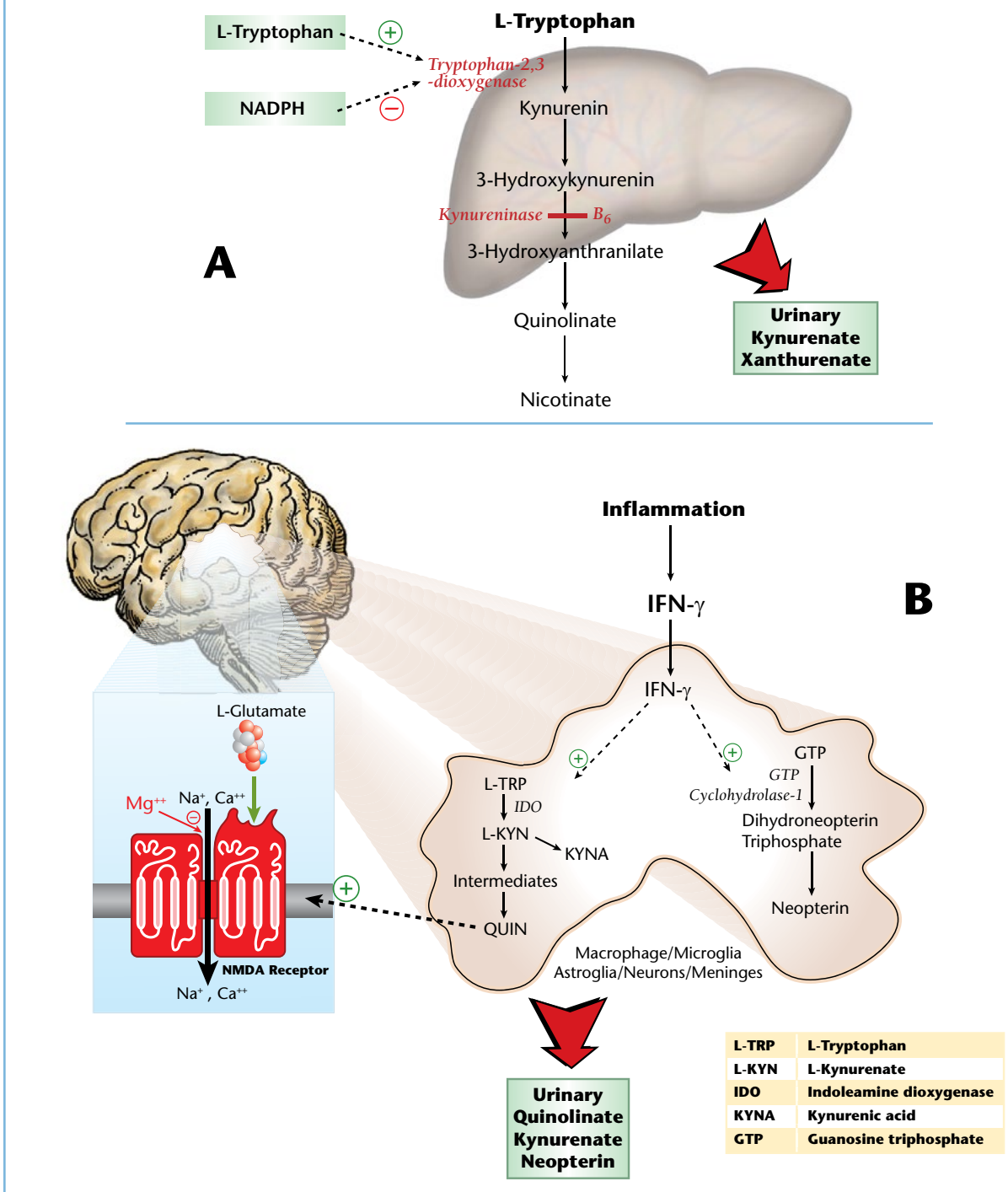


FIGURE 6.15 — Hepatic and CNS Kynurenin Pathway Products

(**A**) In the liver the kynurenine pathway processes excess tryptophan to form nicotinic acid, sparing dietary intake requirements for vitamin B_3. (**B**) Immune and neuronal cells use the kynurenin pathway to produce quinolinic acid as way of modulating brain activity. Their output of quinolinic acid (and, secondarily, kynurenic acid) interacts with NMDA receptors of glutamatergic neurons in the hippocampus and other regions of the brain to increase activity by agonizing response to glutamic acid and glycine.

and sparing tryptophan. One net result of estrogen's action is increased plasma tryptophan concentration compared with the estrogen unstimulated state.[178] The glucocorticoid effect is apparently reversed with chronic exposure, since repeated administration of hydrocortisone causes decreased QUIN in rat brain.[307] Further differentiation of kynurenin pathway activity in macrophages and microglial cells is at least partly achieved through variation of responses to nitric oxide. In IFN-γ-primed macrophages, nitric oxide negatively modulates the expression of IDO activity, whereas no such response is found in microglial cells.[308] Cytokines other than INF-γ, especially tumor necrosis factor-α and interferon-α, may influence the magnitude and duration of response of the macrophage kynurenin pathway.[309]

Since the gut is frequently a source of chronic inflammatory signal induction via INF-γ, there is reason to suspect that QUIN elevation may indicate both inflammatory bowel conditions and neuronal degeneration. Within the brain, the hippocampus is an area rich in NMDA receptors, and it is very sensitive to the neurotoxic effects of QUIN. Because modulation of glutamatergic activity affects firing patterns of dopaminergic neurons, there is potential for QUIN excursions to disrupt the primary learning system.[295] These responses suggest that QUIN elevation following episodes such as

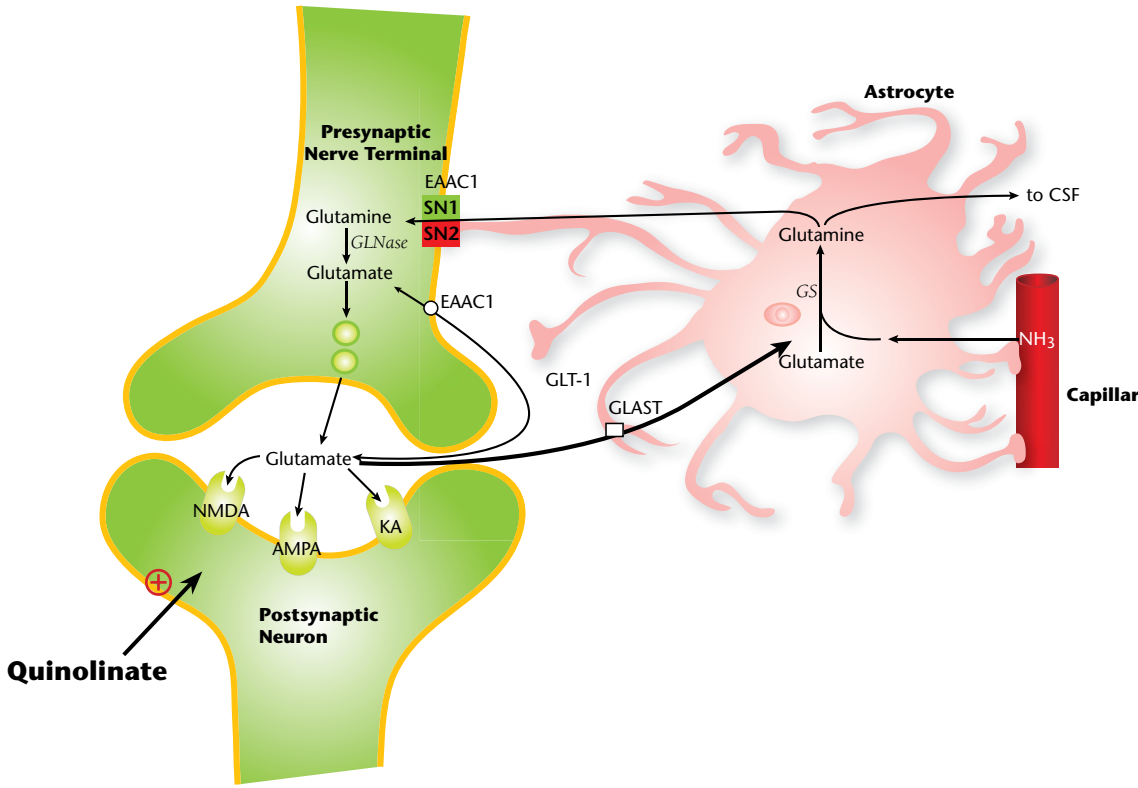


FIGURE 6.16 — **Quinolinate Impact on Glutamatergic Neurons**

This figure was first presented as part of the discussion of glutamate and glutamine in Chapter 4, "Amino Acids." The stimulation by quinolinate is added here to draw attention to the conjunction of factors that contribute to the overall clinical outcome in a patient with both inflammatory and amino acid regulatory issues. The inflammation will agonize NMDA glutamatergic receptors via quinolinate while fluctuations in neuronal glutamate will produce varying symptoms of hyperexcitability. The familiar scenario of a viral infection that produces generalized pain and hyper-sensitization to light is one reference point regarding the type of symptoms experienced by patients when these metabolic disorders are present. NMDA, AMPA and KA are synaptic glutamate receptors. EAAC1 and GLT-1 and GLAST are high affinity glutamate transporters. SN1/SN2 is a glutamine transporter system and GS is astrocyte glutamine synthetase.

viral infections is a metabolic event with potential for precipitating brain developmental disruption of the type seen in regressive autism.

Because of the tight, positive association between QUIN and IFN-γ, immune stimulation and increased QUIN production may also be a key feature in the progression of events that lead to chronic fatigue syndrome. An immunological dysfunction in patients with chronic fatigue syndrome has been demonstrated by decreased monocyte response to IFN-γ.[310] In one set of patients with chronic fatigue syndrome, CD4 T lymphocytes have decreased IFN-γ production.[311] However, during acute and convalescent parvovirus B19 infection, circulating IFN-γ is associated with prolonged and chronic fatigue.[312] Elevated QUIN may be an indication of the immune-mediated form of chronic fatigue. A patient with particularly low INF-γ-stimulated QUIN production is shown in Case Illustration 6.6.

KYNA, a biochemical precursor to QUIN, is frequently found to be simultaneously elevated by inflammatory responses. KYNA production via the kynurenin pathway is characteristically altered in relapsing-onset multiple sclerosis.[313] Patients in remission or not progressing for at least 2 months have lower rates of CSF KYNA formation.[313] Early work showed that the gateway enzyme for the hepatic kynurenin pathway, tryptophan-2,3-dioxygenase, is highly inducible by corticosteroids, and urinary kynurenin excretion increases in direct proportion to activity of this enzyme in the liver.[314] Patients with diseases like rheumatoid arthritis that are characterized by chronic corticosteroid stimulation or those under hydrocortisone therapy may show patterns of elevated kynurenate with normal or low quinolinate.

Refer to Case Illustration 6.6

Because QUIN is a powerful agonist of the NMDA receptors, and KYNA antagonized this effect, the relative amounts of the two metabolites should relate to the potential for neuronal degeneration. In inflammatory diseases, the ratio of QUIN/KYNA is frequently found elevated (> 2.0), so that neurotoxicity must be suspected.[315] Efforts have been intensified to develop kynurenic acid analogues as neuroprotectants for the treatment of stroke and neurodegenerative disease. Any agent that can change the balance of synthesis of kynurenic and quinolinic acids away from the excitotoxin and toward the neuroprotectant, has anticonvulsant and neuroprotective properties.[316] The laughing gas nitrous oxide is such a powerful NMDA antagonist that it has potential neurotoxic effects that are managed by coadministration of GABAergic-inhibiting drugs.[317] Magnesium effectively competes for NMDA receptors to prevent calcium entry into the neuron. Thus, magnesium sulfate infusion can be an effective and inexpensive way to reduce the morbidity and mortality associated with acute glutamate toxicity in conditions like aneurysmal subarachnoid hemorrhage.[318] A similar protective effect of magnesium is found in protecting against death due to birth asphyxia.[319] Otherwise, the alternatives for offsetting QUIN toxic effects are opioid drugs.[320]

Picolinate

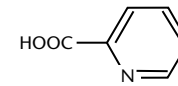


Figure 6.15 shows an alternative route just prior to QUIN formation in the kynurenin pathway that leads to the formation of picolinate instead of nicotinate in hepatic tissue. Dietary protein and fatty acids modulate the enzyme α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase (ACMSD) that controls this branch point.

High-protein diets induce large increases in ACMSD activity through a mechanism unrelated to increased tryptophan in the diets.[321] Thus high-protein intake shifts the processing of tryptophan away from nicotinate production to picolinate. Polyunsaturated fatty acids, on the other hand, divert flow through the kynurenin pathway from picolinate to quinolinate by inhibiting ACMSD.[322] Diets high in omega-3 PUFAs have the largest effects, suppressing transcription of the ACMSD gene and decreasing picolinate production.[323] The PUFA-mediated suppression of ACMSD resulted in increased flow of tryptophan into QUIN and the raising of serum QUIN concentrations by approximately two-fold.

Notes:

Picolinate is a potent activator of inflammatory chemokines.[324] Picolinate and QUIN are thought to act in a concerted way to regulate leukocyte recruitment and distribution into damaged tissues during inflammatory responses.[325] Anti-*Candida* activity of neutrophils is augmented by adding 2 mM picolinate to the culture medium, especially in the presence of INF-γ.[326] Mouse macrophage ability to restrict the growth of *Mycobacterium avium* was augmented by coadministration of picolinate and INF-γ. The bacterial cells showed acquisition of metabolic changes typical of apoptosis. Similar results were found for antifungal activity when macrophages were pretreated with picolinate.[327] One possible mechanistic link between picolinate and macrophage activity is its ability to up-regulate inducible nitric oxide synthase in activated cells.[308]

We may conclude that low-protein diets and high intake of omega-3 fatty acids tend to divert tryptophan degradation from picolinate to nicotinate production in the liver. Independent mechanisms regulate macrophage picolinate formation in order to modulate responses to infectious agents.

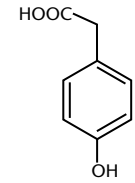
Since quinolinate is a normal physiological regulator of immune and neuronal responses, one might ask whether quinolinate could be too low. This question has not been addressed in current literature. From what we know about the origin of quinolinate, a patient with an undetectable level might be suspected of low immune response to normal inflammatory signals that would produce interferon-γ. A case where this situation may be present is discussed in Case Illustration 6.6. Such scenarios emphasize the tissue differentiation of kynurenin pathway responses. The pathway intermediates that reflect vitamin B_6 status originate from hepatic kynurenin pathway flux, whereas accumulation of quinolinate is a phenomenon of cytokine-stimulated neurons and immune cells. The case shows how information from fatty acid testing can provide insight about potential origins and treatments in such cases.

Notes:

Oxidative Damage and Antioxidant Markers

Cell Proliferation

p-Hydroxyphenyllactate (HPLA)



p-Hydroxyphenyllactate (HPLA) is a metabolite of tyrosine. Elevated HPLA is associated with tumor growth and leukemia.[328] HPLA is also a marker of hepatic encephalopathy in patients with hepatic cirrhosis,[329] and urinary levels may be elevated in some inborn errors of metabolism.[330, 331] The parasite *Trypanosoma cruzi* has been shown to secrete HPLA when grown in culture, but no evidence has been presented to demonstrate elevated levels in human urine due to *T. cruzi* infection.[332]

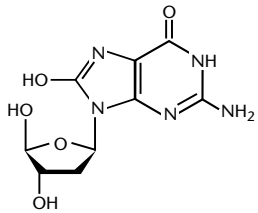
As early as 1975, studies in mice established the blastomogenic activity of HPLA. Twice weekly 1.5 mg doses induced leukemias, adenomas, hepatomas, tumors of the vascular tissue, and benign and malignant tumors and precancerous conditions of the urinary bladder.[333] The methyl ester methyl-p-hydroxyphenyllactate (MeHPLA) is an important cell growth-inhibiting agent. Tumor cells contain esterase activities that hydrolyze the compound to the free acid HPLA. Thus, the effects of elevated HPLA may be due to depletion of MeHPLA. MeHPLA binds strongly to nuclear sites, blocks estradiol stimulation of uterine growth, and inhibits growth of human breast cancer cells.[334] HPLA, on the other hand, binds more weakly to the same nuclear site and does not block uterotropic responses to estradiol or inhibit growth of breast cancer cells. Thus, HPLA is an important regulator of normal and malignant cell growth, and it may mediate the cancer-promoting effects of estrogen. MeHPLA is deficient in a variety of rat and mouse mammary tumors and human breast cancer preparations, and this deficiency correlates with the loss of regulatory control.[334] Other estradiol-potentiating effects are mediated by toxins such as pertussis[335] and cholera toxin.[336] These effects are worsened by high HPLA because of the loss of regulatory control when MeHPLA is depleted. The

cell proliferative stimulation of hepatic encephalopathy is manifested as elevated urinary p-hydroxyphenyllactate.[329] This effect may be mediated by HPLA inhibition of brain Na+, K+-dependent ATPase.[337]

Elevated levels of HPLA result in a dramatic decrease of ascorbic acid concentration in the liver, adrenal glands, and blood.[338] High doses of ascorbic acid (100 mg/kg body weight daily) were shown to arrest or significantly inhibit the excretion of HPLA in patients with hemoblastoses and nephroblastoma.[339] Thus, high urinary HPLA signals an increased tissue growth response that may be associated with neoplastic disease or increased growth of normal tissue. The dramatic responses to ascorbic acid suggest its involvement in mediating this level of cell growth. Tissue saturating levels of vitamin C should be considered in patients with elevated HPLA.

DNA Oxidative Damage

8-Hydroxy-2'-deoxyguanosine (8-OHdG)



When inflammatory markers like quinolinate or metabolic enhancers like p-hydroxyphenyllactate are elevated, there is a need to know the overall impact of increased production of reactive oxygen species (ROS).[138] Sustained inflammatory responses and increased cell proliferation rates cause increased production of reactive oxygen species.[340] When local antioxidant protection fails to keep reactive oxygen species in check, there is increased threat of clinical consequences from damage to cell membranes, enzymes or structural proteins, and DNA or RNA. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is a product of oxidative damage to DNA.[341] 8-OHdG is formed in a pro-mutagenic DNA lesion due to reaction of oxygen radicals with guanosine groups in DNA. It is recognized as a useful marker in estimating DNA damage induced by oxidative stress. Oxidative DNA damage is common in various forms of chronic liver disease, suggesting a link between chronic inflammation and hepatocarcinogenesis.[341] The uniform increases of 8-OHdG with the stages of Parkinson's disease led to suggesting its use for evaluating the progression of this disease.[342] Elevations of 8-OHdG have been reported in diabetic nephropathy[343] and chronic obstructive pulmonary disease.[344] Case Illustration 6.7 shows actual laboratory results in a patient with COPD. In studies published in 2006, human conditions where oxygen radical damage has been assessed by measuring urinary 8-OHdG include ultraviolet protection of skin,[345, 346] bladder cancers,[347] hemodialysis,[348] cardiomyopathy,[349] insomnia,[350] environmental toxin exposures,[351] diabetes,[352] effects of cigarette smoke,[353] arsenic poisoning,[354] and depression.[355] The potential for genotoxicity from sidestream cigarette smoke was also demonstrated by finding increased 8OHdG production.[356] Most studies measure 8-OHdG in urine. Leukocyte 8-OHdG was found to be lower in lifelong smokers, indicating the inappropriateness of this specimen for assessing oxidative stress.[357]

The antioxidant protection in young children was evaluated by urinary 8-OHdG.[358] Formation of 8-OHdG is sufficiently sensitive to reveal even mild chronic effects of ROS. The association of cancer with chronic psychological stress and perceived overwork may be via the formation of 8-OHdG.[359] Increases in 8-OHdG with cigarette smoking is associated with aging and enhancement of oxidative damage in human lung tissues.[360] 8-OHdG levels rise with age in adults with mild hypercholesterolemia and/or mild hypertension.[361]

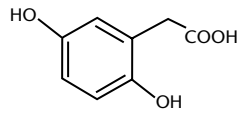
Lower levels of antioxidants may predispose to oxidative stress, which is manifested by higher levels of 8-OHdG. Levels of 8-OHdG were significantly higher in atherosclerotic patients, and vitamin C levels were significantly lower. This oxidative stress may promote and worsen atherosclerosis.[362] Exposure to organochlorines does not result in elevated 8-OHdG, indicating that the genotropic effects of this compound are exerted through mechanisms other than ROS formation.[363] Moderate alcohol consumption seems to have the overall effect of reducing DNA damage in some individuals as shown by the decrease in 8-OHdG levels.[364]

Refer to Case Illustration 6.7

Diabetics tend to have higher urinary 8-OHdG excretion than healthy controls and the levels are related to the severity of tubulointerstitial lesions. Oxidative stress may contribute to the progression

of tubulointerstitial injury in patients with diabetic nephropathy.[365] In a study of the analytical performance of 8-OHdG measurements, no significant difference between the mean group levels of 8-OHdG/creatinine in spot urine and in 24-hour urine was observed.[366] Thus, a first morning urine specimen is adequate for 8-OHdG measurements.

Homogentisate (HGA)



The liver must provide a mechanism for clearing postprandial tyrosine that exceeds utilization demands. This pathway starts with tyrosine participating in the vitamin B_6-dependent hepatic transamination system that converts amino acids to their keto analogues. Tyrosine, thus, is converted to p-hydroxyphenylpyruvate, and a subsequent vitamin C-dependent oxidation produces homogentisic acid (HGA) (Figure 6.17). The third reaction is an oxidation step catalyzed by homogentisate-1,2-dioxygenase, assisted by iron. This enzyme is deficient in people with the condition called alkaptonuria (AKU), and results in an elevated HGA. AKU was first recognized from the characteristic symptom of urine turning from straw colored to black, along with dark blue color development in ears and eyes.[18-20] The pigmentation, called ochronosis, arises from homogentisate polymerization.[21] Arthritis and nephrolithiasis are common clinical features in alkaptonuric patients.

Because the only observable signs of HGA deposition are pigmentation in connective tissues and arthropathy that worsens with age, AKU was initially considered to be an inborn metabolic disease with few clinical consequences.[22] However, more recent discoveries of the insidious metabolic effects of HGA have changed the outlook for AKU patients. Excess HGA is now known to produce oxidative effects on human hemoglobin,[23] to add significant risk of coronary heart disease,[24] and to produce oxidative damage to DNA[24] with potentially mutagenic effects. Thus, elevated HGA is not a harmless metabolite, but one that should be managed at the earliest possible date of detection. AKU is managed by restriction of tyrosine loading using a low-protein diet, the success of which is monitored by a lowering of urinary HGA.[25] In addition, high doses of ascorbic acid lower the binding of HGA to connective tissue, lessening one of the clinical consequences of the disorder.[26] High levels of HGA also result in oxidation to the alternative metabolite, benzoquinone acetic acid that is a likely cause of the toxic consequences of the disorder. DNA oxidative damage with increased excretion of 8-hydroxy-2'-deoxyguanosine has been observed with HGA elevation.[27] Treatment of AKU neonates with high doses of ascorbic acid has been shown to reduce the production of benzoquinone acetic acid.[28] Combination protein-tyrosine restriction and high-dose ascorbic acid can effectively lower urinary homogentisate and relieve the joint pain reported by adults with the disorder.[29, 30] Drugs that inhibit the enzymes of the pathway can successfully lower HGA excretion, but may produce elevations of plasma tyrosine with unknown long-term side effects.[19, 31]

Quantitative clinical laboratory testing for urinary homogentisate is challenging because the compound can

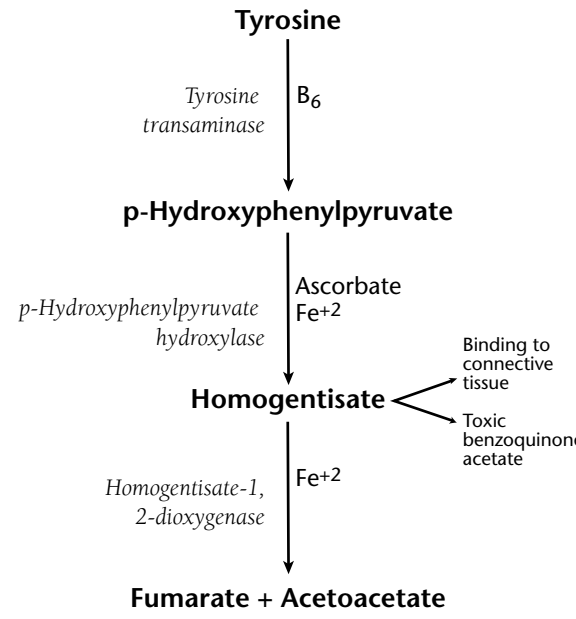


FIGURE 6.17 — Homogentisate Formation in Catabolism of Tyrosine

The catabolism of tyrosine requires the genetically susceptible enzyme homogentisate-1,2-dioxygenase. Deficiency of this enzyme produces alkaptonuria with characteristically high urinary levels of homogentisate, and generation of toxic consequences by direct binding to connective tissue and DNA oxidation due to the alternative product, benzoquinone acetate.

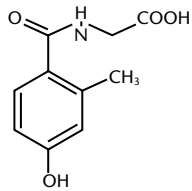
degrade rapidly during transport. For neonatal qualitative detection of alkaptonuria, the specimen should be tested within two hours of collection or frozen and shipped on dry ice and kept frozen until tested. Quantitative testing for lower levels to detect subtle metabolic imbalances is even more susceptible to inaccuracy from degradation. Even under frozen conditions homogentisate degrades so readily that accuracy of measurement may be compromised unless the specimen arrives at the laboratory frozen less than 2 days after collection.

Detoxification Markers

Chapter 8, "Detoxification," is devoted to the subject of evaluating patient detoxification status or biotransformation capacities. The compounds discussed in detail here will be reintroduced there in relationship to tests other than profiling of urinary organic acids. The organic acids of this group serve as biomarkers of distinct parts of the detoxification system, providing insight about both exogenous toxin accumulation and endogenous detoxification responses.

Xylene exposure

2-Methylhippurate



2-Methylhippurate is a by-product of the detoxification of the common solvent xylene, which is first oxidized via hepatic P450 oxidase enzymes to 2-methylbenzoate. This organic acid is then conjugated with glycine to form the peptide product, 2-methylhippurate (Figure 6.18). The conjugation reaction requires formation of an acyl ester with coenzyme A. The potential for enhancing clearance by assuring adequacy of the substrate (glycine) and the essential precursor of coenzyme A (pantothenic acid) is discussed in more detail below under "Benzoate and Hippurate." Although the 2-methyl isomer is the most abundant in most xylene preparations, the common solvent grade of xylene contains

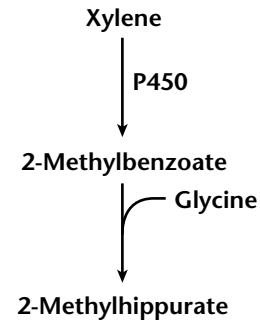


Figure 6.18 — **Metabolism of Xylene**

The highly water-insoluble xylene molecule is oxidized to 2-methylbenzoate by hepatic P450 cytochrome systems (Phase I of detoxification) and then conjugated with glycine by glycine conjugase (Phase II of detoxification) to form 2-methylhippurate that appears in urine.

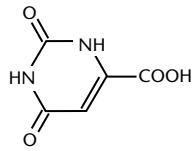
3- and 4-methyl isomers as well. With properly chosen techniques, all of the isomers may be measured, allowing detection of exposures to multiple xylene mixtures.[367, 368]

Xylene is a very common solvent found in paint thinners and building products, fuel and exhaust fumes, and industrial degreasers and solvents. Spray-painting workers show elevated methylhippurate, indicating recent exposure to xylene.[369] Both spot urine specimens collected at the end of the work day and 24-hour urine specimens showed high correlation with time-averaged paint-worker exposures.[370] Patient counseling in avoidance is indicated. Worker habits of smoking and drinking in combination were found to suppress the conversion of xylene to methylhippurates.[371] The importance of screening of methylhippurates in human urine for evaluating occupational exposure is indicated by the appearance of special analytical methods for their measurement, such as micellar electrokinetic capillary chromatography and LC/MS-MS.[17, 372]

Notes:

AMMONEMIA

Orotate



Details of how ammonia is cleared by the action of the urea cycle were presented in detail in Chapter 4, "Amino Acids," where the urea cycle was diagrammed in Figure 4.9. When there is insufficient capacity for detoxifying ammonia via the urea cycle, carbamoyl phosphate leaves the mitochondria and stimulates the synthesis of orotic acid (orotate)[373] (Figure 6.19). Increased orotate production is a sensitive indicator of arginine deficiency.[374] Symptoms that develop following arginine deprivation can largely be accounted for by a decreased efficiency of ammonia detoxification and reduced formation of nitric oxide. The reader may refer to the section "The Urea Cycle and Nitrogen Management" in Chapter 4 for discussion of the common symptoms of ammonemia. Mild or late onset forms of inborn errors of urea-cycle enzyme defects, such as ornithine transcarbamylase (OTC) deficiency, may also lead to increased orotate and ammonia levels.[375, 376] Increased orotate biosynthesis is observed with increasing ammonia concentrations in rat, mouse and human liver. Orotate production is reduced by in vitro arginine supplementation due to stimulation of urea cycle activity.[121,377] Hyperammonemic attacks and urinary orotate excretion were both decreased significantly following arginine supplementation in patients with late-onset ornithine transcarbamylase deficiency.[374] Magnesium deficiency can also have adverse effects on urea cycle enzymes[378] and can be a cause of reversible renal failure.[379] In addition, orotate requires magnesium for its metabolism. Although a normal level in a urinary organic acid panel may not necessarily indicate magnesium sufficiency, high levels should alert one to a significant possibility of intracellular magnesium insufficiency, especially in patients with kidney failure.[380, 381]

Intestinal bacterial overgrowth should always be suspected in cases of chronic ammonemia. Although cases of overgrowth severe enough to produce cirrhosis or coma are rare,[382-384] patients with mild, transient ammonemia may be detected on routine urinary orotate assessment. Oral glutamine supplementation at levels above 10 g/d in adults can result in elevation of urinary orotate due to increased ammonia generated from hepatic oxidative deamination reactions.[385] Although high-protein diets might be suspected of contributing to ammonemia, studies in rats have shown that, in these animals, high-protein intake induces sufficient glutamate synthesis in perivenous hepatocytes to assist with the increased ammonia production.[386] To the extent that a patient with high urinary orotate is able to mount such responses, they may tolerate high-protein diets. However, genetic polymorphic effects predict that such ability may not be found in all patients.

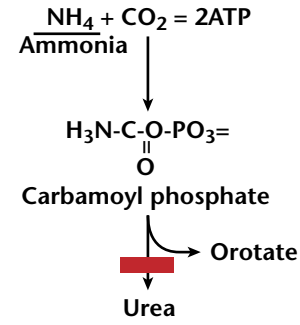
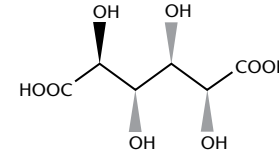


FIGURE 6.19 — **Orotate and Ammonia**

When the capacity of the major ammonia clearance system, the hepatic and renal urea cycle, is exceeded, the optional conversion of carbamoyl phosphate to orotate increases to clear the toxic ammonia.

HEPATIC PHASE I AND II ACTIVITY

Glucarate



Various aspects of major detoxification pathways are discussed in more detail in Chapter 8, "Toxicants and Detoxification." Glucaric acid (glucarate) is a by-product of the predominant liver hepatic phase I detoxification reactions involving cytochrome P450 oxidation of glucose to glucuronic acid, the substrate for phase II conjugation reactions. Hepatic output of glucarate is accurately reflected by urinary levels, and glucarate

Table 6.10 — Classes of Compounds Cleared by Glucuronidation[401]

Class	Examples
Polycyclic aromatic hydrocarbons	Benzo(a)pyrene, benzanthracene, naphthalene
Various nitrosamines	Cured meats, tobacco products, rubber products, pesticides
Fungal toxins	Aflatoxin
Steroid hormones	Estrogen, testosterone
Heterocyclic amines	Well-done, fried, or barbequed meats
Pharmaceutical drugs	Aspirin, lorazepam, digoxin, morphine
Vitamins	Vitamins A, D, E and K

excretion is an indicator of overall hepatic detoxification demand.[387]

The clinical significance of endogenous glucarate production should not be confused with the cancer-protective role of oral supplementation with glucarate salts, which decreases the enterohepatic circulation of carcinogens.[388] β-Glucuronidase produced by intestinal bacteria can increase the enterohepatic circulation of carcinogens.[389] Oral D-glucarate is converted into the potent β-glucuronidase inhibitor D-glucaro-1,4-lactone under the influence of stomach acid. Urinary glucarate is influenced by oral glucarate supplementation (usually as calcium D-glucarate), because most absorbed glucarate is cleared in urine.[390]

The liver produces glucuronate for use in phase II conjugation reactions.[391, 392] The best-known example is glucuronate conjugation of the hemoglobin degradation product bilirubin as a final preparation for urinary excretion. A great variety of drugs, food components and products of gut microbial metabolism are prepared for excretion by glucuronidation (Table 6.10). The by-product glucarate can become elevated as an indication of enzyme induction due to such potentially toxic exposures that induce greater rates of glucose oxidation to glucuronic acid.[393-395] Metabolic challenges that result in stimulation of hepatic P450 activity tend to produce increased excretion of glucarate. Urinary D-glucarate, for example, is elevated during rifampicin/streptomycin treatment[396] and in pesticide-exposed groups.[397] Both of these exposures induce mixed function oxidase activity. Glucarate measurements have been advanced as useful biomarkers to xenobiotic exposure, being particularly useful as a screening tool for xenobiotic exposure in reproductive epidemiology.[398]

Long-term exposure to environmental pollutants and continued toxic load to the detoxifying systems may lead to oxidative stress, high levels of P450 activity, and reduced capacity for phase II conjugation reactions. Patients suffering from toxic burdens may experience a wide range of symptoms, among them fatigue, headaches, muscle pain, mood disorders and poor exercise tolerance. Researchers have reported that many chronic fatigue syndrome patients have disordered liver detoxification ability and show signs of increased toxic exposure.[399]

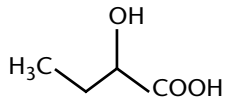
Hepatic phase II conjugation reactions convert fat-soluble substances to water-soluble forms for elimination. The major phase II pathways generate mercaptan (glutathione), methyl, sulfate, glycine, and glucuronide conjugates. Markers for all of these pathways may be found in a profile of organic acids in urine. Assessment of the metabolic status of these major detoxification processes assists in understanding the body's capacity to detoxify foreign substances, and thereby prevent long-term damage from their continued exposure. Measurement of glucarate in urine serves as a specific biomarker for glucuronidation.[400] Elevations in urinary glucarate specifically suggest exposure to pesticides, herbicides, fungicides, petrochemicals, alcohol, and drugs.

The ability of the laboratory to measure glucarate is determined by the type of sample preparation chosen. Glucarate is extremely water soluble and does not move into the organic solvent layer when methods requiring solvent extraction are used. Newer methods utilizing LC-MS/MS technology may report glucarate because no solvent extractions are performed.

Notes:

GLUTATHIONE STATUS

α-Hydroxybutyrate (2-Hydroxybutyrate)



α-Hydroxybutyrate (AHB) was mentioned briefly in Chapter 4, "Amino Acids," in the discussion of threonine catabolism. It was pointed out that the contribution of the threonine pathway constitutes a fairly constant background amount of AHB because threonine intake is small compared with most other amino acids, and the alternative pathway of oxidation of the precursor α-ketobutyrate to propionate is favored over reduction to AHB under most conditions.[402] Although there is little direct evidence to date, the dominant source of urinary AHB seems to be the conversion of cystathionine to cysteine (see Chapter 4, "Amino Acids," Figures 4.13 and 4.20). The activity of this pathway is highly variable, changing in response to demands for protection against oxidative stress. As oxidative stress increases, the flow of homocysteine shifts away from transmethylation to methionine toward transsulfuration to cystathionine in order to increase the flux of cysteine into glutathione synthesis.[403] Thus, AHB production can be directly related to the rate of hepatic glutathione synthesis. This reciprocal regulation of homocysteine flux is illustrated in Figure 4.20.

Myocardial tissue has high activities of the enzyme α-hydroxybutyrate dehydrogenase (AHBD), which catalyzes oxidation of AHB. The activity of AHBD on the second day after a myocardial infarction is a marker for estimates of infarct size and a measure of reperfusion effectiveness.[404] The intense energy demand of cardiac muscle is likely the reason for such high concentrations of AHBD in that tissue, because AHB strongly inhibits mitochondrial energy metabolism as measured by CO_2 production.[405] Studies on rat liver mitochondria show strong responses of enzyme activities that maintain a high NADPH/NADP+ ratio needed to recycle glutathione to the reduced state.[406] Elevation of this ratio has been proposed as the explanation of high AHB excretion in extreme situations such as heavy alcohol consumption.[407] Any conditions that result in high activity of carbohydrate oxidation can raise the NADH/NAD+ ratio, because the extramitochondrial oxidative pathways tend to load reducing equivalents (-H) onto NAD. Alcohol consumption and insulin-stimulated glucose uptake raise this ratio. See Chapter 9, "Oxidative Stress and Aging," for further discussion of the novel concept of reductive stress.

Smoking, poor diet, and lack of exercise significantly inhibit the activity of AHBD, suggesting that urinary elevation of AHB may be related to these factors.[408] High AHB is also found during phases of increased lymphocyte destruction in infectious diseases such as measles.[409] Elevated AHB is found in birth asphyxia and in the inherited metabolic diseases such as "cerebral" lactic acidosis, glutaric aciduria type II, dihydrolipoyl dehydrogenase (E3) deficiency, and propionic acidemia.[405] All of the conditions that have been associated with increased AHB excretion may be related to increased rates of hepatic glutathione synthesis from methionine (Figure 6.20).

Elevated AHB, thus, shows increased flow through the transsulfuration pathway as required during times of increased demand for glutathione for meeting oxidative stress or for detoxification functions. Other signs, such as urinary pyroglutamate and sulfate, discussed below, must be assessed to determine a patient's ability to

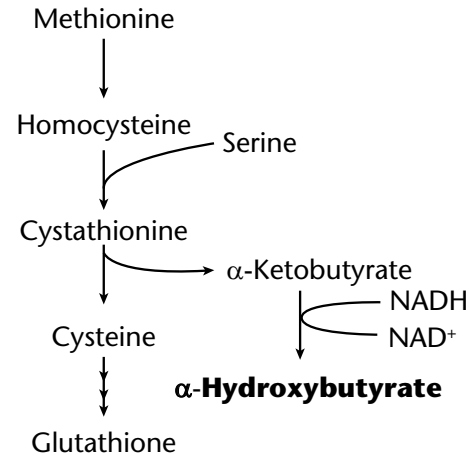
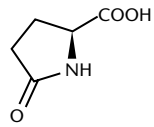


FIGURE 6.20 — **Alpha-hydroxybutyrate Formation From Glutathione Biosynthesis**

As the rate of hepatic and small intestinal cysteine formation for glutathione synthesis increases, the by-product α-hydroxybutyrate can be passed into urine at higher concentrations. When the conversion is limited by availability of methionine (and homocysteine) the lower rate of glutathione production can be reflected by falling α-hydroxybutyrate in urine.

sustain this flow. It is also possible to find some patients in late stages of chronic glutathione and methionine depletion who have such low capacity to generate the transsulfuration flow that their AHB is normal. These patients generally show low plasma methionine and taurine. Three scenarios of glutathione markers are shown in Case Illustration 6.8.

Pyroglutamate (5-Oxoproline)



Pyroglutamate is created in the γ-glutamyl cycle (GGC), a pathway that is highly active in renal tubules and anywhere there is a high demand for glutathione (Figure 6.21). When cytosolic glutamate is abundant, the GGC functions without forming γ-glutamyl amino acid bonds to achieve net glutathione synthesis. The amino acid reabsorbing function of the GGC may be unique to renal tissue. When the γ-glutamyl peptide bonds are broken, instead of releasing glutamate, the reaction produces the cyclic pyroglutamate that must be opened by 5-oxoprolinase to regenerate glutamate. In healthy individuals, a very modest amount of pyroglutamate is spilled in the urine because the rate of pyroglutamate conversion to glutamate for glutathione reformation keeps pace with the rate of γ-glutamyl amino acid formation.[410] The enzyme that initiates the cycle, γ-glutamyl transpeptidase (GGT) is very abundant in hepatocytes, and this fact has led to the use of serum GGT activity as a primary differential diagnostic tool for liver disease because damage to hepatocytes causes release of the enzyme into blood.[411, 412] Although liver pathology is indicated when serum GGT is elevated, other signs appear in cases of GGT deficiency.

Gene defects are known to cause deficiency of GGT. Cell regulation by leukotriene production from polyunsaturated fatty acids is affected in individuals with GGT deficiency because cysteinyl-leukotriene C4 (LTC[4]) fails to be cleaved to LTD(4), producing a total lack of LTD(4).[413] Growth failure, shortened life spans, and infertility are found in mice with genetic GGT deficiency.[414] The enzyme 5-oxoprolinase that converts the ring form to the open-chain glutamate form may also be defective. Hereditary 5-oxoprolinase deficiency can produce profound pyroglutamic aciduria. Developmental delay has been reported in cases of human of 5-oxoprolinase deficiency.[415, 416] Glutathione synthase deficiency is the most common genetic defect seen in the GGC pathway.[417] The autosomal recessive form of this defect in the γ-glutamyl cycle causes recurrent kidney stones, whereas the homozygous expression results in vomiting, diarrhea, and abdominal pain.[418, 419] Defects in the enzyme that accomplishes the final reaction for reformation of glutathione produce quite different clinical outcomes. Extreme pyroglutamic aciduria (also known as 5-oxoprolinuria) is found in patients with a polymorphism in glutathione synthase characterized by metabolic acidemia, hemolytic anemia, and central nervous system damage.[420] The enzymatic defect causes decreased levels of cellular glutathione that greatly stimulates the synthesis of γ-glutamylcysteine that cannot be utilized because the defective enzyme drives the final glutathione reforming step. Cysteine is released for further protein and peptide synthesis along with pyroglutamate that simply spills into the urine. Enzyme polymorphisms can often be mild enough to escape detection until late in life, if at all. Complaints of malaise and anorexia by a 48-year-old man were idiopathic until organic acid profiles revealed the cause of recurrent high anion gap as consistent pyroglutamic aciduria, indicating a genetic alteration in the γ-glutamyl cycle.[421] Other cases of mild impairment may become noticed only when drugs like acetaminophen place stress on hepatic glutathione pools and pyroglutamic aciduria causes elevated anion gap.[422]

As glutathione fails to be recovered in the γ-glutamyl pathway, a concurrent reduction in total body sulfate may be found (see below). Studies showing an age-related decline in expression of glutathione synthase in rat liver suggests that this problem may be more prevalent in older patients.[423] Preformed glutathione or

Notes:

supplemental NAC may be used along with antioxidant supplementation to build glutathione levels.[424-426] Additionally, as shown in Figure 6.21, ATP (as Mg^{++}-ATP) is required in three steps. If any compromise in energy production occurs, an increase in pyroglutamate could theoretically be noted. Alternatively, drugs that require glutathione conjugation can cause failure to sustain glutathione adequacy. This effect has been reported in acetaminophen toxicity.[427] Elevated pyroglutamate following such normal drug use indicates a mild polymorphism that is revealed only under increased detoxification stress. In mammary tissue the γ-glutamyl cycle may serve as a signaling pathway where pyroglutamate stimulates amino acid metabolism. As usually is found in studies of antioxidant systems, the over-use of NAC and other glutathione inducers can cause adverse metabolic stress, so monitoring of progress with laboratory evaluation is warranted.[428]

Burn patients excrete higher than normal amounts of pyroglutamate, whereas their blood levels of glutathione are lowered and their rate of glycine synthesis is decreased.[429] The possibility that glutathione reformation by glutathione synthase can be limited by the supply of glycine has been demonstrated in several ways. Dietary glycine restricts glutathione formation on the order of the effect produced by limiting sulfur amino acid availability.[430] Urinary pyroglutamic aciduria has been proposed as a marker for glycine deficiency.[431] During dietary protein restriction, glutathione synthesis is limited. If glycine conjugation demand concurrently is increased in healthy human subjects by methionine loading, then pyroglutamate excretion increases because of lowered availability of glycine. Under these conditions urinary pyroglutamate is positively related to urinary sulfate in a linear manner over the entire sulfate concentration range.[432] These results indicate the diversion of

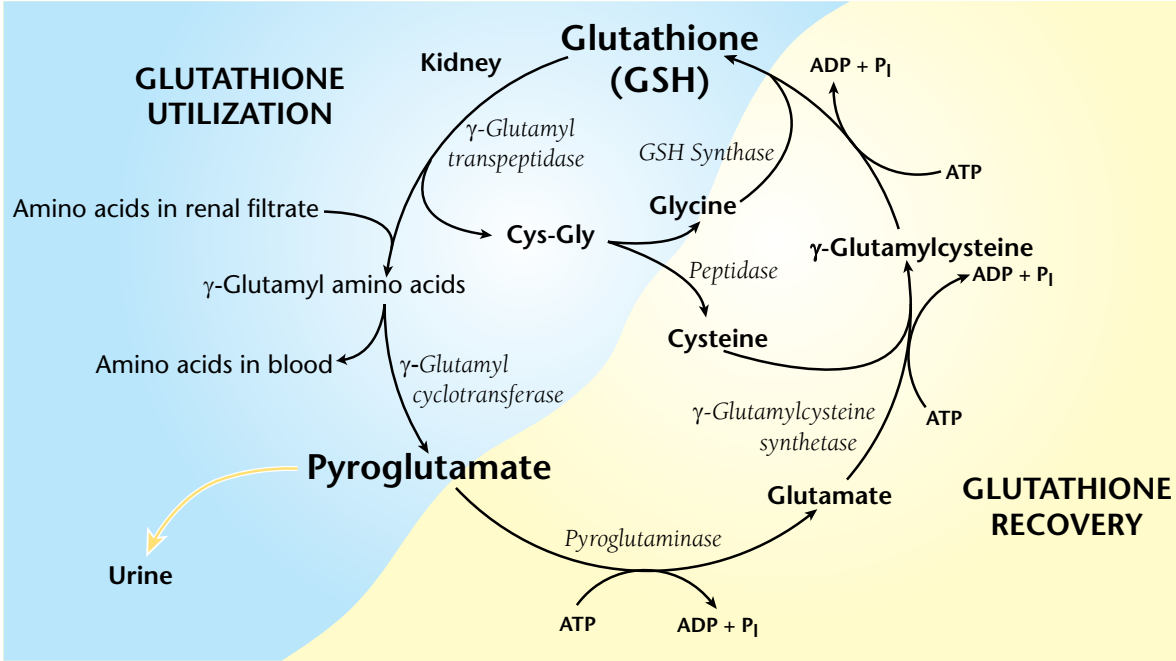


FIGURE 6.21 — **The γ-Glutamyl Cycle**

Rather than the usual alpha carboxyl peptide linkage, the gamma linkage of the glutamyl residue in glutathione is very unique among peptide structures in human tissue. One consequence is to make oral glutathione persist in the presence of pancreatic enzymes that degrade other peptides and proteins. Another is to make γ-linked amino acids unsusceptible to proteolytic attack and therefore to allow their safe transport. The γ-glutamyl cycle steps that link glutamate, cysteine and glycine serve to achieve net synthesis of glutathione, especially in the liver. In the kidney, γ-glutamyl transpeptidase generates γ-glutamyl amino acids for transport from glomerular filtrate back into blood in the tubules of the kidney. The transport process results in degradation of the glutathione peptide that must be reformed to sustain body glutathione levels. Restricted mitochondrial ATP generation or low levels of available cysteine or glycine are factors that prevent the efficient reformation steps, leading to elevated urinary pyroglutamate.

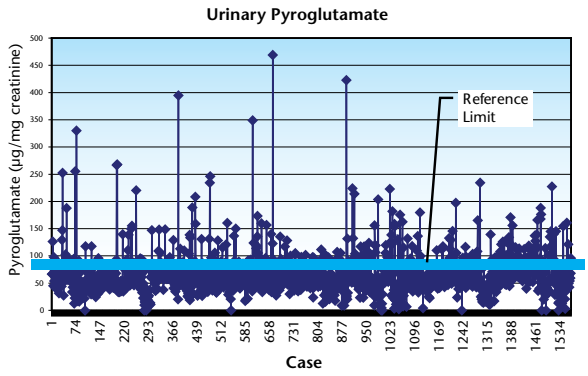


FIGURE 6.22 — Distribution of Pyroglutamate Results

The cutoff of 80 µg/mg creatinine is indicated by the shaded bar. A large number of individuals have some degree of wasting of glutathione and some individuals are in a state of severe depletion, which can effect detoxification and protection against free radical damage.

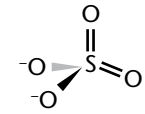
methionine-derived cysteine from glutathione to sulfur synthesis as glycine limitation worsens. See Chapter 4, "Amino Acids," for more in-depth discussion of sulfur amino acid relationships.

Small amounts of pyroglutamate are always present in overnight urine because it is produced as an intermediate in a cycle used in the active transport of amino acids in renal tubules.[433] Since the appearance of a micromole of pyroglutamate in urine is accompanied by the recovery of a micromole of amino acids into the renal blood supply, we might think of the situation as one where glutathione is wasted in order to prevent massive essential amino acid loss. Up to one-third of the glutathione circulating in blood may be used in this amino acid recovery process.

Since pyroglutamate may be formed by heating of foods that contain high amounts of glutamic acid, urinary pyroglutamate may have dietary origins. Foods high in glutamic acid include artificial diets where glutamate is used as a flavor enhancer and high-protein foods, such as meats and eggs and dairy products. This effect is of particular concern in neonatal diagnosis where normal infants consuming breast milk are compared with those on formulas that are heated.[434] Reference ranges established on large out-patient adult populations will reflect normal intake of dietary pyroglutamate.

Figure 6.22 shows results for urinary pyroglutamate in 1,500 sequential cases submitted to a clinical laboratory. Relative frequencies of moderate-to-severe occurrences of elevated pyroglutamate are apparent from inspection of the figure. N-acetylcysteine (NAC) is an effective oral agent for rebuilding total body glutathione, and oral taurine spares sulfur amino acids while providing an effective antioxidant.

Sulfate



Sulfate is the ionic form of an inorganic rather than organic acid. It may be included on panels of urinary organic acids because of the important information it provides about sulfur metabolism. For example, the protein synthesis disruption of zinc deficiency causes decreased incorporation of cystine into proteins with concurrent large increases in urinary sulfate and taurine from cysteine degradation.[435] The rise in sulfate parallels the depth of zinc deficiency, indicating that, if other sulfate sources are normalized, urinary sulfate can be a metabolic marker of the severity of zinc deficiency.

The sulfation pathway is used in phase II liver detoxification for biotransformation of many drugs, steroid hormones, phenolic compounds, and others. The addition of a sulfate group increases water solubility of hydrophobic compounds in preparation for their excretion in urine (see Chapter 8, "Toxicants and Detoxification"). The ratio of urinary sulfate to creatinine has been used to assess total body reserve of sulfur-containing compounds (especially glutathione) used in phase II pathways.[436] When the ratio of sulfate to creatinine is low, these stores need replenishment. Glutathione administration with oral N-acetylcysteine, taurine, and salts of sulfate are used in combinations to replenish sulfur pathways and restore the hepatic supply of inorganic sulfate.[437]

REFER TO CASE ILLUSTRATION 6.8

Severe depletion of organic sulfur sources will cause simultaneous high pyroglutamate and low sulfate excretion. High pyroglutamate with normal sulfate indicates inadequate organic sulfur sources for production of cysteine required for glutathione synthesis. Only

organic sulfur in the form of compounds such as N-acetylcysteine or methionine along with adequate glycine will restore normal glutathione levels. Normal urinary pyroglutamate with low sulfate levels can occur in individuals with impaired sulfate activation. In these cases, rapid replenishment of hepatic sulfate may be accomplished with either sulfur donors like N-acetylcysteine or inorganic sulfate such as sodium sulfate.[436] On the other hand, a well-nourished patient under temporary metabolic stress of detoxification from, for example, use of acetaminophen, may have elevated α-hydroxybutyrate, signaling the increased rate of hepatic glutathione synthesis, but have no need for amino acid or glutathione therapy due to a normal sulfate level. An individual with limited ability to produce glutathione may show the glycine depletion sign of high pyroglutamate and low sulfate. This patient is a candidate for glutathione administration along with glycine and N-acetylcysteine or methionine, and their taurine status should be monitored, since depletion of other sulfur-containing compounds is likely. Urinary sulfate has also been shown to reflect intake of sulfiting agents widely used as food additives.[438]

Intestinal Dysbiosis Markers

The abnormal overgrowth of microflora in the small and large intestine is sometimes referred to as "gut dysbiosis" in order to distinguish this clinical condition from that of infection. Dysbiosis has been related to a wide variety of symptoms due to pathogenic toxins produced by the populations of microflora. Many high molecular weight products such as cholera toxin can exert toxic consequences directly in the intestinal cells. Small molecular weight products can appear in urine, revealing metabolic activities of the microbes that inhabit the mucosal layer and lumen of the gut (Figure 6.23). The compounds have a wide range of relative toxicities, with cresol near the upper end and hippurate and benzoate at the lower end.

Among the factors that lead to dysbiosis, importance of mealtime habits should not be overlooked because of the power to restore intestinal health by allowing normal stomach emptying rate and adequate flow of gastric and pancreaticobiliary fluids. A much more extensive discussion of tests that may be used for evaluating digestive and microbial status of the gastro-

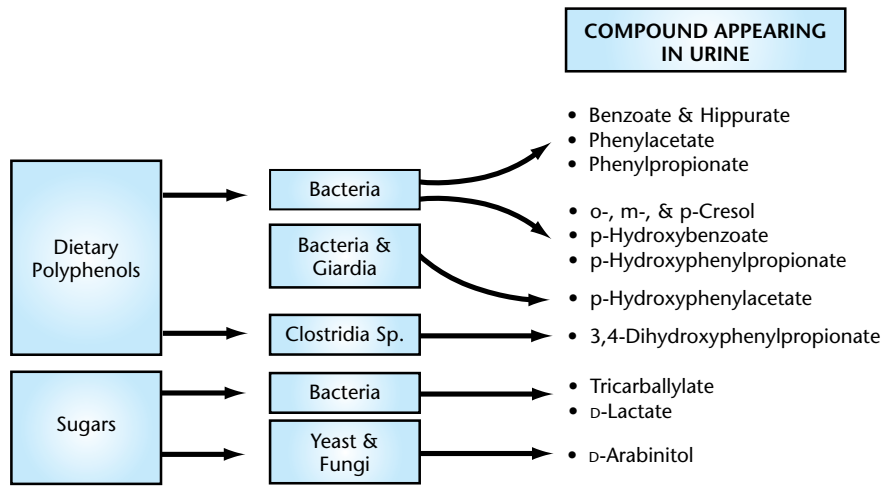


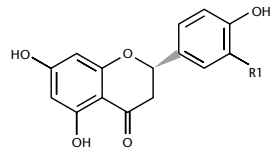
FIGURE 6.23 — **Origins of Urinary Dysbiosis Markers**

Undigested dietary polyphenols are the predominant substrate for growth of most intestinal microbes. Each species has adapted a metabolic preference that sustains its growth while producing compounds that suppress the growth of competing species. Certain species of bacteria and most yeast prefer carbohydrate substrates that are limited in most individuals by rapid uptake in the upper small intestine. Restricted uptake found in small bowel syndrome or genetic carbohydrate absorptive defects enhance the potential for overgrowth of the carbohydrate-preferring species.

intestinal tract is found in Chapter 7, "Gastrointestinal Function," where the urinary organic acid biomarkers of overgrowth are included in a more general context.

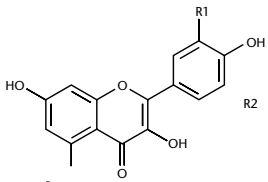
Since the writing of the previous version of this book, a great deal of new information has emerged regarding urinary microbial products. Markers of yeast growth have required total reevaluation as described below under "Products of Fungi." Much more information has been reported about the unique overgrowth scenario revealed by high urinary D-lactate. The origin of phenyl compounds has been greatly clarified by studies on microbial conversions of dietary polyphenols. The phenyl compound emphasis from early reports of human conditions of microbial overgrowth places great importance on this area. Therefore, a more detailed description of dietary polyphenols and their microbial products is provided to allow clinicians to develop a clear picture of how abnormalities of the compounds should be interpreted.

Dietary polyphenols, amino acids, and sugars that are not transported from the gut lumen into enterocytes can be used by gut microbes for growth.[439] A summary of some important dietary polyphenol structures and dietary sources is shown in Figure 6.24. The wide range of polyphenol food sources means the great majority of patients will have sufficient intake for overgrowth of bacterial microbes to be revealed. Only a small fraction of dietary polyphenols are converted into products that appear in the urine of healthy individuals. For example, the total output of metabolic products from chlorogenic



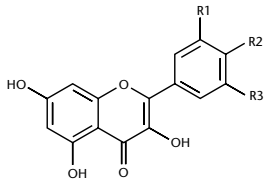
Flavones

Example	Sources	R1
Apigenin	Parsley	H
Luteolin	Celery, Peppermint	OH



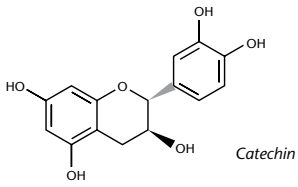
Flavonols

Example	Sources	R1	R2
Quercetin	Cranberries, Onion, Peppers, Tomatoes	OH	H
Kaempferol	Broccoli	H	H
Isohamnetin	Parsley	OCH_3	H



Flavonones

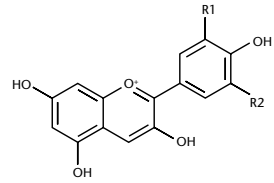
Example	Sources	R1
Hesperetin	Oranges, Lemons	OH
Naringenin	Grapefruit	H



Catechin

Catechin

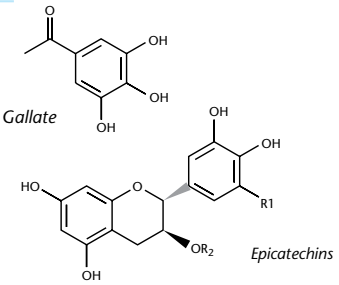
Example	Sources
Catechin	Grapes, Plums



Anthocyanidins

Anthocyanidins

Example	Sources	R1	R2
Cyanidin	Cherries, Raspberries,	OH	H
Malvadin	Blueberries	OCH_3	OCH_3



Epicatechins

Epicatechins

Example	Sources	R1	R2
Epicatechins	Chocolate, Apples, Blackberries	H	H
EGC*	Black tea	OH	H
EGCG**	Green tea	OH	Gallate

**** Epigallocatechin (EGC)***
***** Epigallocatechin-3-gallate (EGCG)***

FIGURE 6.24 — **Dietary Polyphenolic Compounds That Serve As Substrates for Intestinal Bacterial Metabolism**

The six classes of compounds shown represent the great majority of dietary polyphenols. The tables show members of each class and common dietary sources. The designations of substituent groups (R) are also shown in the tables. Microbes in the human gut metabolize these compounds, releasing the aromatic organic acids discussed in this section.

TABLE 6.11 — CHRONOLOGICAL SUMMARY OF MICROBIAL SUBSTRATE AND URINARY PRODUCT REPORTS

Year	Substrate and Protocol Sketch	Urinary Products*
1957	Identify products in human urine after administration of caffeic acid.[459]	**m-Hydroxyhippurate +++**
1964	Adding 50 mg catechin to rats on low catechin diet caused appearance of urinary products that fell with administration of antibiotics.[460]	**m-Hydroxyhippurate +++** **m-Hydroxyphenylpropionate +++**
1968	Caffeic acid (chlorogenic acid) challenge in rats and rabbits.[461]	**p-Hydroxyphenylpropionate**
1969	Anaerobic culture of entire rat intestinal content with and without added catechins.[462]	p-Hydroxyphenylpropionate m-Hydroxyphenylpropionate δ-(3,4-Dihydroxyphenyl)-g-valerolactone
1970	Caffeic acid (chlorogenic acid) challenge in conventional and germ-free rats.[464]	**m-Hydroxyphenylpropionate** (complete absence in germ-free rats)
1971	Bacterial metabolite excretion in a patient with cystic fibrosis and severely impaired amino acid absorption.[463]	**p-Hydroxyphenylacetate** **p-Hydroxybenzoate**
1976	Deuterated tyrosine metabolites found in in vivo and in culture medium after 1 week of human fecal specimen anaerobic incubation.[465]	Phenol p-Cresol p-Hydroxybenzoate p-Hydroxyhippurate p-Hydroxyphenylacetate p-Hydroxymandelate p-Hydroxyphenyllactate
1986	Products of normal rat cecal content action on catechins in anaerobic batch culture.[466]	Phenylacetate o-, m-, and p-Hydroxybenzoate p-Hydroxyphenylacetate Phenylpropionate o-, m-, and p-Hydroxyphenylpropionate 3,4-Dihydroxyphenylpropionate p-Hydroxyphenyllactate
1994	Urinary products from germ-free rats on standard chow before and after fecal bacterial introduction.[13]	**m-Hydroxyphenylpropionate +++** **p-Hydroxyphenylpropionate +++** **Benzoate ++** **m-Hydroxyphenylacetate ++** **Phenylacetate ++** **p-Hydroxymandelate +** **p-Hydroxyphenylglycol +** **o-Hydroxyphenylacetate +** Homovanillate Hydroxyphenylacetate p-Hydroxyphenylacetate p-Hydroxyphenyllactate m-Hydroxyphenylhydracrylate 4-Hydroxy-3-methoxyphenylglycol
1997	Urinary metabolites from oral administration of *Ginkgo biloba* to humans.[467]	Hippurate p-Hydroxybenzoate p-Hydroxyhippurate 3-Methoxy-4-hydroxybenzoate 3-Methoxy-4-hydroxyhippurate 3,4-Dihydroxybenzoate
2000	Metabolites produced by human colonic microflora grown in culture on C14-labeled proanthrocyanidin-enriched chow under anoxic conditions.[468]	**Phenylacetate +++** **Phenylpropionate +++** **p-Hydrophenylacetate +** **m-Hydroxyphenylacetate++** **m-Hydroxyphenylpropionate +++** **m-Hydroxyphenylvalerate ++**
2000	Urinary products from consumption of brewed black tea by human subjects.[469]	**Hippurate +++**

Table 6.11 continued on following page..

Table 6.11 continued from previous page...

Year	Substrate and Protocol Sketch	Urinary Products*
2002	Comparison of rat urinary products on control diet and after addition of wine extract.[470]	**3,4-Dihydroxyphenylacetate +++** **3,4-Dihydroxyphenylpropionate +++** **p-Coumarate ++** **Caffeate ++** **Ferulate ++** **o-Hydroxyhippurate ++** **Hippurate ++** **o-Hydroxybenzoate +** **p-Hydroxybenzoate +** **o-Hydroxyphenylacetate +** **Vanillate +** p-Hydroxyhippurate Phenylacetate
2003	Comparison of urinary products from humans on a polyphenol-free diet and after addition of 80 g chocolate /d, supplying 439 mg proanthocyanidins and 137 mg catechin.[455]	**Vanillate ++** **Ferulate +** **m-Hydroxybenzoate ++** **3,4-Dihydroxyphenylacetate +** **m-Hydroxyphenylacetate ++** **m-Hydroxyphenylpropionate +** p-Hydroxybenzoate p-Hydroxyhippurate 3,4-Dihydroxyphenylpropionate **Phenylacetate -** **Hippurate --**
2004	Changes in human subject excretion with introduction of grape seed extract supplement that supplies 1000 mg of polyphenols.[471]	**m-Hydroxyphenylpropionate ++** **m-Hydroxyphenylacetate ++** **p-O-Methylgallate +** p-Hydroxyphenylacetate
2005	Randomized, crossover design used for 17 humans to detect urinary effects of two days dosing with 6 g green tea, 6 g black tea.[472]	**Hippurate ++**

** Compounds in bolder blue color showed significant increases as a result of the challenge described in the column labeled "Protocol Sketch." Relative rankings of the response magnitudes are shown as high (+++) to low (+). Compounds showing no significant urinary response are in black and those with negative response are in bolder red color. For these compounds, the intervention suppressed either bacterial or host tissue reactions required for their production. Compounds from studies that identified but did not quantitate the products are also shown in black.*

acid, one of the most abundant dietary polyphenols, has been reported to be less than 58% of ingested amounts.[440] Thus, a significant degree of variability in urinary products among individuals may be ascribed to varying rates of intestinal microbial growth. Differences in gut microbial action explains why some people who consume soy protein produce significant amounts of equol, whereas others do not.[441] However, patients under treatment with medical foods or total parenteral nutrition may not be candidates for urinary phenolic bacterial product assessment because of inadequate polyphenol intake to allow microbial metabolic product formation. Since these patients are at risk of being malnourished, there is potential for eventual interpretation of low fecal phenolics[442] or urinary polyphenol products[443] as assessments of polyphenol inadequacy. The other microbial products derived from non-phenolic sources discussed below are not dependent on polyphenol intake. Table 6.11 summarizes many of the studies reporting evidence that supports the microbial origin of human urinary metabolites, and the foods or components that contribute to the wide variety of patterns appearing for individual patients.

Many bacteria that inhabit the human gut have highly adaptable metabolic responses to the food sources provided by their environment.[444] Scientist are beginning to think of the 2 to 3 pounds of microbial mass as an organ with vast metabolic potential that expands human adaptability and that is required for human health.[445] Microbial catabolic pathways generally stop short of full oxidation of substrates to carbon dioxide and water to yield organic acids and other classes of compounds that can be absorbed from the gut and excreted in urine. Early in vitro work demonstrated various metabolic potentials for specific bacteria.[446-450] Measuring products formed by ruminants when dietary intake of specific compounds was varied yielded information about rumen bacteria. The proximity of the

rumen to the oxygen content of swallowed air means that organisms thriving there are generally aerobic and microaerophilic, in contrast to the highly anaerobic environment below the stomach.[451-454] This ruminal stage of microbial activity is most closely approximated in the human jejunum. Absorbed bacterial metabolic products may undergo further metabolism in host tissues, mainly liver and kidney. For example, urinary benzoic acid was found in ruminant urine as the result of hepatic action on the phenylpropionate, a principal product of microbial metabolism of dietary cinnamic acid.[452] These data explain the failure of phenylpropionate to appear in appreciable quantities in urine of most patients. Normal hepatic oxidase activity converts any absorbed phenylpropionate into benzoate (and hippurate). The specific compounds that are excreted by a given individual depend on the available substrates and the species of organism present.[443, 455] Some of the compounds discussed in this section are exclusively produced by such intestinal microbial metabolism, whereas others may have small contributions from human metabolic pathways. Benzoate is a common dietary component. Nevertheless, since benzoate is also potentially produced by intestinal bacteria, elevation of benzoate and hippurate can add strength to conclusions when a general pattern of elevated bacterial products is found.

High predictive values of urinary markers have been established for some conditions. In one study of 360 acutely ill infants and children, small bowel disease and bacterial overgrowth syndrome was predicted with no false-negative results, and only 2% false-positive results from urinary p-hydroxyphenylacetate.[433] Three of the patients who tested positive for this compound were diagnosed with *Giardia lamblia* infection, giving evidence of the potential for revealing certain types of protozoal overgrowth.

The extent to which future work on urinary microbial markers may allow more detailed prediction of pathogenic intestinal microbe growth needing specific interventions is difficult to predict. As knowledge of the range of products that might be measured increases and connections with specific microbial overgrowth consequences are understood, the time may come when either single or multiple timed urine collections will be able to more accurately show the status and clinical consequences of intestinal dysbiosis. Recent combinations of HPLC-MS and nuclear magnetic resonance-based metabolomic studies have revealed great differences between individuals, whereas single patients show quite stable patterns across all types of molecules, including microbial products.[456, 457]

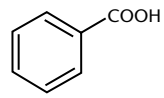
Notes:

Bacterial and Protozoal Phenolic Products

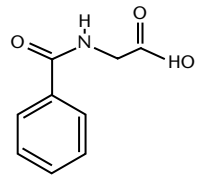
By acting on various dietary or endogenous substrates, bacteria or parasites can generate metabolic products that are absorbed and excreted in urine with or without further modification in the liver and kidney. Dietary polyphenols have been shown to be one of the dominant substrates for yielding phenolic compounds, whereas dietary simple sugars lead to generation of others. Some studies that have contributed to our knowledge of these products are summarized in Table 6.11. Although there are numerous polyphenolic chemical structures contained in foods, it appears that a relatively small number of phenolic products are formed.[443] This feature of the system means that variations in specific foods consumed from one patient to the next may have only small effects on the potential for generating phenolic products. The greater factor is the type and activity of the microbes that are present.

The anatomical region of the gut that is most likely to yield bacterial metabolites is the middle or transitional gut, including the terminal ileum and the ascending colon because the passing of chyme to the lower ileum corresponds to the lag phase for the onset of logarithmic growth rates characteristic of most bacteria.[458] It is during this most intense growth phase when the microbial counts rise from 10^5 to 10^{11}/g that metabolic products are most actively produced. Thus, by measuring their products in urine, information principally about the mid- or transitional-gut microbial mass is obtained. These microbial populations that may produce toxic metabolites may be detected more accurately by their urinary markers than by attempting to detect the organisms directly through stool specimens. See Chapter 7, "Gastrointestinal Function," for further discussion of microbial populations in the gut and stool testing interpretation.

Benzoate



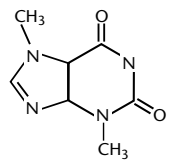
Hippurate



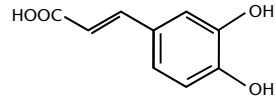
Benzoate was one of the compounds first found to be elevated in urine from patients with intestinal bacterial overgrowth of various origins. Many patients with intestinal bacterial overgrowth resulting from cystic fibrosis or unclassified enteritis, celiac disease or short-bowel syndrome were found to have elevated benzoate along with various degrees of elevated phenylacetate, p-hydroxybenzoate, and p-hydroxyphenylacetate, as described below.[473] These products were thought to be derived from unabsorbed phenylalanine or tyrosine from dietary protein.[473] Later reports have demonstrated that bacterial catabolism of dietary polyphenols may be the predominant origin of benzoate, which is normally conjugated with glycine in the liver to form hippurate.[13] Dietary polyphenols generally persist into the lower small intestine because they are resistant to degradation by digestive fluids[474].

Coffee, fruits and vegetables are sources of beneficial chlorogenic acid, over 57% of which is recovered in urine as organic acids, mainly benzoate and hippurate.[440] See Figure 6.25 for differentiation of caffeic acid from caffeine that is also present in coffee and tea, but is not a polyphenolic compound and does not yield any of the compounds currently measured as intestinal microbial products in urine. Quinic acid, a tetrahydroxybenzoic acid compound found in tea, coffee, fruits, and vegetables is also largely metabolized to benzoic acid by intestinal bacteria and excreted as hippurate.[475] When humans were changed from a low-polyphenol diet to one including 6 g of green tea or black tea solids, they started excreting more hippurate.[472] The conversion of catechin from tea is thought to involve bacterial release of 3-phenylpropionate with subsequent absorption and hepatic oxidation to benzoate and conjugation to yield hippurate (Figure 6.26). Consumption of 6 g of green tea solids by healthy male volunteers produced an approximate doubling of urinary hippurate from 1.9 to 4.0 mmol/24 h.[472] A change of 2 mmol/24 h corresponds to approximately 120 µg/mg creatinine, a small effect compared with a typical abnormal cutoff of 800 µg/mg creatinine. Similar results from ingestion of brewed black tea had been reported before the tea extract study was done.[469] In addition, other studies found that the measured levels of phenolic compounds from green tea are lowered by administration of antibacterial agent to a human subject, confirming the microbial contribution to the appearance of urinary products.[476]

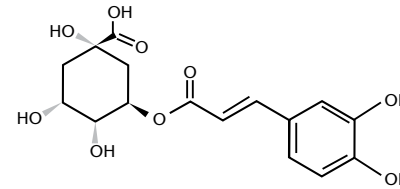
Benzoic acid is also a common food component. It is used as a preservative in packaged foods such as pickles and lunch meats, and it occurs naturally in cranberries and other fruits.[477] This should be taken into account when interpreting elevated hippurate levels in urine. Whether the source is dietary intake or jejunal



Caffeine



Caffeic Acid



Chlorogenic Acid

FIGURE 6.25 — Differentiation of Caffeine, Caffeic Acid and Chlorogenic Acid

Although teas and coffee may contain all three comounds, caffeine and caffeic acid have quite different chemical structures and the metabolism of caffeine by intestinal bacteria and human enzymes is distinctly different from that of the other two compounds. Caffeine is a methylated purine, while caffeic acid is a phenolic organic acid. Chlorogenic acid, the ester of caffeic acid and quinic acid is a major polyphenolic compound in coffee. Intestinal microbial enzymes hydrolyze the ester bond, releasing caffeic acid.

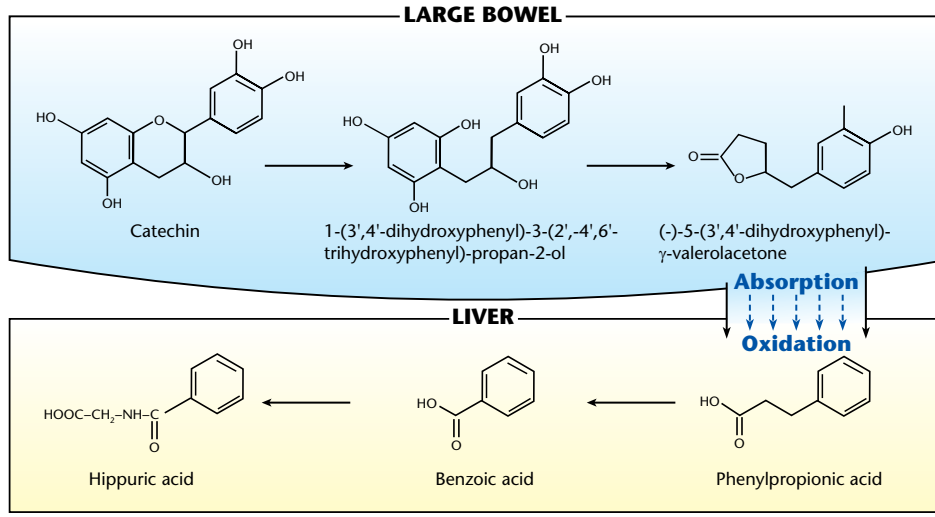


FIGURE 6.26 — **Bacterial and Human Enzymatic Conversion of Catechin to Hippurate**

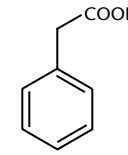
Catechin from dietary green or black tea consumption is converted to 3-phenylpropionate by intestinal bacteria and the absorbed 3-phenylpropionate is oxidized to benzoate and conjugated to hippurate by human intestinal and hepatic enzymes. Higher rates of bacterial conversion cause elevation of urinary hippurate. Abnormally high hippurate is consistent with normal polyphenol intake and abnormally high growth rates of those bacteria that can carry out this conversion.

bacterial metabolism, benzoate should be rapidly converted to hippurate by conjugation with glycine. Glycine and pantothenic acid can be limiting factors in this process. Availability of glycine is easily limited as discussed above ("Pyroglutamate") and in Chapter 4, "Amino Acids." Elevated benzoate is a confirmatory marker for inadequacy of glycine or pantothenic acid for conjugation reactions.[478,479] Abnormalities of urinary benzoate and hippurate may reveal clinically significant detoxification or dysbiosis issues. High benzoate indicates poor detoxification via phase II glycine conjugation. Interpretations of other scenarios are collected in Table 6.12, and Case Illustration 6.9 show scenarios of abnormal benzoate and hippurate.

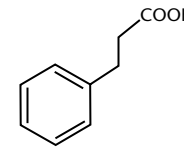
REFER TO CASE ILLUSTRATION 6.9

The organic solvent, toluene, is detoxified by oxidation to benzoic acid and excretion as hippurate.[480] Although some reports have associated hippurate excretion with exposure to toluene, the relationship is weak because of the multiple other sources of hippurate described here. Short-term toluene exposure produces no significant changes in hippurate excretion.[480]

Phenylacetate



Phenylpropionate



Intestinal bacterial action on dietary polyphenols causes the appearance of phenylacetate (PAA) in urine. Excretion of PAA is markedly increased after the gastrointestinal tracts of germ-free rats are inoculated with fecal microorganisms, indicating its microbial origin.[13] Significant PAA has been found in human fecal water, indicating that absorption from the gut is only partial and having potential implications for involvement in colonic function.[442] For individuals with normal, healthy intestinal function, phenylacetate should not appear at more

than background concentrations in urine. However, phenylacetate is a trace product of endogenous phenylalanine catabolism that can accumulate in the phenylalaninemic state found in PKU.[481] Although PAA shows little toxic effect on brain glutamatergic activity,[482] it has significant effects on hepatic flux of glutamate and α-ketoglutarate,[483] indicating that PAA may mediate some of the toxic consequences of PKU. These findings are cause for concern over chronic elevation of PAA due to bacterial metabolism in the gut. Urinary PAA is the product of unidentified, specific strains of bacteria, marking a state of bacterial overgrowth when it is elevated in urine.

A large body of literature has addressed quite a different role of PAA in cancer treatment.[484-492] PAA has been found to modulate early estrogen-mediated events in breast cancer,[484] cell cycle events in prostate cancer,[485] and regression of primary brain tumors.[488] When these effects are considered with knowledge of its intestinal bacterial origin, a symbiotic relationship of novel proportion is suggested. Though little attention has been directed to the subject, it is possible that normal flow of PAA into blood from the gut is protective against neoplastic events similar to the protective effect of colonic butyrate formation.[493] There is potential clinical utility for definition of low reference limits for PAA, and there is high likelihood that future research will reveal other such symbiotic relationships involving products of normal intestinal microbial metabolism.

The similar compound that has two $-CH_2-$ groups instead of one, phenylpropionate (PPA), is also produced by anaerobic gut flora.[494] PPA does not normally appear in human urine, however, because it is metabolized by mitochondrial medium-chain acyl-CoA-dehydrogenase (MCAD).[495] The glycine conjugate of PPA, 3-phenylpropionylglycine, has been proposed as a marker for diagnosing asymptomatic MCAD-deficient individuals who do not sufficiently carry out the oxidative step.[496] This human genetic polymorphism test is unique in being dependent on the production of PPA by gut flora such as *Peptostreptococcus anaerobis*. A rare case of elevated unconjugated PPA is shown in Case Illustration 6.10 where both asymptomatic MCAD deficiency and glycine conjugation deficiency is indicated. Because of the intestinal bacterial requirement, the question of which organisms may be required has been addressed in one study. Of the 67 bacterial and 5 yeast isolates that were examined, only the 3 isolates of *Clostridium sporogenes* and one of *Clostridium difficile* produced PPA.[497] These researchers went on to investigate the effects of antibiotics on PPA production by analysis of stool cultures to produce the findings of Table 6.13. They conclude that use of PPA to screen for asymptomatic MCAD deficiency must be done with caution.

Refer to Case Illustration 6.10

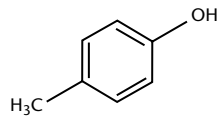
Table 6.12 — Interpretation of Patterns for Urinary Benzoate and Hippurate Abnormalities

Benzoate	Hippurate	Other Bacterial Markers	Interpretation
Low	Low	No elevations	Low intake of benzoate and precursors, plus normal or low dietary polyphenol conversion by intestinal microbes
		Multiple elevations	Low intake of benzoate and precursors with intestinal microbial overgrowth of species that do not metabolize dietary polyphenols (very rare)
High	Low	No elevations	Glycine conjugation deficit (possibly genetic polymorphic phenotype if hippurate is very low); dietary benzoate or precursor intake
		Multiple elevations	Glycine conjugation deficit; presume benzoate is at least partially from intestinal microbial action on dietary polyphenols
Low	High	No elevations	Normal hippurate production via active glycine conjugation; No indication of microbial overgrowth
		Multiple elevations	Normal hippurate production via active glycine conjugation; Presume hippurate is at least partially derived from intestinal microbial action on dietary polyphenols
High	High	No elevations	Very high dietary benzoate or precursor intake with partial conversion to hippurate
		Multiple elevations	Very high benzoate load, some, or all, of which is contributed by intestinal microbial action on dietary polyphenols

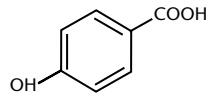
TABLE 6.13 — ANTIBIOTIC ACTIVITY AGAINST CLOSTRIDIA THAT PRODUCE PHENYLPROPIONATE[407]

Intravenous		Oral	
No effect	Inhibition	No effect	Inhibition
Cefazolin Cefuroxime Ampicillin sodium Chloramphenicol Gentamicin	Ticarcillin with clavulonate Oxacillin	Amoxicillin alone Amoxicillin with clavulonate	Metronidazole Clindamycin

Cresol



Hydroxybenzoate

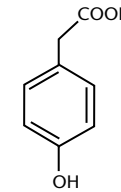


Dietary polyphenols or tyrosine residues from dietary proteins are the parent compounds from which urinary p-cresol, p-hydroxybenzoate, and p-hydroxyphenylacetate are formed (Figure 6.27).[454] Cresol has a chemical structure very similar to phenol and is highly toxic. Cresol excretion is not affected by dietary protein intake, suggesting that the bacteria responsible reside in the lower portions of the small intestine where amino acids from dietary protein rarely penetrate. These bacteria apparently produce cresol from intestinal secretions as well as from dietary sources.[498] Mammalian tissues have negligible metabolic activity toward absorbed cresol according to studies in sheep, where 95% of cresol infused into the rumen appears in urine.[454] Production of cresol in humans may be dependent on small intestinal populations of aerobic or microaerophilic bacteria because, in sheep, its production is almost exclusively confined to the rumen.[453]

A large majority of adult celiac disease patients were found to excrete unusually high amounts of p-cresol.[499] Due to the loss of renal function, uremic patients accumulate cresol, which may contribute to toxic effects. The resultant increase in serum cresol can be prevented by the use of non-absorbed oral sorbents, demonstrating that the origin of the p-cresol is the bowel. Finely powdered, activated charcoal is a generally available sorbent, but newer synthetic compounds such as AST120 may be more effective.[500] Cresol excretion was found to be lowered by administration of prebiotic substrate (oligofructose-enriched inulin) along with *Lactobacillus casei, Shirota* and *Bifidobacterium breve* to human subjects.[501]

Strains of *Escherichia coli* can produce p-hydroxybenzoate from glucose.[502] Other studies showing intestinal microbial production of p-hydroxybenzoate are summarized in Table 6.11. Esters of p-hydroxybenzoate, called parabens, have antibacterial activity[503] and they are part of the mechanism for establishing bacterial dominance in intestinal populations. This phenomenon is discussed further in Chapter 7, "Gastrointestinal Function."

Hydroxyphenylacetate



No other species has a digestive tract exactly like humans. The one that has the closest resemblance is swine. Studies in newly weaned pigs have revealed specific microbes that carry out tyrosine degradation (Figure 6.27).[504] Both the transamination to form p-hydroxyphenylacetate (HPA) and the decarboxylation to p-cresol are carried out by *Clostridium difficile*. Since *Proteus vulgaris* can do only the first of these steps, HPA will increase in urine if *P. vulgaris* is the predominant organism. When *P. vulgaris* is accompanied by overgrowth of a newly identified strain of *Lactobacillus*, however, p-cresol will be the major product to accumulate. The lactobacillus was not given species identification, but the characteristics eliminate the species listed in the legend of Figure 6.27. Such studies illustrate the potential for more specific bacterial identifications based on patterns

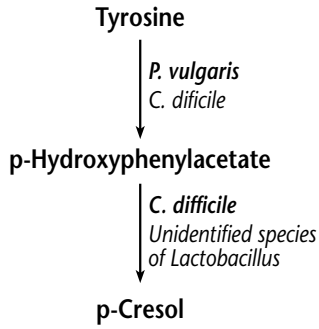


FIGURE 6.27 — **Cresol from Bacterial Metabolism of Tyrosine**

Unassimilated dietary tyrosine is metabolised by *P. vulgaris* and *C. difficile* to p-hydroxyphenylacetate. This product can be further metabolised to p-cresol by *C. difficile* and by a *Lactobacillus* species other than *acidophilus, leichmannii, delbrueckii, plantarum, brevis, minutus* or *fermentum.*[504] Exact microbial origins of compounds found in urine are difficult to establish.

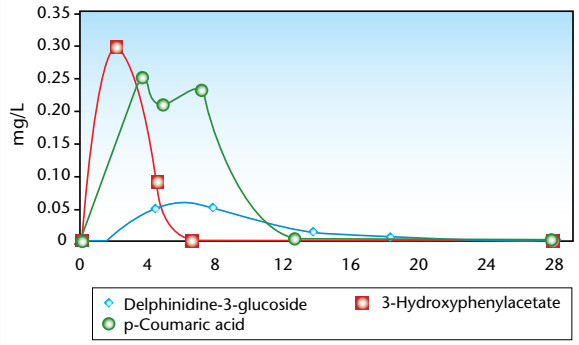


FIGURE 6.28 — **Timed Appearance of Black Currant Juice Metabolites In Human Urine**[517]

Ingestion of 330 ml of black currant juice after 2 days of low polyphenol diet resulted in the appearance of small concentrations of unaltered anthocyanidin glycosides and numerous products of their bacterial metabolism. Hippurates (not shown) and unchanged parent compounds like delphinidin-3-glucoside appear at peak concentraions within one hour, showing how that they have been delivered to absorptive regions of the gut. Others like p-coumaric acid show second peaks at about six hours, and still others like p-hydroxyphenylacetate have peaks at 10 and 24 hours. The longer times of appearance indicate activity in lower regions of the gut where different microbes dominate.

of products appearing in urine. To achieve more detailed assignments of origin, however, urine collections may need to be timed following intake of specific sources. As shown in Figure 6.28, a bolus of black currant juice can cause the appearance of different products as it passes from one region of the gut to the next.

p-Hydroxyphenylacetic aciduria has been found useful in detecting small bowel disease associated with *Giardia lamblia* infestation, ileal resection with blind loop, and other diseases of the small intestine associated with anaerobic bacterial overgrowth. [433] Use of antibiotics that act primarily against aerobic bacteria (such as neomycin) can encourage the growth of protozoa and anaerobic bacteria that then produce greater amounts of these compounds.[505] A clostridial species isolated from swine feces carries out the further metabolism of p-hydroxyphenylacetate to p-cresol.[504]

Patients with cystic fibrosis or other conditions that severely impair amino acid absorption can demonstrate the potential for intestinal bacterial conversion of phenylalanine and tyrosine to phenyl compounds that appear in urine. These patients tend to excrete very high levels of phenylacetate and p-hydroxyphenylacetate.[463] However, since tyrosine released from dietary protein is rapidly absorbed in most individuals; conversion of tyrosine to p-hydroxyphenylacetate may be a rarely observed sign of dysbiosis in humans. However, the other isomers, o- and m-hydroxyphenylacetate, may be derived from dietary polyphenols that are unaffected by digestive enzymes and they are normally abundant dietary components. Of the three isomers, the most likely bacterial dysbiosis marker is m-hydroxyphenylacetate, which appears when bacteria are introduced to germ-free rats and increases markedly when humans are fed catechin and proanthocyanidins-rich chocolate, as shown in Table 6.11 (see row "2003"). In experiments conducted with a human anaerobic fecal fermentation device, quercetin was found to be metabolized within 2 hours to 3,4-dihydroxyphenylacetate, which, over the next 8 hours, was converted to m-hydroxyphenylacetate.[506]

Notes:

HPA is elevated in a wide variety of conditions involving direct intestinal pathology or digestive organ failure (Figure 6.29), which have obvious potential for dysbiosis. Although treatments vary greatly depending on the nature of the disorder, the lowering of elevated urinary HPA reveals a normalized intestinal bacterial population.[473] Some microbial compounds are absorbed and enter the detoxification pathways of the liver to be excreted as modified products that can serve as indicators of gastrointestinal activities. For example, bacterial amines are converted to piperidine, a sensitive biochemical index of gastrointestinal flora changes in celiac disease.[507] Other compounds appear due to genetic traits that affect how bacterial products are metabolized. The anaerobic bacterial product, 3-phenylpropionate mentioned previously, for example, is normally converted to common hippuric acid, but is excreted as 3-phenylpropionylglycine in individuals with a relatively common inborn error of fatty acid oxidation.[497] Some compounds excreted in these instances are not organic acids, so they must be analyzed in separate assays to enhance the interpretation of origins for microbial compounds in urine.

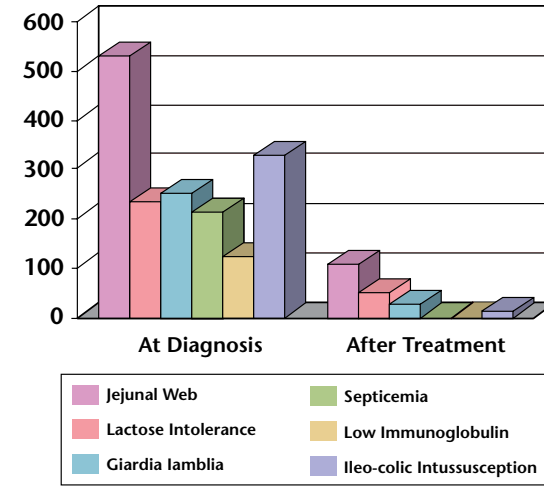
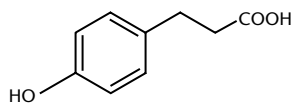


FIGURE 6.29 — **p-Hydroxyphenylacetate Response to Treatment**

The dramatic lowering of HPA after treatments commensurate with six different clinical presentations demonstrates the removal of bacterial overgrowth in every case, even when the cause varied from lactose intolerance to Giardiasis.

Hydroxyphenylpropionate



The o- (or 2) and m- (or 3)-hydroxyphenylpropionates can reveal specific types of intestinal bacterial activity. When germ-free rats are given feed that is contaminated with feces from standard rats, they begin to excrete m-hydroxyphenylpropionate (m-HPPA).[13] Subsequent studies showed that m-HPPA is absent from the urine of germ-free rats, whereas it is the principal product that appears from conventional rats when caffeic acid is introduced.[464] Increased excretion of m-HPPA was found in healthy human volunteers who consumed 1,000 mg of polyphenols as grape seed extract (Figure 6.30).[471] Low levels of urinary m-HPPA, therefore, can indicate low intake of caffeic acid and the proanthocyanidins found in grapes and other foods. High levels of m-HPPA, on the other hand, may indicate increased intestinal bacterial metabolism of dietary catechins and caffeic acid. m-HPPA systematically increases in rat urine when catechin is added to their chow, and its excretion in urine drops from around 200 µg/24 h to 10 µg/24 h after administration of a combination sulfathiozole + auromycin antibiotic.[460] A close inspection of the data from the chlorogenic acid study reveals a wide range of individual variation in responses, as shown in Figure 6.32. Such responses could be attributed to variation in intestinal bacterial conversion potential from the normal rates exhibited by most people.

Unlike the other two positional isomers, p (or 4)-hydroxyphenylpropionate (p-HPPA) is produced in human cells by tyrosine transamination. The further conversion of p-HPPA to common energy pathway intermediates is inhibited in genetic disorders that produce elevated tyrosine and p-HPPA in blood and urine. Tyrosine transaminase is deficient in type II tyrosinemia that may be controlled with a low phenylalanine and tyrosine diet.[508] The removal of p-HPPA is under the control of the enzyme p-hydroxyphenylpyruvate dioxygenase that is deficient in type III genetic tyrosinemia.[509] The reaction also requires participation of ascorbic acid. Type III tyrosinemia has been studied in a mouse model system to elucidate the nature of the genetic disorder.[510] A nucleotide substitution has been identified that generates a termination codon resulting in failure to express the gene in mice.[511]

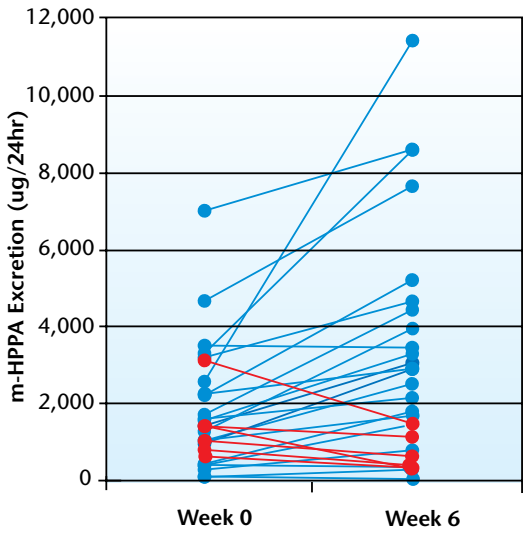


FIGURE 6.30 — Individual Subject Variation in Response to Grape Seed Extract

Each line represents one individual. Although the average response is an approximate doubling of m-hydroxyphenylpropionate (m-HPPA) excretion, four individuals show negative response while three others show several fold increase. These data suggest large intra-individual differences in microbial populations that are capable of releasing m-HPPA from ingested polyphenols.

In vitro bacterial growth experiments indicate that, in the gut, p-HPPA is metabolized by bacteria but not by protozoa. Bacterial action converts p-HPPA into p-hydroxybenzoate, p-hydroxyphenylacetate, phenylpropionate, phenyllactate, and phenylpyruvate.[512]

When p-HPPA is elevated without concurrent elevation of tyrosine, then the possibility of intestinal clostridial production from dietary tyrosine should be considered. Under in vitro conditions, where L-tyrosine is supplied as a growth substrate, p-HPPA is a major product of *Clostridium sporogenes*, *Clostridium botulinum A*, *C. botulinum B* and *Clostridium caloritolerians*.[513] Such growth conditions also result in the appearance of even greater concentrations of phenylpropionate, but insignificant amounts of phenylacetate, phenyllactate, p-hydroxyphenylacetate, and indole. Human fecal bacteria grown with the polyphenol naringin as a substrate show predominant production of phenylpropionate or p-HPPA as shown in Figure 6.31. These results help to explain the varied patterns of urinary products that appear with individual patients.

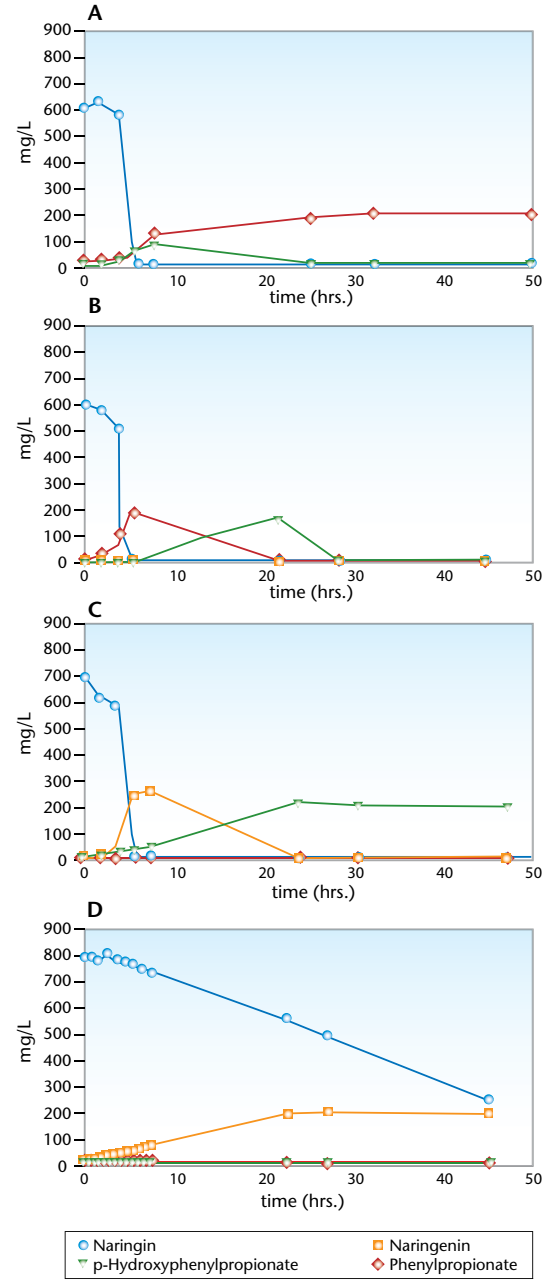


FIGURE 6.31 — Individual Patterns of Naringin Degradation

Graphs A, B, C and D are from four individuals. Fecal specimens were inoculated on growth media containing added naringin. After 10 hours, subjects may be defined as predominant producers of both phenylpropionates (A and B), p-hydroxyphenylpropionate (C), or neither phenylpropionate (D). The differences are due to variations in dominant bacterial types. These products are from growth in culture, so no effects of host metabolism are seen.[443]

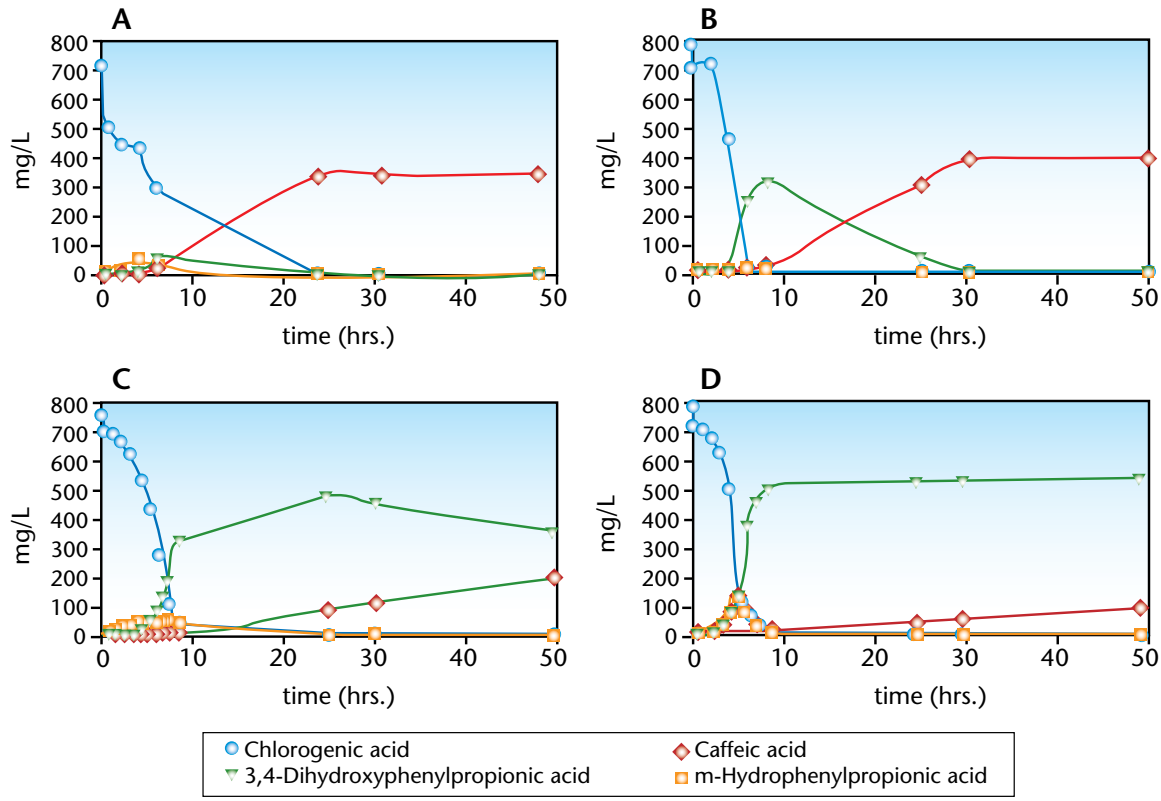


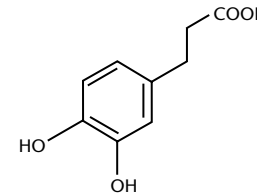
FIGURE 6.32 — **Individual Differences in Microbial Product Formation From Chlorogenic Acid**

Graphs A, B, C and D are from four individual, healthy fecal donors. Individual fecal slurries were introduced into growth media containing the polyphenol chlorogenic acid. Compounds plotted are chlorogenic acid and its degradation products, caffeic acid, 3,4-dihydroxyphenylpropionic acid, and m-hydroxyphenylpropionic acid. Subject A has the slowest rate of chlorogenic acid (the dietary component) removal and least appearance of 3,4-DHPP, while subjects C and D have very active rates of 3,4-DHPP production.[443]

Abnormal appearance of the o- or m- isomers indicates the more common bacterial overgrowth utilizing dietary polyphenols, whereas high p-hydroxyphenylacetate may be due to type III tyrosinemia or bacterial conversion of unabsorbed tyrosine. Patients with the genetic trait will present with characteristic signs of type III tyrosinemia, whereas those with chronic maldigestion of protein will generally show gastrointestinal signs.

Notes:

3,4-Dihydroxyphenylpropionate



Several clostridia are known to cause human disease, for example, *Clostridium difficile*-associated enteric disease epidemics[514] and *Clostridium perfringens*-associated food borne infectious illness outbreaks from eating cooked beef.[515] However, many species of the genus *Clostridium* make up a major portion of the bacterial population in the normal human gut, with *Clostridium coccoides* frequently found as the most

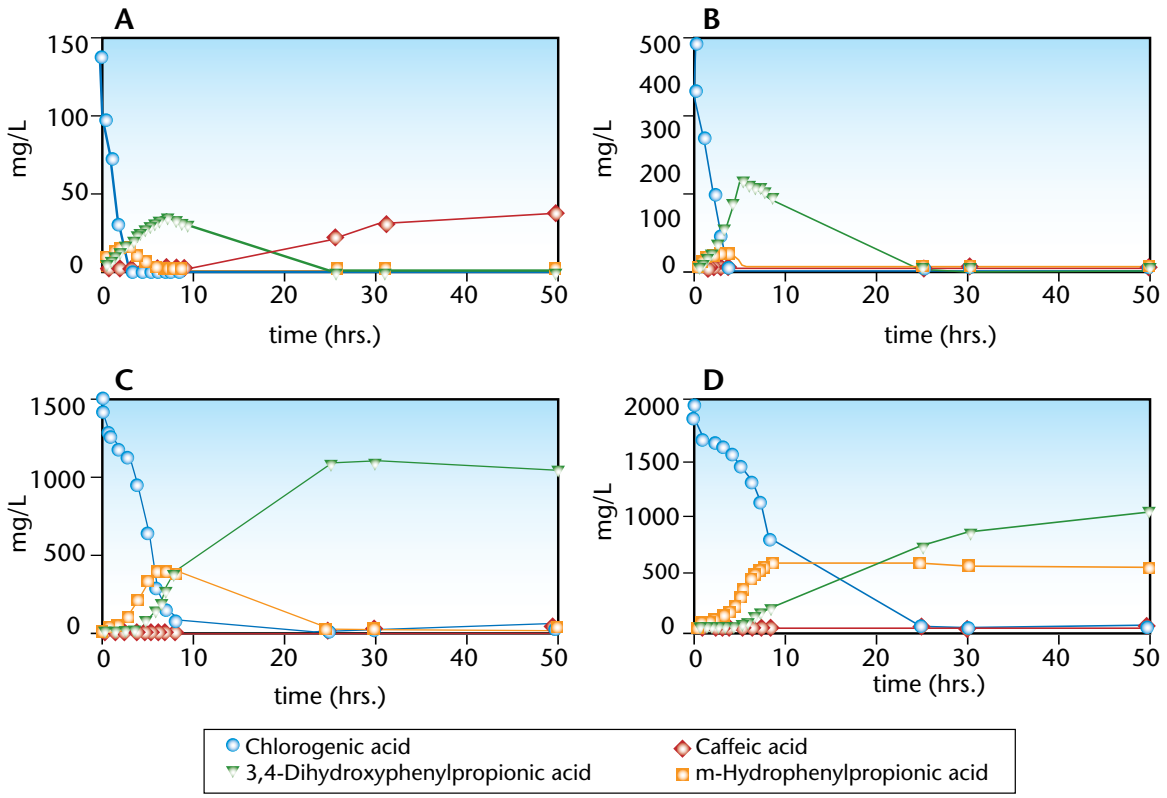


FIGURE 6.33 — **Individual Fecal Bacterial Activity at Different Chlorogenic Acid Concentrations**

The fecal slurry from a single individual was incubated at (A) 125 mg/l, (B) 500 mg/l, (C) 1500 mg/l and (D) 2000 mg/l concentrations of chlorogenic acid. Curves are shown for concentrations of chlorogenic acid and its degradation products, caffeic acid, 3,4-dihydroxyphenylpropionic acid, and m-hydroxyphenylpropionic acid. The conversion of caffeic acid to 3,4-DHPP is highly dependent on concentration, with much greater accumulation of 3,4-DHPP at high chlorogenic acid concentrations. These data may simulate the effect of more vigorous bacterial activity at the same concentration.[443]

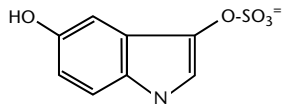
abundant species.[516] The importance of clostridia to urinary product formation is both their abundance and their metabolic diversity.

The full designation of the compound discussed here is 3-(3,4-dihydroxyphenyl)-propionic acid, which we shorten to 3,4-dihydroxyphenylpropionic acid and abbreviate as 3,4-DHPP. The appearance of 3,4-DHPP is highly variable from one individual to the next as shown in Figures 6.32 to 6.33.

Numerous reports have been received of patients with *Clostridium* overgrowth confirmed by stool culture, where elevated levels of 3,4-DHPP have fallen to baseline with Flagyl, but were unaffected by nystatin.[596] Although other organisms may produce 3,4-DHPP, clostridia is the most commonly encountered genera among those susceptible to Flagyl. In vitro studies have confirmed the production of 3,4-DHPP from dietary quinolines by various species of clostridia.[518, 519] Rats excrete 3,4-DHPP when they are fed the naturally occurring flavonoid hesperetin.[520] Depending on the species, clostridia excrete various other organic acids as the end products of aromatic amino acid metabolism.[513] Cytotoxic quinoid metabolites that require glutathione conjugation for removal may be formed from 3,4-DHPP.[521] Various compounds closely related to 3,4-DHPP are also produced by the genus *Clostridium*.[513] In addition, 3,4-DHPP has been found as a product of metabolism of quinoline by *Pseudomonas stutzeri*.[522, 523] Intestinal 3,4-DHPP is degraded by an enzyme produced by *E. coli*, thus helping to insure its survival in the presence of intestinal clostridial growth.[524, 525]

BACTERIAL PRODUCTS FROM TRYPTOPHAN

Indican



Bacteria in the upper bowel produce the enzymes that catalyze the conversion of tryptophan to indole. Absorbed indole is converted in the liver to indoxyl, which is then sulfated to allow for urinary excretion. Indoxyl sulfate (also known as indican) can be measured colorimetrically by conversion to colored oxidation products or directly by liquid chromatography with a UV absorption or mass spectrometric detector.

Because the upper bowel is sparsely populated with bacteria, indican is present in urine at low levels in health. An elevated level of urinary indican is an indication of upper bowel bacterial overgrowth. Certain patients, such as those with celiac disease may be at greater risk.[499] Bacterial overgrowth, utilizing urinary indican, was demonstrated in 8 out of 12 patients following jejuno-ileal bypass surgery.[526]

Oral, unabsorbed antibiotics reduce indican excretion.[527] Indican excretion is also reduced when the gut is populated with strains of *Lactobacillus* at levels above 10^5 organisms/g.[527] *Lactobacillus salivarius, Lactobacillus plantarum,* and *Lactobacillus casei* were more effective in achieving reduced indican than were two strains of *Lactobacillus acidophilus.* In patients with cirrhosis of the liver, tryptophan loading can produce neuropsychiatric manifestations due to intestinal bacterial production of tryptophan metabolites.[528] The symptoms are reduced by antibiotic therapy, demonstrating the bacterial origin of the metabolites.

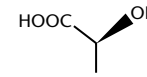
Indican testing can aid in differentiating pancreatic insufficiency from biliary stasis as the cause of steatorrhea (fatty stools).[529] Patients with steatorrhea due to pancreatic insufficiency show a rise of indican from low values to above normal when they are treated with pancreatic enzyme extract.[529] Urinary indican does not rise in patients with steatorrhea not due to pancreatic insufficiency, nor in the normal subjects who receive pancreatic enzymes. This scenario demonstrates how bacterial populations respond to increased concentrations of luminal amino acids. Large shifts in bacterial populations induced by the artificial sweetener, saccharin, have also been demonstrated by changes in indican excretion.[530]

No age adjustment for reference limits is necessary, since excretion has been shown to be constant for young and elderly control subjects.[531] The test sensitivity may be enhanced by oral loading of 5 g tryptophan.[532] The number of false-positives may be reduced by including elevations of other bacterial metabolites with that of indican as criteria of abnormal bacterial colonization of the small intestine.[533]

The interpretation of indican results is complicated by impaired protein digestion, which increases the tryptophan available for bacterial action. Even patients with normal intestinal bacterial populations can show increased postprandial indican excretion when they fail to digest dietary protein. The relationship between increased indican and incomplete digestion might be utilized as a measure of protein digestive adequacy. Indican evaluation has been used to assess intestinal absorption of tryptophan in scleroderma.[534] Increased urinary indican has been shown to correlate with enteric protein loss.[535] Indican elevation has revealed that impaired protein digestion and increased bacterial conversion of tryptophan is a complication of cirrhosis of the liver.[536] Some degree of malabsorption was found in 30% of an elderly population by combinations of indican with the Shilling and other tests.[537]

PRODUCTS OF DIETARY CARBOHYDRATE

D-Lactate



Nanomolar concentrations of D-lactic acid may be produced by human tissues,[538] but it is a major metabolic product of several strains of bacteria that inhabit the human gut.[539] D-lactate is frequently detected in patients with short-bowel syndrome (SBS) due to poor dietary carbohydrate absorbtion because of impaired absorptive regions in the upper small intestine. Many genres of bacteria can convert simple sugars into D-lactate. However, *Lactobacillus acidophilus* is uniquely adapted to withstand the dramatically lowered intestinal pH resulting from massive accumulation of luminal D-lactate and other organic acids. Under conditions of

carbohydrate malabsorption, D-lactate is simultaneously increased in blood and urine.[540] Some D-lactate entering portal circulation can undergo hepatic conversion to carbon dioxide, but this pathway has limited capacity. This limitation is in contrast to the extremely large capacity for metabolism of the L-lactate isomer produced in skeletal muscle and other tissues. With continued increases in intestinal output, rising blood levels are reflected in urinary output of D-lactate.[541] When intestinal production rates exceed the capacity for clearance, D-lactic acidosis is produced.[542] Intestinal symptoms of diarrhea are frequently present due to the disruption of bowel flora.[543, 544]

D-Lactic acidosis due to overgrowth of *Lactobacillus plantarum* was reported in a child who developed an unusual encephalopathic syndrome due to neurotoxic effects of D-lactate.[545] D-Lactic acidosis may be accompanied by any of the various neurological symptoms listed in Table 6.14.[540, 546, 547] Attacks are usually episodic, lasting from a few hours to several days. Direct toxic effects of D-lactate in the brain are suspected.[546, 548]

Jejunoileostomy patients have the highest risk of developing D-lactic acidosis and the accompanying encephalopathy because they usually have some degree of carbohydrate malabsorption.[549,550] Procedures as mild as stomach stapling may lead to D-lactic acidosis.[542] Precipitating factors include use of antibiotics[554] and medium-chain triglycerides.[555] Carbohydrate malabsorption associated with pancreatic insufficiency can also induce D-lactic acidosis.[556] Elevated levels of D-lactate were found in blood samples of 13 out of 470 randomly selected hospitalized patients.[552] Studies in cattle have confirmed that increases in D-lactate following overloading of grain in the diet corresponded to growth of lactobacilli rather than coliform bacteria.[553]

The specificity and sensitivity of urinary D-lactate has led to the test being proposed for routine diagnosis of bacterial infections.[551] D-Lactate has also been reported to be a marker for diagnosis of acute appendicitis,[557] and for differentiating perforated from simple appendicitis.[558] Whatever the origin, patients are managed with antibiotics and probiotics,[559] including *Saccharomyces boulardii*.[540]

During acidotic episodes in patients with SBS, 24-hour urinary excretion of D-lactate may rise to levels above 600 µg/mg creatinine, far higher than concurrent L-lactate concentrations of around 24 µg/mg creatinine.[547] D-Lactic acidosis has also been reported in a patient with chronic pancreatitis and renal failure rather than short-bowel syndrome.[556] Compared to controls, significant elevations of D-lactate were reported for

Table 6.14 — Neurologic Signs & Symptoms in 29 Patients With D-Lactic Acidosis[508]

Symptom	% of Patients
Altered mental status ranging from drowsiness to coma	100
Slurred speech	65
Disorientation	21
Impaired motor coordination	21
Hostile, aggressive, abusive behavior	17
Inability to concentrate	14
Nystagmus	14
Delirium	10
Hallucinations	10
Irritability	3
Excessive hunger	3
Headache	3
Partial ptosis	3
Asterixis	3
Blurred vision	3

Notes:

ischemic bowel, small bowel obstruction, and acute abdomen, with a negative predictive value of 96% and a positive predictive value of 70%.[561]

The phenomenon of D-lactic acidosis has been described as turning sugar into acid in the gastrointestinal tract.[562] D-Lactate is not the only organic acid produced from simple carbohydrates, they are also turned into p-hydroxybenzoate and tricarballylate but those compounds are never absorbed at rates that can produce the systemic effects found with D-lactate. When D-lactate is elevated, supplementation with D-lactate-producing species of *Lactobacillus* is contraindicated, and steps to reduce bacterial populations should be considered. Not all species of *Lactobacillus* produce significant D-lactate, as shown in Table 6.15. Once the carbohydrate excess in the small intestine is controlled, a recommended approach to managing recolonization with probiotic species is to supplement with species that do not produce D-lactate.

Urinary D-lactate reference values of 5.9 and 13.7 µg/mg creatinine for adults and children less than one year old, respectively, have been reported.[546, 547, 563] Studies that have performed simultaneous plasma and urine specimen collections show that urinary

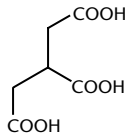
Notes:

TABLE 6.15 — LACTATE ISOMERS PRODUCED BY INDIVIDUAL SPECIES OF LACTOBACILLUS[565]

Producers of Only D(-)-Lactate
Lactobacillus delbrueckii subsp. *delbrueckii*
Lactobacillus delbrueckii subsp. *lactis*
Lactobacillus delbrueckii subsp. *bulgaricus*
Lactobacillus jensenii
Lactobacillus vitulinus
Producers of Racemate DL-Lactate
Lactobacillus acidophilus
Lactobacillus amyiovorus
Lactobacillus aviarius subsp. *aviarius*
Lactobacillus brevis
Lactobacillus buchnari
Lactobacillus crispatus
Lactobacillus curvanus
Lactobacillus formentum
Lactobacillus gasseri
Lactobacillus graminis
Lactobacillus hamsteri
Lactobacillus helviticus
Lactobacillus homohiochii
Lactobacillus pentosus
Lactobacillus plantarum
Lactobacillus reuteri
Lactobacillus sake
Producers of Only L(+)-Lactate
Lactobacillus agilis
Lactobacillus amylophilus
Lactobacillus animalis
Lactobacillus bavaricus
Lactobacillus casei
Lactobacillus mali
Lactobacillus maltaromicus
Lactobacillus murinus
Lactobacillus paracasei subsp. *paracasei*
Lactobacillus paracasei subsp. *tolerans*
Lactobacillus ruminis
Lactobacillus salivarius
Lactobacillus sharpeae
Lactobacillus rhamnosus

concentrations can frequently be 10-fold higher than plasma.[559] An advance in analytical sensitivity has recently been achieved in which a single chiral chromatographic separation allows resolution and low-level accuracy for simultneous, quantitative analysis of D- and L-lactate by tandem mass spectroscopy.[564] Since independent enzymatic methods frequently have varying calibration errors and efficiencies of recovery, the simultaneous determination of both isomers allows more accurate detection of patients predominantly excreting the D-isomer. In summary, urinary D-lactate elevation may predict bacterial overgrowth as a result of: carbohydrate malabsorption, ischemic bowel, certain types of pancreatic insufficiency, acute appendicitis, and surgical procedures that compromise upper gastrointestinal function. Diagnosis and treatment of D-lactic acidosis can significantly improve patient outcomes.

Tricarballylate



Tricarballylate (tricarb) is produced by a strain of aerobic bacteria that quickly repopulates in the gut of germ-free animals.[566] As its name implies, tricarb contains three carboxylic acid groups that are ionized at physiological pH to give a small molecule with three negative charges akin to the structure of the powerful chelating agent EDTA. Magnesium is bound so tightly by tricarb that magnesium deficiency results from overgrowth of tricarb-producing intestinal bacteria in ruminants.[567] This condition, known as "grass tetany," is also accompanied by lower levels of calcium and zinc, all of which can form divalent ion complexes with tricarb (Figure 6.34).

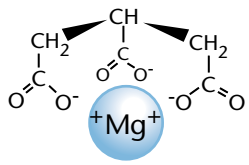
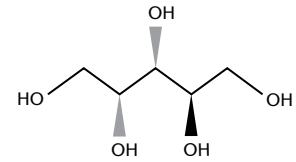


Figure 6.34 — **Tricarballylate-magnesium Complex**

Products of Fungi (Yeast)

D-Arabinitol



D-Arabinitol (DA) is a metabolite of most pathogenic *Candida* species, in vitro as well as in vivo. D-Arabinitol is a five-carbon sugar alcohol that can be assayed by enzymatic analysis. It is important to distinguish the sugar alcohol from the sugar D-arabinose that is unrelated to any yeast or fungal condition in humans. A single report of two autistic brothers who were found to have significant concentrations of arabinose in their urine has led to claims about possible associations of yeast infections and autism,[568] though no further evidence in support of this association has been reported. DA, on the other hand, has long been known to be associated with candidiasis in a variety of clinical situations.[569-571] The enzymatic method using D-arabinitol dehydrogenase is precise (mean intra-assay coefficients of variation [CVs], 0.8%, and mean inter-assay CVs, 1.6%) and it shows excellent recovery of added DA.[572]

Among pathogenic yeasts and fungi, *Candida* spp. are of widest clinical concern, because of their transmission by direct invasion of the gastrointestinal and genitourinary tracts and their ability to rapidly overwhelm immune responses in many hospitalized patients. Most species of *Candida* grow best on carbohydrate substrates. Activities of the enzymes aldose reductase and xylitol dehydrogenase are induced in *Candida tenuis* when the organism is grown on arabinose.[573] The rate of DA appearance in the body equals the urinary excretion rate and is directly proportional to the concentration ratio of DA to creatinine in serum or urine.[574]

Measuring serum DA allows prompt diagnosis of invasive candidiasis.[575] Immunocompromised patients with invasive candidiasis have elevated DA/creatine ratios in urine. Positive DA results have been obtained several days to weeks before positive blood cultures, and the normalization of DA levels has been correlated with therapeutic response in both humans and animals.[576,577] Elevated DA to creatinine ratios were reported in 69, 36,

and 9% of patients with *Candida* sepsis, *Candida* colonization, and bacterial sepsis, respectively.[578] In another study, when patients were divided into categories of superficial candidiasis, possible deep, invasive candidiasis, and definite, deep invasive candidiasis, all three groups showed significant DA elevations.[579] Another group reported highly elevated, slightly elevated, and normal DA levels in 2, 2, and 3 patients, respectively, with superficial *Candida* colonization.[580] Yet a fourth independent group has reported the appearance of DA in both disseminated and simple peripheral candidiasis.[581] The somewhat more discriminating elevated urine D-arabinitol–L-arabinitol (DA/LA) ratio has been found to be a sensitive diagnostic marker for invasive candidiasis in infants treated in neonatal intensive care units. Eight infants with mucocutaneous candidiasis were given empiric antifungal treatment, but had negative cultures; five of these had repeatedly elevated DA/LA ratios. Three infants with suspected, and four with confirmed invasive candidiasis experience normalized ratios during antifungal treatment.[582] The ratio of D- to L-arabinitol in serum reveals the presence of disseminated candidiasis in immunosuppressed patients.[576]

Putative Yeast Markers and Promising Bacterial Markers

Tartarate, citramalate, and other compounds were found at high concentrations in two brothers who had conditions thought to be associated with intestinal yeast overgrowth.[568] However, no evidence has appeared, that supports the contention that tartarate and citramalate are products of intestinal yeast overgrowth. Furthermore, the large dietary intake effects on urinary tartarate were not controlled in the single previous study.[583] D-Arabinitol is the only urinary biomarker of invasive *Candida* sp. overgrowth that has reliable scientific support.

Early studies on bacterial isolates showed that various strains of coliform bacteria can decarboxylate amino acids to their amine forms. Thus, *Bacterium coli* decarboxylated arginine, lysine, ornithine, histidine, and glutamic acids to agmatine, cadaverine, putrescene, histamine, and γ-aminobutyric acids, respectively.[446-450] These data must be viewed with caution, however, because they do not reveal the extent to which the products may be further metabolized by other microbial species in the gut or by human tissues. The amino acid product γ-aminobutyrate is discussed in Chapter 4, "Amino Acids," where no association with bacterial overgrowth in humans is mentioned because of the absence of such observations in the scientific literature. Tyrosine decarboxylation was found to be characteristic of seven strains of *Streptococcus faecalis* that had no activity toward other amino acids.[447]

Actions to Consider for Elevated Dysbiosis Markers

There are multiple negative impacts on nutrient status caused by intestinal dysbiosis. Lowered levels of vitamins B_1, B_2 and B_6, and the minerals magnesium and zinc are found in patients with elevated nascent production of ethanol due to gut fermentation[584] (Figure 6.35). Therefore, evaluation of micronutrient status and selection of appropriate repletion dosages of nutrients is a key to successful management of patients with significant dysbiosis.

Further discussion of stool microbial analysis and other methods for GI function testing is found in Chapter 7, "Gastrointestinal Function." Although species identification is not possible from the limited number of urinary markers currently detected, and no antibiotic sensitivity testing can be done, the information available is sufficient for clinical decisions about appropriate interventions. The susceptibilities of various bacteria known to reside in the upper jejunum to five antibiotics are shown in Table 6.16. Many bacteriostatic herbal preparations and natural compounds inhibit growth of bacteria and yeast. A summary of interventions commonly used is presented in Table 6.17. Additional insight may be gained from studies of the antibiotic sensitivities of organisms present in cases of small intestinal bacterial overgrowth syndrome. Since the specimen

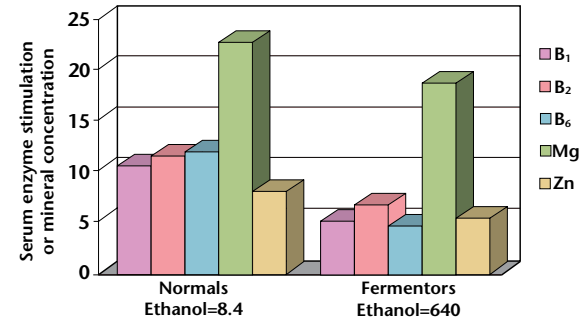


FIGURE 6.35 — Nutrient Deficiency Due to Gut Fermentation

Table 6.16 — Antibiotic Sensitivities of Upper Jejunal Bacterial Strains in Small Intestinal Bacterial Overgrowth Syndrome[532]

		Antibiotic Sensitivity (% of Susceptible Strains)				
Bacteria	**Prevalence (%)**	**Amoxicillin**	**Amoxicillin-Clavulanic Acid**	**Cephalothin**	**Erythromycin**	**Trimethoprim-sulfamethoxazol**
Microaerophilic	100					
Streptococcus	71	85	85	80	57	43
E. coli	69	63	76	76	?	?
Staphylococcus	25	38	62	69	46	100
Micrococcus	22	75	75	75	87	50
Klebsiella	20	0	100	100	?	100
Nisseria	16	100	100	100	100	30
Proteus	11	100	100	100	?	100
Acinetobacter	9	0	0	0	?	100
Enterobacter	7	0	0	0	?	100
Anaerobic	93					
Lactobacillus	75	89	100	?	72	28
Bacteroides	29	16	100	16	42	63
Clostridium	25	90	100	?	52	52
Veillonella	25	89	92	?	13	61
Fusobacterium	13	80	100	?	73	20
Peptostreptococcus	13	80	90	?	60	5

used for the data in Table 6.17 was taken in the upper jejunum,[585] the data represent only the proximal part of the small bowel where microaerobic species are most likely to persist. The more distal regions of the jejunum and the entire ileum are less favorable for aerobes.

Since the microbial compounds that appear in urine originate in the lumen of the gut, oral sorbents that bind them and prevent their absorption can be used. This approach is especially helpful for those compounds of high toxicity like cresol. The absorbent, AST-120 (3–7 g/d), has been shown to effectively lower p-cresol accumulation in uremic patients.[500,586] Uremic coma and bleeding tendency is attributed to the accumulation of p-cresol, and p-cresol is a co-carcinogen in the mouse skin test. The fact that an oral sorbent lowers levels of cresol in uremia demonstrates that the gut is a significant source of the toxin. Activated charcoal may be used for this purpose, but there is greater tendency for constipation than with AST-120. The generally sound advice of using a high-fiber diet rich in legumes and other whole foods is an effective way of achieving lowered transit times and less exposure to intestinal toxicants.[587-589] Fiber simultaneously encourages favorable colonic species that degrade cellulose. Because of the increased microbial metabolism, dietary fiber can increase intestinal short-chain fatty acids and lactate in urine.[590]

Fasting is an effective way to reduce microbial populations in the gut.[591] If the resident microbes do not receive a relatively constant infusion of substrate for growth, such as carbohydrates or amino acids, they die. A discussion of the various clinical ramifications

Notes:

of fasting is beyond the scope of this book. Although many patients may be candidates for fasting,[592] the vast majority are reluctant to apply the discipline required to properly execute severe food restriction.

Regular oral dosing with organisms favorable to the human gut (probiotics) and use of substrates that encourage their growth, such as dietary fiber and fructo-oligosaccharide (pre-biotics) are generally implemented to achieve long-term control of intestinal microbes.[593] When aggressive intervention is warranted, use of amoxicillin-clavulanic acid is a suitable candidate, because offending microaerophilic bacteria are very susceptible to its antibacterial action, and the anaerobic populations decline as well, possibly due to the more oxygen-rich environment produced by declining populations of the oxygen-consuming species. The rising oxygen content can have bactericidal effects on strictly anaerobic species.

TABLE 6.17 — INTESTINAL OVERGROWTH INTERVENTIONS

Class of Intervention	Examples
General	Encourage high fiber diet, remove mucosal irritants such as allergenic or IgG-positive foods, alcohol, and NSAIDs
Antibacterial	Pharmaceutical: Amoxycillin/Clavulanic Acid
	Natural: Goldenseal or other berberine-containing herbs, citrus seed extract, garlic, uva ursi, aloe vera, glycyrrhiza, olive leaf extract, garlic
Anti-fungal	Pharmaceutical: Nystatin
	Natural: See natural antibacterials plus oil of oregano, undecylenic acid and caprylic acid
Anti-protozoal	Pharmaceutical: Flagyl
	Natural: See natural antibacterials
Probiotic	*L. acidophilus, L. sporogenes, Bifidobacteria* sp., *S. boulardii,* soil organisms
Prebiotic	Fructo-oligosaccharide (FOS), increase use of raw and cooked vegetables
Mucosal regeneration	Glutamine, pantothenic acid, deglycyrrhizinated licorice, slippery elm, oligopeptide preparations

Notes:

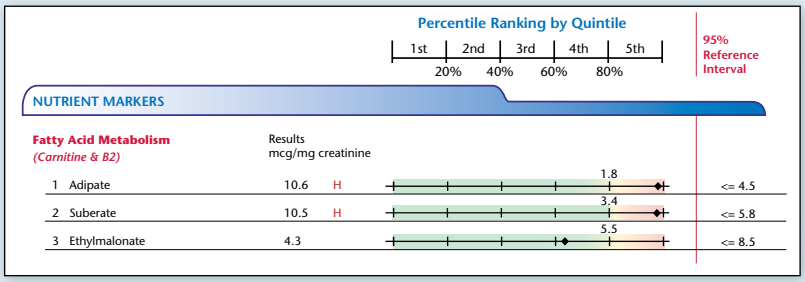
Case Illustrations

Case Illustration 6.1 — Carnitine Insufficiency in Schizophrenia

This data is from a woman who had her first psychotic episode at 21-years-old. She presented for this metabolic profile twelve years later exhibiting a severe form of schizophrenia. Her plasma amino acid profile shows very low lysine along with low values for the branced chain amino acids (BCAA). A urinary organic acid profile done at the same time showed positive biochemical markers for carnitine insufficiency. The results justify essential amino acid therapy with a custom formula that enhances lysine and BCAA levels. Initial use of carnitine can help to assure metabolic demand for energy production from fatty acid oxidation is met while lysine status is enhanced gradually by twice daily dosing of free form amino acids.

Note that the long chain fatty acid metabolism makers, adipate and suberate are very high while the marker derived largely from leucine catabolism is only in the 4th quintile, presumably because of the concurrent depleted status of leucine exhibited in the amino acid data. ❖

(See also Chapter 4, Case Illustration 4.6, Essential Amino Acid Support in Schizophrenia.)



Case Illustration 6.2 — α-Ketoglutarate Dehydrogenase Deficiency[139]

Profound alpha-ketoglutaric aciduria was found in a patient with progressive extrapyramidal tract disease. The 4½-year-old boy was referred for evaluation of chronic, relentless and progressive loss of milestones. The neurological impairment was observed to be primarily motor with a lesser degree of behavioral and cognitive delay. Development had been normal until an infection at 1.5 years of age that resulted in seizures 1 to 2 times daily that were treated with valproic acid. His speech was normal, but he had difficulty climbing up and down stairs and throwing a ball. Another infection at age 3 resulted in spasticity of the lower, and then the upper extremities, along with ataxic gait. Three months later another upper respiratory infection with fever, diarrhea and vomiting resulted in increased tremors in extremities, difficulty swallowing and inability to walk.

Urine organic acid excretion (mcg/mg creatinine)

Compound	Patient	Reference Limit
Lactate	458	25
β-Hydroxybutyrate	108	—
3,4 Dihydroxybutyrate	23	166
Glycolate	36	103
Adipate	67	12
Aconitate	**174**	**< 2**
Citrate	609	770
α-Ketoglutarate	**417**	**< 2**
α-Hydroxyglutarate	94	16
p-Hydroxyphenylacetate	64	28

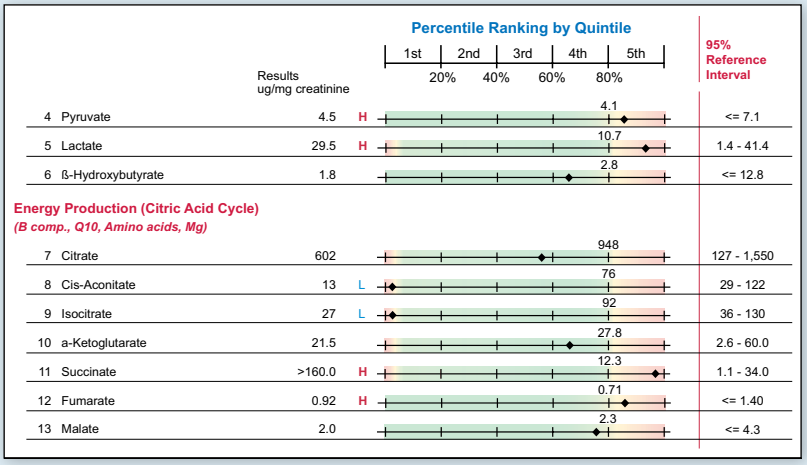
His urinary organic acid profile is shown. The bold fonts emphasize the profound *cis*-aconitate and α-ketoglutarate elevations. Moderately elevated adipate, β-hydroxybutyrate, and p-hydroxyphenylacetate are also found.

The activity of α-ketoglutarate dehydrogenase in fibroblasts was found to be ~27% of normal, revealing the major metabolic lesion in this case. Normal response to thiamin pyrophosphate was demonstrated, allowing elimination of thiamin insufficiency as a reason for the low enzyme activity. The absence of citrate elevation shows the responsiveness of citrate synthase to the high concentrations of downstream products, controlling even more severe accumulation of the pathway intermediates.

After the patient was placed on thiamine 25 mg/kg/d, biotin 80 µg/kg/d, L-carnitine 50 mg/kg/d, riboflavin 15 mg/kg/d, and the antipsychotic, thioridazine 15 mg/kg/d, he was stabilized and discharged to a chronic care facility. ❖

Case Illustration 6.3 — Apparent Succinate Dehydrogenase Deficiency

This report presents an unusual pattern of very low isocitrate and very high succinate. The degree of succinate elevation (too high to measure) is suggestive of an inborn error of metabolism. The low levels of precursors, *cis*-aconitate and isocitrate, as well as failing to find low downstream products, fumarate and malate tend to be confusing. However, the citric acid cycle is not an isolated biochemical pathway. The intermediates may be drawn from multiple sources as shown in Figure 6.1. *cis*-Aconitase is inhibited by elevated succinate, limiting precursor formation and restricting the further accumulation of succinate. Note the elevations of pyruvate and lactate that have also been reported in cases of diagnosed succinate dehydrogenase deficiency.

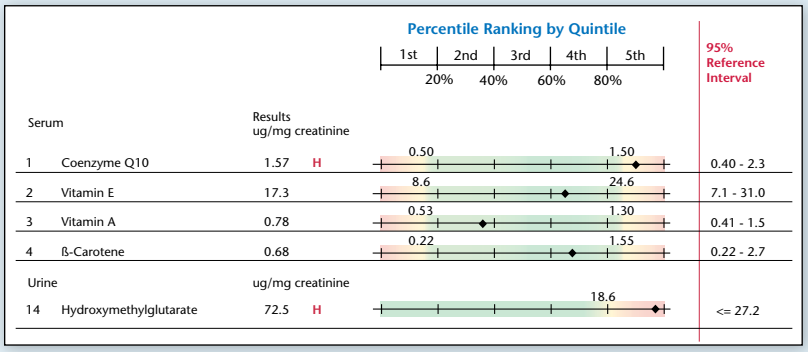


Of course we would expect rather profound clinical effects if succinate dehydrogenase is significantly impaired because this enzyme is otherwise known as Complex II in the electron transport system of the mitochondrial membrane.

Succinate dehydrogenase deficiency is known to cause encephalomyopathy in childhood and optic atrophy or tumors in adulthood.[148] Some variants respond to riboflavin and CoQ_{10}. ❖

Case Illustration 6.4 — Signs of HMG-CoA Lyase Deficiency in Autism

The laboratory results shown below were found in a 3-year-old boy with autistic features. No supplementation of coenzyme Q_{10} had been done to explain the slightly elevated serum CoQ_{10}.

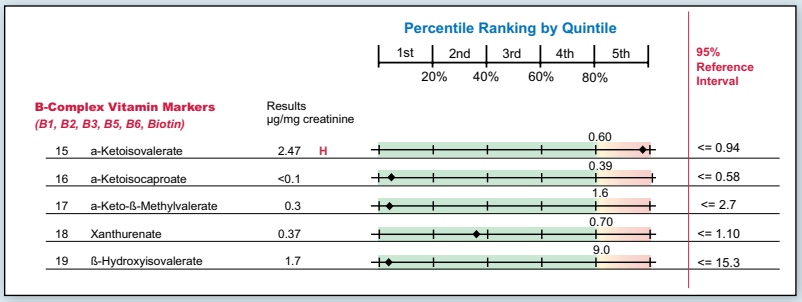


The urinary organic acid profile showed an extremely elevated level of hydroxymethylglutarate (HMG), indicating a potential deficiency of HMG-CoA lyase. The elevated HMG can accelerate the biosynthetic pathway leading to CoQ_{10} and cholesterol, although that pathway is tightly controlled in most individuals. Serum cholesterol was found to be 164 mg/dL in this 3-year-old.

This situation is in contrast to that seen when statin drugs operate to inhibit HMG-CoA reductase preventing the conversion to mevalonate and cholesterol. In that scenario, CoQ_{10} biosynthesis is also inhibited producing lower serum levels. ❖

Case Illustration 6.5 — Specific Ketoisovaleric Aciduria as a Sign of Genetic Abnormality

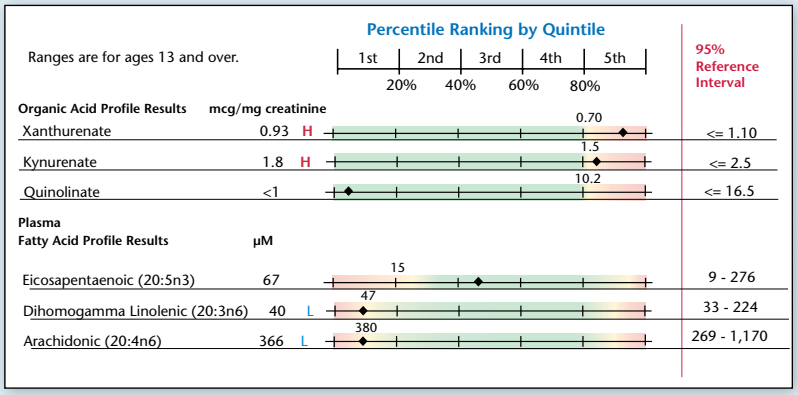
This 10-year-old male has an extreme aversion to cold environments and his urine was producing foam. A urinalysis showed greatly elevated total protein and moderately elevated creatinine. His serum BUN and creatine was also elevated. A urinary organic acid profile revealed severe α-ketoisovaleric aciduria with 1st quintile α-ketoisocaproate and α-keto-β-methylvalerate as shown in the inset. Such an imbalance of the branced chain amino acids indicates factors other than thiamin or general B-complex vitamin insufficiency that would affect all of them. Thus, the results support a presumptive diagnosis of genetic polymorphism of the isovaleryl-CoA dehydrogenase enzyme.



A low protein diet is indicated along with supplementation of carnitine and glycine. A trial of thiamin supplementation effect on urinary α-ketoisovalerate will establish whether the enzyme variant in this patient is responsive to cofactor concentration. Plasma amino acids may be monitored to assess essential amino acid adequacy. ❖

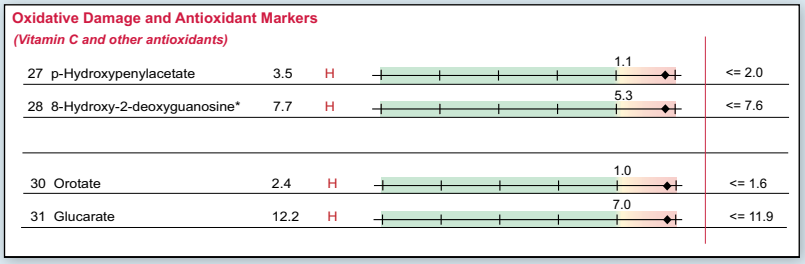
Case Illustration 6.6 — Undetectable Quinolinate

A 67-year-old woman who had exhibited chronic signs of thyroid insufficiency with refractory responses to various administration of thyroid hormone was tested for urinary organic acids and plasma fatty acids. She exhibits orange coloration of the skin suggesting failure of β-carotene conversion to vitamin A which is stimulated by thyroxine. Testing confirmed an elevated β-carotene along with low normal vitamin A. Her urinary quinolinate was less than the quantitative limit of detection (< 1.0 µ/mg creatinine). This result is emphasized by the elevation of xanthurenate and kynurenate, metabolites produced in the hepatic kynurenin pathway. Insufficiency of inflammatory cascade initiators such as the eicosanoid products of essential fatty acids could lead to poor cytokine stimulation of the neuronal kynurenin pathway activity (see Figure 6.15). The data has some support for such a guess. This patient's levels of polyunsaturated fatty acids show an unusual pattern of mid-normal omega-3 levels together with low levels of omega-6. The dominant omega-6, series 2 eicosanoid precursor, arachidonic acid, and the series 1 precursor, dihomogammalinolenic acid, are below their first decile cutoff values. This pattern suggests that inflammatory cascade initiators may be insufficiently available to maintain normal regulatory cytokine control. The thyroid gland is responsive to eicosanoid signaling mechanisms via cyclooxygenase enzymes present in thyroid epithelial cells.[594] The laboratory data shown suggests that this patient is a candidate for aggressive supplementation with vitamin B6 and oils rich in gamma-linolenic acid which may assist in increasing DGLA and AA. A positive response in thyroid status would be supportive of the hypothesis. ❖



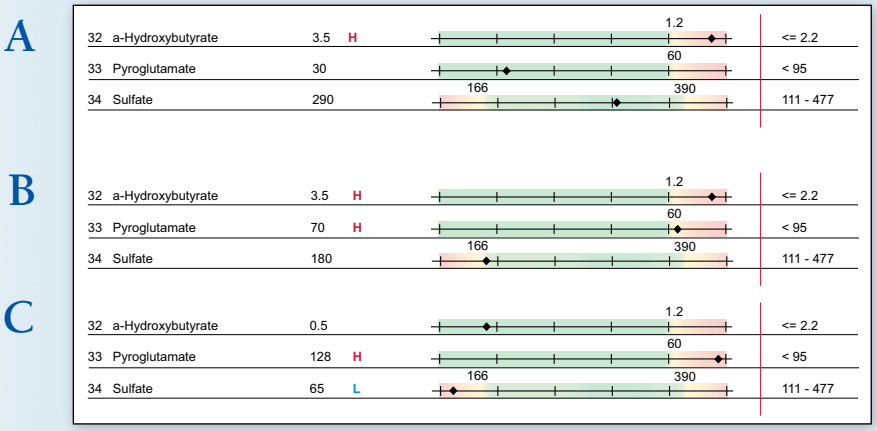
Case Illustration 6.7 — Redox Stress in COPD

This 72-year-old female has chronic obstructive pulmonary disease, emphysema, severe fatigue, congestive heart failure and is a former smoker. She has been on prescriptions of Mucinex, Actonel, Spiriva, Coreg and Xanax The organic acid profile shows elevated HPLA and 8-OHdG, indicating stimulation of cell division and oxidative stress. Concurrent orotate elevation indicates difficulty with meeting hepatic demands for ammonia clearance through the urea cycle. The high glucarate signals stress on the hepatic glucuronidation pathway. Combination therapy with high antioxidant-content foods and supplements and arginine stimulation of urea cycle activity is suggested by the data. ❖



Case Illustration 6.8 — Three Glutathione Demand Scenarios

These three patterns represent the situation that might be found in patients with mild, transient glutathione demand (A), moderate threat of glutathione depletion needing glycine support (B) and chronic, severe glutathione deficiency where low sulfate suggests poor total-body glutathione status and first quintile α-hydroxybutyrate indicates inability to up-regulate glutathione synthesis (C). Fasting plasma amino acid profiles provide very helpful additional information about these patients abilities to sustain methionine and taurine sulfur-containing amino acid levels. ❖



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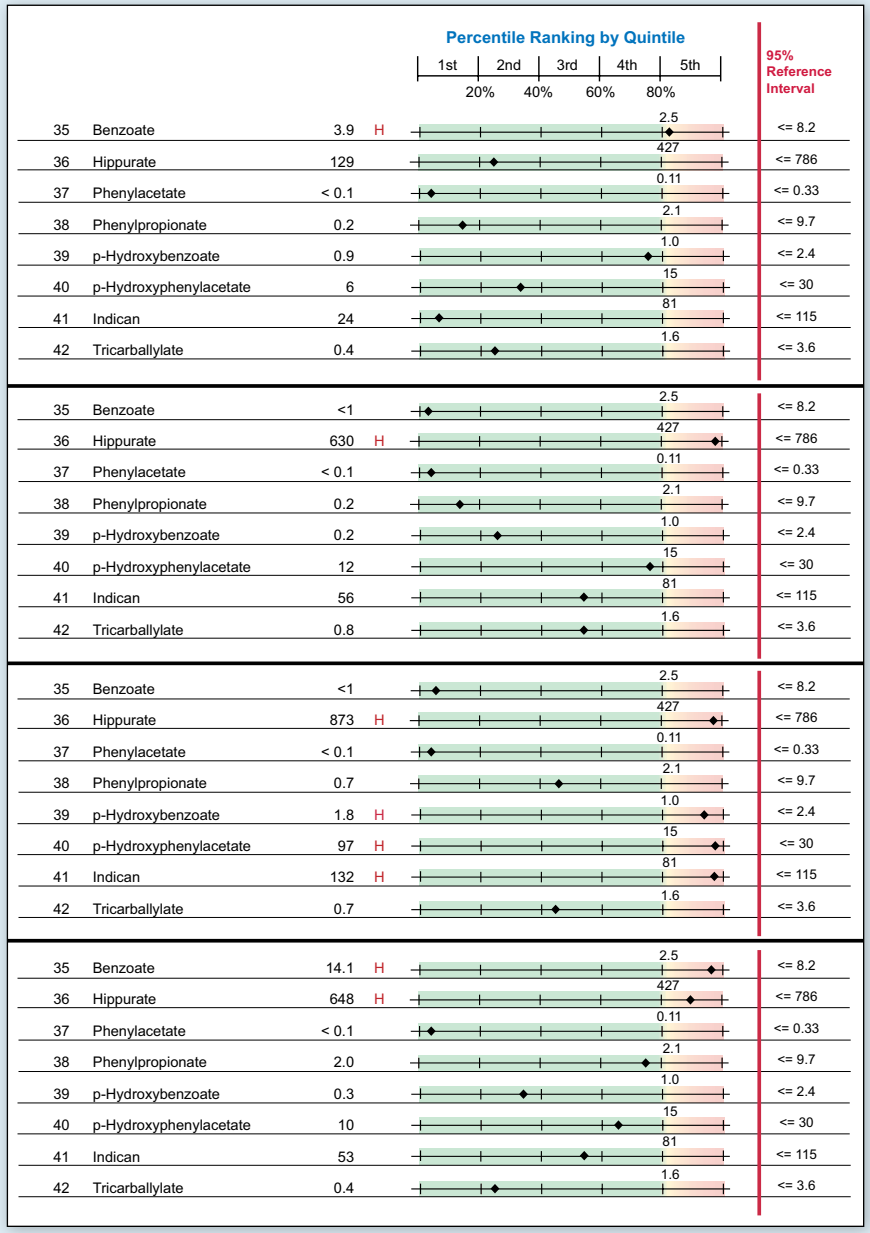
Case Illustration 6.9 —
Scenarios for Abnormal Benzoate and Hippurate



A- Detoxification Phase II glycine conjugation insufficiency without bacterial overgrowth—only benzoate high.

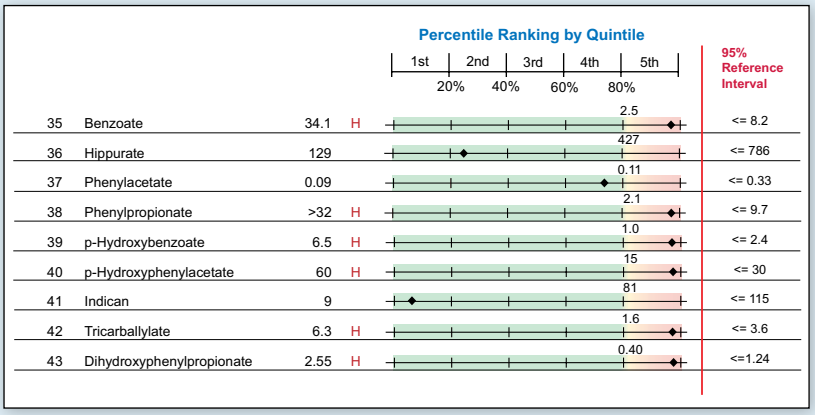
B- Normal glycine conjugation with absence of bacterial overgrowth—only hippurate high. Bacterial origin of the hippurate cannot be ruled out, but dietary benzoate is the more common explanation.

C- Normal glycine conjugation with presence of bacterial overgrowth—hippurate plus other bacterial markers high.

D- Insufficient glycine conjugation with absence or restricted type of bacterial overgrowth—benzoate and hippurate high. The more severe levels raise the probability that specific bacteria overgrowth is contributing benzoate and hippurate, even though other products of polyphenol metabolism are not abnormal. ❖

Case Illustration 6.10 —
An Unusual Microbial Phenyl Compound Pattern

This 58-year-old woman has a pattern of multiple elevations for the phenyl bacterial products in her urinary organic acids profile. Phenylpropionate is greatly elevated, indicating lack of medium-chain acyl-CoA dehydrogenase. Even in the absence of this enzyme, however, glycine conjugation enzymes should form the glycine conjugate, 3-phenylpropionyl glycine for elimination. The pattern shows great elevation of benzoate and low normal hippurate that only occurs when the glycine conjugation system is severely compromised (by a second genetic polymorphism in most cases). The data indicate the presence of dual genetic defects affecting MCAD and glycine conjugase enzymes. Further complicating the picture is the unique absence of phenylacetate, indicating that bacterial species with ability to produce this compound are strongly suppressed.



Regarding phenylacetate: Although elevated urinary phenylacetate (PA) may be ascribed to the presence of intestinal bacterial growth, a distinctly different indication may be drawn from finding abnormally low levels. Since the section of the laboratory report shown here is presenting the group of bacterial markers, it does not have low limits for PA. Methods that are optimized to accurately measure PA in the low physiological range can reveal cases of abnormally low values.

Mammalian brain can convert phenylalanine to 2-phenylethylamine (PEA)[597], and PEA is degraded to phenylacetate in the liver.[598] Wine is a notable dietary source of PEA formed by *Lactobacillus brevis* or *L. hilgardii* during fermentation.[599] PEA is a neuromodulatory agent that induces dopamine release. Combined dosing of mice with PEA (100 mg/kg, i.p.) and l-deprenyl (10 mg/kg, s.c.) produces stereotypic behavior, following which brain dopamine levels are reduced.[600] Urinary PA was found to be low in unipolar depression[601] and mania.[602] In a separate study, patients with severe depression (Hamilton Depression score of 17 or higher) were shown to have lower plasma and urinary PA levels than those with less severe scores.[603] These studies provide a basis for the elevation of mood by supplemental phenylalanine. Raised PA excretion has also been proposed to explain the antidepressant effects of exercise.[604] Thus, in addition to the potential protective effects of PA against cancer as discussed in this chapter, the compound has further potential as a marker that shows metabolic effects of PEA on mood and behavior. In both instances, increased risk is indicated by abnormally low urinary PA. ❖

Notes:

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Chapter 7

Gastrointestinal Function

David M. Brady, J. Alexander Bralley and Richard S. Lord

CONTENTS

Notes:

Gastrointestinal Impact on Nutrient and Toxicant Status

Proper gastrointestinal (GI) function is critical to adequate nutritional status and can impact all aspects of body function. Approximately one-third of daily caloric expenditure is required to drive the digestive, assimilative and immune functions while maintaining the gastrointestinal tract.[1] A large amount of the body's total lymphatic tissue is located in the gut, and the gastrointestinal system is the only organ system of the body with its own independently working lymphatic and nervous systems. Only a system of prime importance to overall health would have such a large number of total-body resources dedicated to it.[2-6]

Failures of the gastrointestinal system manifests as various digestive diseases, such as gastroesophageal reflux disease (GERD), irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), non-alcoholic

Table 7.1 — Summary of Laboratory Evaluations for Gastrointestinal Function

GI Aspect	Function	Testing	Abnormal	Intervention
Stomach	Gastric acid, Pepsin	Heidelberg capsule Direct pH readings	↓ pH	– Mucosal building protocol – Betaine HCl – Free-form amino acids (see Chapter 4, "Amino Acids") – B-vitamins – Trace elements (see Chapter 3, "Nutrient and Toxic Elements")
		Indirect indicators	Multiple ↓ trace elements or amino acids	
Pancreas	Protease	Fecal chymotrypsin	↓ Activity	Pancreatic replacement enzymes (proteolytic, lipolytic and amylytic) and essential fatty acids
		PABA index	↓ Index	
	Lipase	Plasma fatty acids	↓ PUFA	
		Fecal fats	↑ Fat	
Liver/ Gallbladder	Bile acid secretion	Fecal fatty acids	↑ Fatty acids	Ox bile, choleretic herbs (milk thistle) and essential fatty acids
Small intestine	Absorption	Schilling test	↓ Urinary B_{12}	B_{12} by injection or ≥ 1,000 µg/d sublingual
		Lactulose-Mannitol challenge	↓ Urinary mannitol	Mucosal restoration
		Fasting plasma amino acids	Multiple low values	Essential amino acid mixtures
		Food-specific IgG	Multiple elevations	Food elimination/Rotation diets
Colon	Water resorption, Microbial containment	Fecal butyrate or other SCFA	↓ Butyrate	Increase dietary fiber
			↑ Isobutyrate	Butyrate enemas
Immune barrier	Glycocalyx antigen binding Allergy-antigen elimination	Serum, urinary or fecal IgA	↑ Food-specific IgA	Eliminate offending antigens
		Serum IgE	↑ Total IgE	Immune-support nutrients such as *Glycerrhiza glabra* (licorice) root or L-glutamine 3,000–6,000 mg daily
Physical barrier	Regulate nutrient admission and restrict toxicant and microbial access	Serum IgG	Many + foods	Eliminate + foods by group (Rotation Diet) Add free-form amino acids and glutamine Zinc 50–100 mg/d, B_5 100–200 mg/d
		Lactulose-Mannitol challenge	↑ Urinary Lactulose	Eliminate + foods
			↓ Mannitol	Mucosal restoration
Microbial populations	Normal: nutrient delivery	Urinary metabolic markers	↑ Bacterial markers	Herbal or pharmaceutical antibioitics (e.g., berberine alkaloids, etc.)
	Pathogen: toxin production	Hydrogen-Methane breath test	↑ Protozoal markers	Prebiotics and probiotics with antiprotozoals
			↑ Yeast markers	Restrict simple sugars with antifungals
			↑ Expired gases	Herbal or pharmaceutical bacteriostatic agents
		Stool microbial DNA quantititation or culture & sensitivity	↑ Growth	Specific antibiotics

steatohepatitis (NASH) and colorectal cancer.[7–9] Disorders of atopic immune origin, such as allergy and asthma, or of autoimmune dysfunction, such as rheumatoid arthritis, type 1 diabetes, autism and Hashimoto's thyroiditis can originate in gastrointestinal disturbances.[10–12] Morbidity and mortality from these diseases can be reduced by identification of antecedent gastrointestinal dysfunction.

Every disorder that pertains to the gut has the potential to manifest as a factor in nutritional deficiencies. The tests described in this chapter reveal the functional status of the organs required for the digestion and assimilation of food and some of the toxicologic and immunologic consequences of failure to properly carry out those functions. The subjects of leaky gut and the associated immunologic challenge are discussed with special reference to ways of testing for clinically relevant factors. Testing also is discussed relevant to stomach, pancreatic, hepatic and intestinal function. The detection of abnormal microbial growth within the gut and the impact of the various toxins produced by their growth is an important and relatively new aspect of gastrointestinal assessment, especially in the analysis of urinary compounds that reveal the presence of various classes of microbes.

From our embryologic beginnings, the inside surface of our mouth, esophagus, stomach and intestines—everything we call our digestive tract—is contiguous with the outside surface of our bodies that we call our skin. Thus, the epithelial surfaces of our skin and digestive tract face outward, serving as barriers to the outside world. In the GI tract the outside world is made up of things we ingest, chemicals that our bodies secrete to act on them or secretions that simply need to be removed from the tissues, all of which support and, to some extent, govern the growth of a large microbial mass. Hundreds of species of bacteria, protozoa and fungi occupy every region of the GI tract in numbers that range from a few hundred to a several million per gram of content. This subject is considered in more detail later in this chapter. Various types of breach in the gastrointestinal barrier frequently become underlying factors in chronic illness.

The proper function of the GI tract depends to a large degree on a myriad of autonomic processes that regulate peristalsis and coordinate it with the opening and closing of the appropriate sphincter valves. These actions control the unidirectional movement of food through the gut at a pace slow enough to allow for proper absorption, but fast enough to continuously bring new nutrients as needed by the body. Digestive secretions, formed principally in response to the presence of food in the GI tract, vary according to the amount and type of food present. The amount of each digestive secretion must be accurately modulated to provide just the amount required for proper digestion. The nervous system that regulates these activities (the enteric nervous system) is to a large degree independent of the brain, although it responds to signals from the sensory organs and the immune system.

Large amounts of digestive secretions are required to process the kilograms of food consumed daily. Based on a typical dietary composition of 55% carbohydrate, 15% protein and 30% fat, a standard 2000 calorie diet delivers 275 g, 75 g, and 67 g, respectively of the macronutrients plus 2 or more liters of water. Establishing a clear picture of a patient's functional adequacy for extracting usable nutrients from the mass of food is a challenge for which laboratory evaluations offer only partial assistance. The principal difficulty is that direct sampling of intestinal contents is impractical for routine clinical practice. Analysis of specimens such as peripheral blood, urine and feces may give results that indirectly reflect difficulties with digestion and absorption. Due to the critical role of the gastrointestinal tract in nutrient supply, even partial answers can be of great value. Invasive procedures for observation or luminal specimen retrieval can be highly informative, but they can be performed only by trained specialists and are often expensive and uncomfortable. Such procedures are beyond the scope of this book. This chapter will deal with non-invasive laboratory evaluations of gastrointestinal function as summarized in Table 7.1.

Notes:

THE STOMACH

Standard medical treatments focusing on the gastrointestinal tract most often involve treating stomach issues. Proton pump inhibitors, histamine-2 (H_2) receptor antagonists and other types of antacid drugs are among the most frequently prescribed medications. The use of these medications can generate other risk factors originating from altered GI function, including bacterial overgrowth,[13, 14] interstitial nephritis[15] and hip fracture.[16] By frequency of medical treatment and by scope of effects, the stomach becomes primary for evaluating gastrointestinal function. The primary function of the stomach is receiving and retaining food for the secretion of gastric acid to initiate the digestive process.

Gastric secretion of hydrochloric acid (HCl) is essential for proper assimilation of elements such as calcium and zinc, digestion of protein (proteolytic activity of pepsin requires a pH of less than 5) and for adequate sterilization of stomach contents. Low pH is also required for proper release of vitamin B_{12} from food sources. When stimulated, the parietal cells of the stomach secrete an isotonic solution containing enough HCl to make the gastric fluid pH less than 1 (see Figure 7.1). At this extreme acidity, the hydrogen ion concentration of gastric fluid is about 3 million times greater than that of arterial blood. It takes a tremendous amount of cellular energy to maintain this gradient because of the high activity of membrane pumps utilizing ATP. Perhaps it is not surprising that problems of intestinal absorption are often due to inadequate secretion of HCl.[17] Depletion of B-complex vitamins may be the precipitating event leading to reduced energy production in cells of the gastric pits. Patients over 50 years of age, who are particularly prone to inadequate secretion of HCl (hypochlorhydria) and intrinsic factor because of degenerative gastric disorders (e.g., atrophic gastritis), are frequently deficient in vitamin B_{12}.[18]

Symptoms of inadequate stomach acid are similar to those of excess acid and are frequently mistaken.

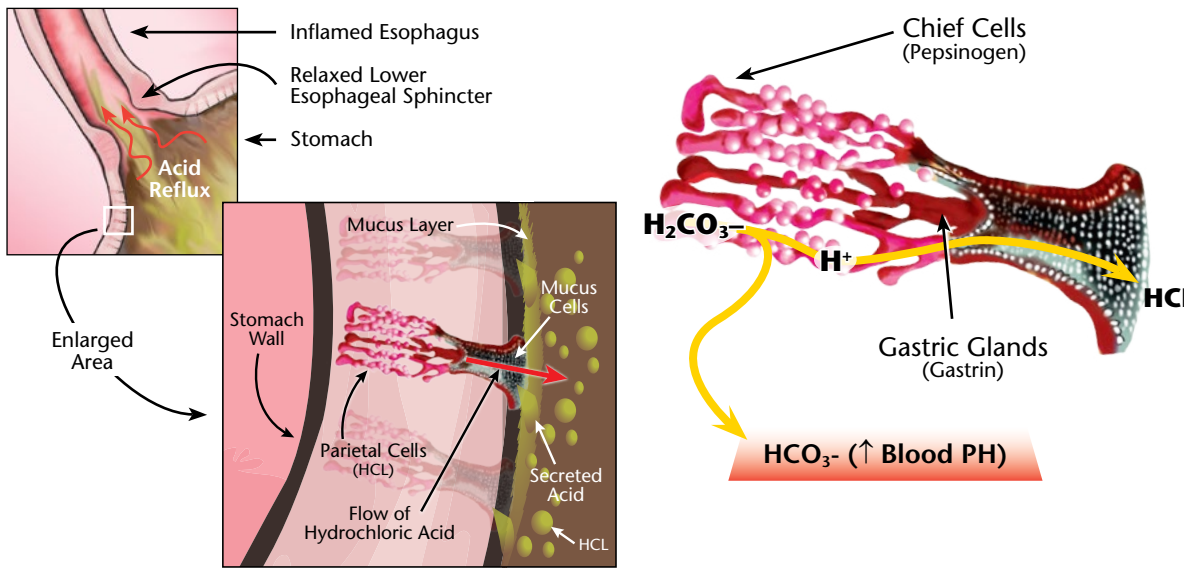

FIGURE 7.1 — **The Gastric Pit**

Overloading the stomach and failure of the lower esophageal sphincter to close cause reverse flow of stomach acid into the unprotected lining of the esophagus. Failure of the pyloric valve to close causes premature emptying and maldigestion. Protein digestion is interrupted and elemental absorption is impaired when food is not adequately acidified. Blocking of stomach acid secretion by pharmaceutical agents designed to prevent heartburn and to treat stomach ulcers can easily cause such interruption of digestion. Laboratory profiles of amino acids and essential elements frequently show low levels of essential amino acids and essential trace elements, respectively, that are warning signs of the long term effects of insufficiency of these critical nutrient factors. See Chapter 3, "Nutrient and Toxic Elements" and Chapter 4, "Amino Acids" for discussion and illustration of those abnormalities.

Upper abdominal burning sensations associated with mealtime are reported in both cases. Excessive acidification of stomach contents leads to burning sensation from exposure of the lower esophagus to highly acidic chyme. With inadequate stomach acid production, regurgitation of chyme into the lower esophagus causes such burning pain. In these cases, advising antacids is contraindicated and counterproductive because they neutralize the acid that is produced, further interfering with digestion. Long-term effects of inadequate stomach acid are much more difficult to study. However, it is now established that absorption of calcium is decreased[19] and subsequent risk of hip fracture is significantly increased in patients who have used proton pump inhibitor medications for one year or more.[16] These results indicate that absorption of other elements is similarly affected. See Chapter 3, "Nutrient and Toxic Elements," for discussion of element dependence on stomach acid.

Stomach acid secretion is a principal line of defense against infection of the GI tract.[20] The proteolytic enzyme activity and low pH in normal gastric secretions kill most of the bacteria and parasites that contaminate food. Chronic hypochlorhydria, whether induced by habitual use of antacids or due to gastric disorders, increases the risk of infection and intestinal microbial overgrowth.[21] The risk of infection is multiplied when hypochlorhydria simultaneously reduces zinc and other trace elements critical for immune system function. Patients with malabsorptive disease, in particular, are at risk of various mineral deficiencies. Inhibition of gastric acid secretion reduces absorption of most trace elements[22] because gastric acid serves to release minerals from their complexed native forms in the stomach.[23] Variations in stomach acid production can explain why the absorption efficiency for dietary calcium varies widely from individual to individual, ranging from 15 to 45%, even with adequate calcium intake. Thus, some individuals seem inherently to be better absorbers of calcium.[24]

Determination of Stomach Acid

The Heidelberg capsule test is considered to accurately assess stomach acid, though it is time consuming for the patient, taking up to 90 minutes to complete.[25] It will be described here because this type of test can give definitive answers about the adequacy of gastric acid secretion. The test uses a tiny plastic encapsulated pH probe that is swallowed by the patient. The capsule, small enough to safely pass the circuitous course of the GI tract, contains a miniature radio transmitter that continuously measures gastrointestinal pH and transmits the data to a waistband antenna connected to a bedside receiver. The pH readings are recorded for a permanent record. The capsule can either be tied to a thin string for retrieval or swallowed untethered. The latter approach allows additional measurements of upper intestinal pH to be gathered. After swallowing the capsule, pH readings typically start around 7.0, and then drop toward 1.0 as the capsule settles toward the stomach bottom. The patient then drinks a challenge solution consisting of concentrated sodium bicarbonate (baking soda), which has strong buffering capacity. Within half a minute, the pH will normally rise to approximately 7. If acid secretion is normal, the pH will fall again, returning to between 1.0 and 2.0 within 20 minutes. The challenge solution is given again and repeated up to four times, as long as the pH response time is less than 20 minutes. Hypochlorhydria is indicated for a patient requiring more than 20 minutes to re-acidify. With achlorhydria, the patient's stomach secretes little acid, and the pH will not fall below 4.0, even on the first challenge.[25] Experienced technologists must administer this test because of factors, such as the timing of bicarbonate solutions that are critical for accurate, reproducible results.

Direct sampling of stomach contents by retrieving an absorptive string swallowed by the patient can provide evidence of stomach pH and bacterial levels. The protocol is less invasive than intubation, but it depends on the tube reaching the surface of the stomach when swallowed. Some evidence indicates quite good results

Notes:

for assessment of patients with gastroesophageal reflux disease (GERD)[26] and for specimen collection to culture *Helicobacter pylori*.[27-29] Even duodenal sampling with the string test has been reported to be helpful for evaluating cholestatic jaundice in infancy.[30, 31] The string test was found to be unacceptable for detection of small bowel bacterial overgrowth.[32]

Other laboratory abnormalities are frequently related to or caused by inadequate stomach acid production. Multiple trace element deficiencies are sometimes found in individuals who have apparent adequate dietary intake. Because of the acid dependency of trace elements, evidence of multiple trace element deficiencies has been used as an indirect measure of adequacy of gastric acid flow. For example, simultaneous low levels of iron, zinc, copper and manganese in serum, erythrocytes or hair is often due to gastric acid inadequacy, especially when intake of trace elements is normal. The critical function of low pH in the stomach is required to set up mineral absorption (see Chapter 3, "Nutrient and Toxic Elements"). High levels of ammonia produced by bacterial action on amino acids are even more directly associated with inadequate hydrochloric acid. The loss of bactericidal action and the failure to digest protein due to low stomach acid simultaneously lead to higher bacterial populations and greater availability of unassimilated amino acids for bacterial conversion.

The Pancreas and Gallbladder

Pancreaticobiliary fluid composition can be highly variable. The pancreas contributes to digestion by secreting alkaline bicarbonate and a variety of digestive enzymes. Secretion of pancreatic fluid is controlled in part by vagus nerve stimulation. A more important regulatory mechanism of pancreatic secretion is the control exerted by the hormones secretin and cholecystokinin (CCK). Both are synthesized in the duodenum (upper small intestine) and secreted in response to the presence of acidified chyme in the small intestine. Additionally, CCK stimulates the contraction of the gallbladder, causing the release of bile into the duodenum. Secretin stimulates the flow of bicarbonate-rich pancreatic fluid that serves to raise the pH of normal chyme from below 4 to above 7, allowing trypsin and other pancreatic digestive enzymes to reach their maximal activities (Figure 7.2).

Prior to 1997, numerous reports had appeared regarding assessment of pancreatic exocrine insufficiency by the PABA test, in which serum or urinary p-aminobenzoic acid (PABA) is measured following oral administration of the dipeptide N-benzoyltyrosyl-p-aminobenzoic acid (BT-PABA) to a fasting patient.[33] A dose of 16.7 mg of BT-PABA/kg body weight is administered. Results were usually expressed as a PABA index, and reference limits varying between 55 and 75% of the administered dose appearing in urine over the next 24 hours were used, according to the laboratory performing the test. Results below the reference limit are evidence of impaired pancreatic protease secretion.

Chyme in the upper portions of the small intestine is also a stimulus for the release of bile, necessary for proper digestion of fat. Bile is synthesized in the liver and stored in the gallbladder until release. Bile, which contains no digestive enzyme activity, is important for digestion due to its high content of bile salts. Bile salts emulsify fat into smaller-sized globules that can be digested by pancreatic lipase.

Fecal Chymotrypsin

The fecal chymotrypsin test is a useful non-invasive test to determine chronic pancreatic insufficiency.[34] False-positive results of up to 10% have been reported in normal individuals. Greater reliability for diagnosing chronic pancreatitis can be obtained by performing the fecal chymotrypsin test in combination with the bentiromide (N-benzoyl-L-tyrosyl-p-aminobenzoic acid) test.[35] The bentiromide test measures the amount of p-aminobenzoic acid (PABA) appearing in urine following an oral bentiromide loading, indicating successful chymotrypsin cleavage at the tyrosyl peptide bond. PABA excretion rates are lower in patients with compromised pancreatic function than in healthy control subjects.[36]

Fecal Pancreatic Elastase

A newer alternative or companion test to the fecal chymotrypsin assay is fecal pancreatic elastase. Human pancreatic elastase, a member of the acidic elastase family, was first detected by Sziegoleit as a new endoprotease and sterol-binding protein present in both human pancreatic secretions and feces.[37-43] Elastase, unlike chymotrypsin, has been found to remain unaffected during intestinal transit and to be stable in stool samples for up to a week at room temperature.[37, 41, 42] Elastase cannot be detected in bovine or porcine pancreatic

enzyme preparations. Therefore, unlike chymotrypsin, it is not affected by oral pancreatic enzyme replacement therapy (see Figure 7.3).[37] Elastase is also not affected by previous gastrointestinal surgery, gastric dysmotility or mucosal disease of the small intestine. Fecal elastase is a simple, non-invasive, relatively inexpensive and accurate functional test for patients with suspected pancreatic exocrine insufficiency and/or chronic pancreatitis, and is superior to the fecal chymotrypsin determination and the more complicated pancreolauryl test for pancreatic cholesterol hydrolase enzyme activity.[37, 38, 44]

Pancreatic exocrine dysfunction has been described frequently in both insulin- and non-insulin-dependent diabetes patients and is often considered a complication of diabetes. However, diabetes secondary to chronic pancreatitis (often caused by biliary microlithiasis) may also be much more common than was previously believed. Approximately 20 to 40% of chronic pancreatitis patients will develop secondary diabetes, whereas diabetes can impair pancreatic exocrine function at least as often.[45] Pancreatic elastase has been advocated as a screening test for pancreatic insufficiency, chronic pancreatitis, biliary microlithiasis and elevated risk of the development of diabetes. Undiagnosed and untreated pancreatic insufficiency can also result in malabsorption of proteins, fats and, ultimately, the entire spectrum of fat-soluble vitamins.[46-50] Reduced pancreatic elastase, combined with elevations in fecal protein, meat and vegetable fibers, are clinical indications for supplemental pancreatic enzyme and betaine hydrochloride supplementation.

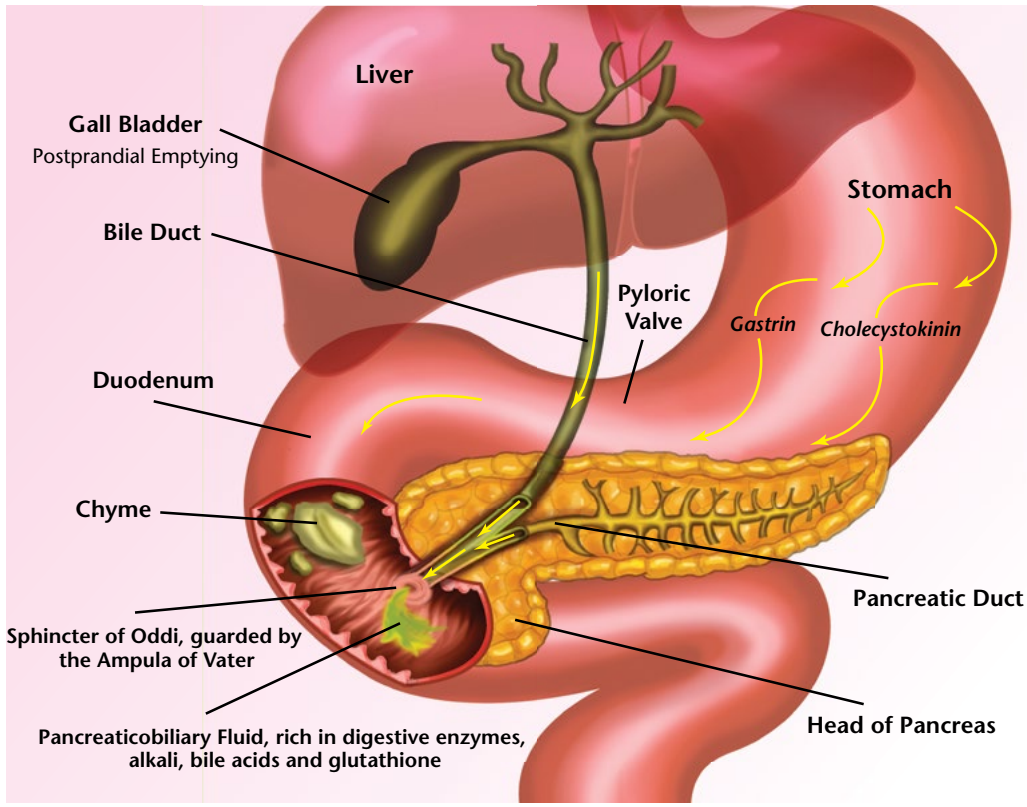

Figure 7.2 — The Pancreaticobiliary System

In order to retain food for adequate generation and mixing of stomach acid, the pyloric valve must remain closed until small (~ 4 cc) boluses of chyme that require pH neutralization by alkaline pancreatic fluid and fat emulsion by biliary flow. The joining of biliary fluid with pancreatic fluid just before entry into the mid-duodenum helps to protect the pancreas from un-neutralized stomach acid and it assures the co-mixing of both fluid types with the chyme. The frequency of allergic reactions to foods such as nuts and milk derives, in part, from the strong requirement of those foods with high fat content and content of proteins that are difficult to digest. The undigested food proteins pass into the small intestine where they can gain entry to the intestinal blood supply, requiring processing by the immune system (see below under "The Immune Barrier").

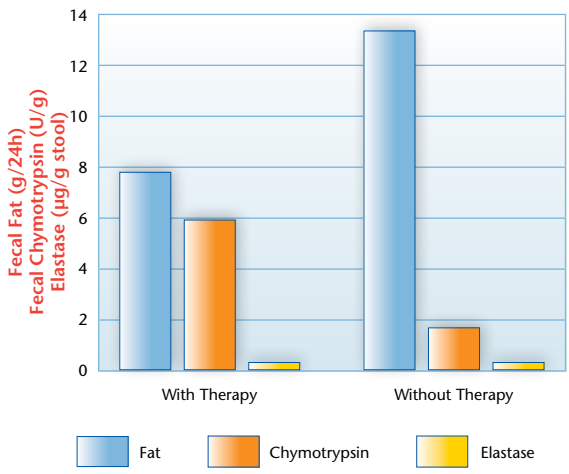

FIGURE 7.3 — Enzyme Replacement Therapy

Twelve patients with cystic fibrosis and steatorrhea were treated with pancreatic enzyme replacement therapy. Their fecal fat, chymotrypsin and immunoreactive elastase were measured before and during therapy. The absence of immunoreactive elastase in bovine and porcine pancreatic enzyme preparations is shown as lack of change while chymotrypsin shows a marked rise. Fecal elastase, therefore, reflects only endogenous pancreatic output.[382]

FECAL FAT

Fat absorption is a passive process. Bile and pancreatic lipases greatly facilitate the rate of fat absorption. Brush-border lipase activity and simple diffusion allow for the absorption of a considerable proportion of fat in the healthy intestine, even in the absence of bile. Oils containing unsaturated fatty acids are absorbed more quickly than saturated fats. Within the intestinal cell, fatty acids are packaged and released into lymphatic circulation as chylomicron particles. Since the lymphatic vessels drain into the arterial system, dietary fat is presented directly to extrahepatic tissues. However, medium-chain fatty acids (6 to 12 carbons) are water soluble and require minimal lipase activity and less bile salt for solubilization. Therefore, they enter portal circulation rather than the lymphatic drainage of the small intestine.

Steatorrhea, defined as the presence of excess fat in the stool, is established by fat-balance studies.[51] Normal fecal excretion of fat is less than 6 g/d. However, this test does not distinguish between fat maldigestion from fat malabsorption. Instead, tests for fecal triglycerides and long-chain free fatty acids can help differentiate between the two disorders. Since most of the dietary fat is composed of triglycerides, excess fecal triglyceride levels indicate incomplete fat hydrolysis (maldigestion). This is possibly due to inadequate pancreatic secretion or activation of pancreatic lipase, which can cause excessive, unhydrolyzed triglycerides to be excreted in the feces.[52] Animal studies using a radio-labeled triglyceride found that significantly greater amounts of radioactivity were present in the triglyceride fraction of the feces from those animals with pancreatic insufficiency compared with controls.[53] On the other hand, if fecal triglycerides are normal but the feces contains excess long-chain free fatty acids (carbon length > 12C), malabsorption is indicated. This is because long-chain free fatty acids are readily absorbed in the presence of a fully functioning intestinal mucosa. Animal research has shown high levels of free fatty acid fractions in the feces of animals with short-bowel syndrome.[53]

FECAL STEROIDS

Fecal steroids come from dietary cholesterol, intestinal mucosal sloughing, and bile acids and cholesterol contained in bile. Fecal cholesterol content can range from 75 to 200 mg/d, most of which comes from bile. The wide variation in fecal steroid content is due to many factors. Especially interesting is the effect of a high dietary polyunsaturated to saturated fatty acid ratio, resulting in increased fecal steroids.[54, 55] These data demonstrate that increasing intake of dietary fat containing polyunsaturated fatty acids is effective for lowering total serum cholesterol without reducing HDL cholesterol. The mechanism involves reduced absorption of cholesterol. Additional reduction in sterol absorption may be obtained by increasing foods high in dietary fiber. Several reports have demonstrated the ability of various dietary fibers, phytosterols and phytostanols to modulate fecal steroid content.[56-59]

FECAL FIBERS

Microscopic inspection of stool can reveal the presence of meat and vegetable fibers. The increase in the amount of these fibers that occurs with impaired digestion is an indirect indicator of hypochlorhydria or insufficient output of pancreatic enzymes.[60, 61]

The Small Intestine: Digestion and Absorption

The intestinal brush border contains various membrane-associated enzymes (e.g., sucrase, lactase, dextrinase, maltase, isomaltase, trehalase) that cleave numerous polysaccharides and disaccharides (sucrose and lactose) to absorbable monosaccharides (glucose and fructose), the products of luminal digestion of dietary starches and fiber. Simple sugars (glucose, fructose and galactose) are principally absorbed by saturable active-transport processes. Impairment of brush-border enzyme activity causes carbohydrate malabsorption, which leads to excess bacterial fermentation of unabsorbed disaccharides and an increase in osmolarity that is accompanied by diarrhea and flatulence.[61] Although most people consume too little dietary fiber, excessive dietary fiber (> 50 g/d), which is resistant to the action of human digestive enzymes, but is broken down by gut bacteria, can lead to diarrhea and gas as well.

The amount of mannitol appearing in urine after a standard oral load of this monosaccharide derivative is used as a measure of small intestinal capacity for monosaccharide (glucose and fructose) absorption (see section below, "Lactulose-Mannitol Intestinal Permeability Challenge Test"). The disaccharidase functions cannot be measured by routine laboratory evaluations, but clinical observations have indicated that the disaccharide enzymes play a role in inflammatory bowel conditions such as celiac disease. Elimination of all disaccharide sources has been helpful in many of these cases.[62] The diet is difficult to maintain because all sources of sucrose, maltose and isomaltose must be eliminated. Maltose and isomaltose are produced in the earlier phases of digestion of starch, so all starch sources must be restricted. Enteral insulin-like growth factor 1 has shown promise for inducing the formation of intestinal disaccharidase enzymes.[63]

Brush-border and cytosolic peptidases hydrolyze small peptides to produce amino acids. Although most proteins are digested to free amino acids prior to absorption, the absorption of large peptides plays a significant role in the assimilation of dietary protein.[64] The transport mechanism for the uptake of peptides by mucosal cells differs from the mechanism for free amino acid uptake, and there is no competition between the two. Nonetheless, because peptide hydrolase enzymes in the brush border and cytosol of mucosal cells hydrolyze small peptides, primarily free amino acids pass into the portal vein and go to the liver.[65, 66] This process is one of the principal dynamic actions affecting the concentrations of amino acids in blood plasma (see Chapter 4, "Amino Acids"). Direct utilization by intestinal cells contributes to the overall dynamic action of amino acids obtained from dietary protein. Up to 50% of orally consumed amino acids may be used by intestinal cells, never becoming available for transport into portal circulation.

Of Further Interest...

As much as a third to half of the protein in the lumen of the small intestine is not of dietary origin, but is protein from sloughed off epithelial cells and digestive enzymes.

Schilling Test

The Schilling test was primarily used to assess the absorptive capacity of the GI tract for vitamin B_{12}. However, the use of the Schilling test for the detection of pernicious anemia has been largely supplanted by serologic testing for parietal cell antibodies or direct assay for intrinsic factor antibodies, the absence of which creates a positive Schilling test.[67]

Notes:

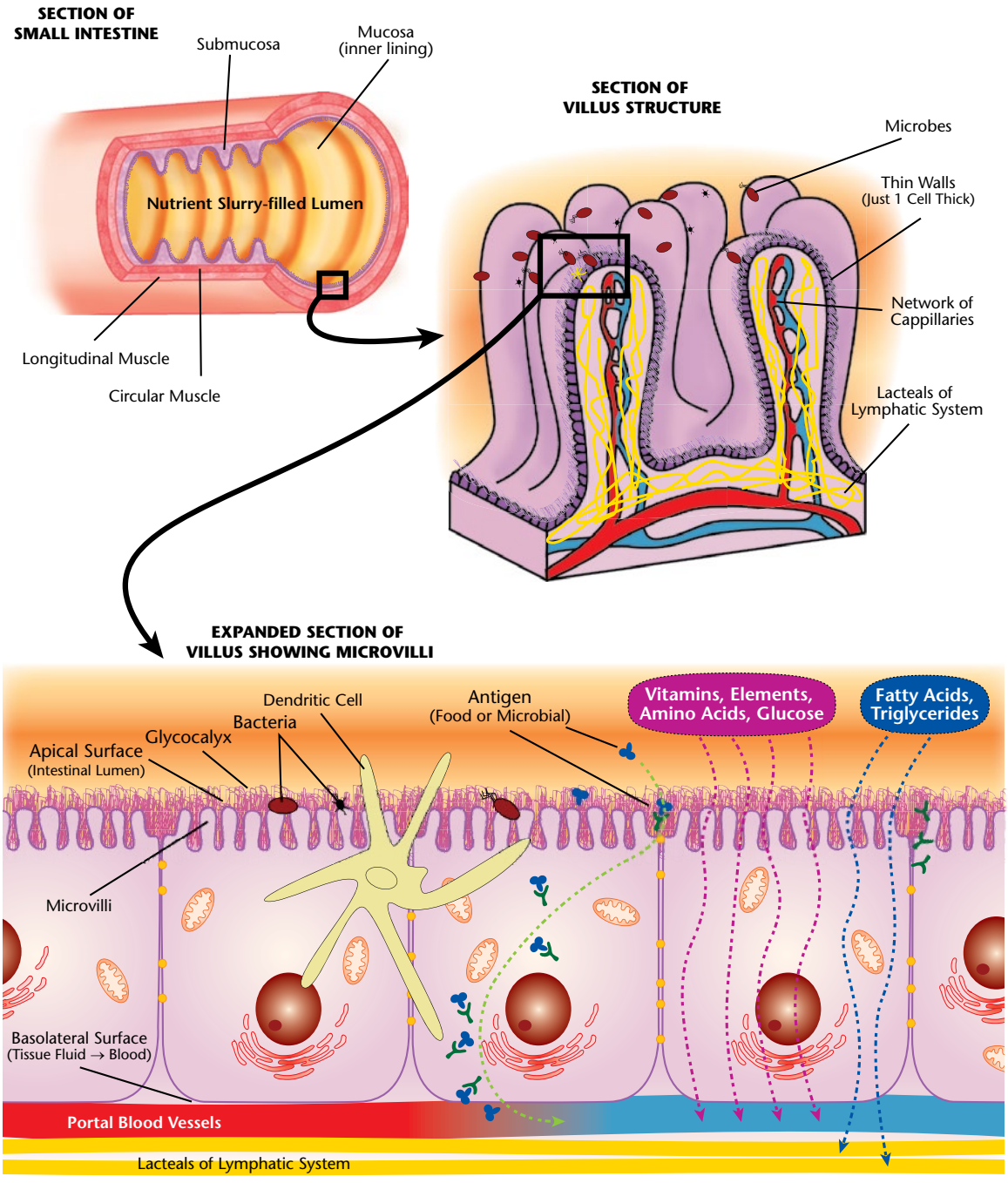

FIGURE 7.4 — Small Intestine Schematic

The closely packed villi of the small intestine are the primary absorptive unit of the digestive system. The villous surface is the interface between systemic fluids and the complex matrix flowing through the lumen where small and large molecules from food components mix with digestive fluids, intestinal secretions and the dynamically changing microbial mass. The tall columnar epithelial cells represented in the lower left are constantly being replaced by cell divisions at the base of the villi. Immune cells are interspersed individually and in groups such as Peyer's patches (not shown). A dendritic cell is shown with a projection into the lumen where bacterial antigens are sampled for presentation to lymphocytes and macrophages.

The Physical Barrier

The lining of the intestinal tract must endure considerable physical, chemical and biological challenges. In response to the entry of harsh gastric juices, the duodenum releases large quantities of mucus that serves to coat and protect the intestinal mucosa from digestion by gastric juice and pancreatic digestive enzymes. The mucus barrier is constantly being eroded, posing challenges to the underlying cells. Turnover of intestinal epithelial cells is among the highest in the body, as new cells must constantly replace those lost to luminal degradation. New cells form at the base of the crypts in the layer of tall columnar epithelial cells that are the outer cellular barrier. The newly formed cells must establish tight junctions to seal off the barrier from passage of large amounts of macromolecular components. The susceptibility of the cellular junctions to penetration by large molecules normally results in about 2% of food proteins getting past the mucosa in a form the immune system can recognize.[68]

Testing for serum IgG concentrations also provides evidence of the integrity of the physical barrier because elevated IgG levels are found only when food antigens can penetrate the physical barrier at an abnormally high rate. The finding of significant IgG elevations toward food antibodies is evidence of increased intestinal permeability and a failure to maintain adequate barrier function because it addresses the central question: Are large, undigested molecules (usually proteins) able to pass into the submucosal layers?

Intestinal Hyperpermeability (Leaky Gut Syndrome)

Leaky gut syndrome is a disorder associated with increased intestinal permeability, subsequent to the loss of intestinal mucosa integrity. Patients with this condition have more than the normal 2% "leakiness" to large molecules. Degradation of the physical barrier often is due to exposure to toxic substances within the intestinal lumen that can damage the "tight junctions" between intestinal epithelial cells, leading to an increase in passive paracellular absorption.[69, 70] Common causes of intestinal hyperpermeability are ethanol consumption,[71] non-steroidal anti-inflammatory drugs (NSAIDs), and viral, bacterial, yeast and protozoan infection.[72-75] Also, elevated levels of reactive oxygen species coming from a variety of sources, such as bile, food, cytotoxic drugs[76] or inflammatory cells,[77, 78] can increase paracellular permeability.

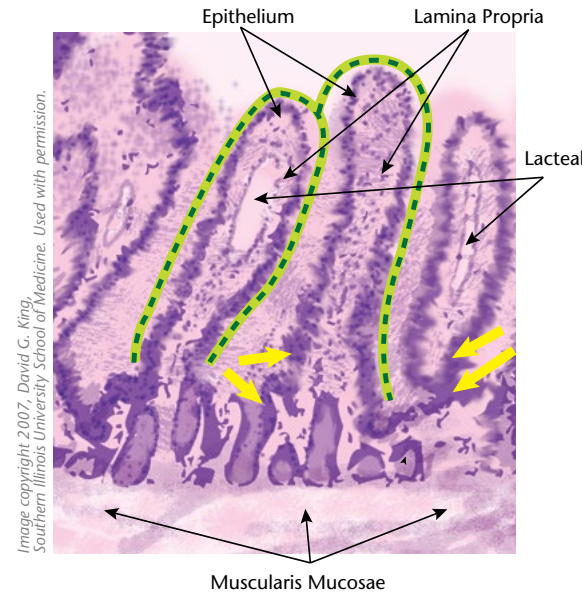

Figure 7.5 — Jejunal Section Photomicrograph

Villi are outlined by tall columnar epithelial cell nuclei which are darkly stained in this image. The mucosal surface evaginates into villi (green outlines) and invaginates into crypts. Though some crypts appear "disconnected" due to the way the tissue was sectioned, the epithelium of villi is continuous with that of adjacent crypts as noted by the yellow arrowheads. The inner villous surface is lined by cells of the lamina propria, and the long, apparent empty spaces are lacteals that empty into the lymphatic system. The muscularis mucosae is only a few muscle fibers in thickness, lying beneath the crypts. Intraepithelial lymphocytes are positioned between epithelial cells, beneath the tight junctions, so they are inside the epithelium, but not inside epithelial cells as the name may incorrectly suggest.

Macromolecules that penetrate the intestinal barrier are carried in the portal vein to the liver, where they are degraded by phagocytic Kupffer cells. Xenobiotics and microbial toxins must be processed by the liver's Phase I and Phase II detoxification systems for excretion into bile or urine (see Chapter 8, "Toxins and Detoxification"). In the colon, where bacterial toxin production is high, the colonocytes carry out the detoxification reactions utilizing the same reactions that occur in the liver.[79] Even for the intact GI tract, the cost of detoxification is high; antioxidants and conjugates such as glutathione are consumed and must be replenished. Compromise of the intestinal barrier can lead to excess production of reactive oxygen species by the hepatic

mixed-function oxidase (cytochrome P450) system. Increased hepatic detoxification causes increased biliary excretion of reactive and carcinogenic compounds. Reflux of this "toxic" bile may be an important cause of chronic pancreatic disease.[80, 81] Intestinal hyperpermeability also causes disorders by immunologic mechanisms. Increased permeability can stimulate hypersensitivity responses to food and components of the gut flora. Dietary gluten and bacterial endotoxin may cause nonspecific activation of the immune system's inflammatory pathways, mediated by complement and cytokines. It has been shown experimentally that chronic low-grade endotoxemia leads to autoimmune disorders.[82-84]

Intestinal hyperpermeability is found in all chronic inflammatory bowel diseases, where it may play an etiologic role, or it may be a secondary consequence due to the vicious cycle involving immune activation, hepatic dysfunction and pancreatic insufficiency.[85] The role of leaky gut syndrome in many diseases is often missed. The availability of non-invasive and affordable methods for measuring intestinal hyperpermeability makes it possible for clinicians to watch for leaky gut syndrome in their patients and to objectively assess the efficacy of treatment.

Lactulose-Mannitol Intestinal Permeability Challenge Test

The lactulose-mannitol protocol was developed to measure intestinal hyperpermeability that could lead to food sensitivity.[86] It is being utilized in assessment of gut permeability in a wide range of conditions, including food sensitivities,[87] pancreatitis,[88] Crohn's disease[89] and cirrhosis. Although the presence of elevated IgG levels to many foods in the test described above indicates hyperpermeability to macromolecular antigenic molecules, the lactulose-mannitol test provides a direct measure of hyperpermeability to the disaccharide lactulose. Lactulose-mannitol is a challenge test in which patients with suspected hyperpermeability ingest 5 g each of the metabolically inert sugars lactulose and mannitol. Since these sugars are not metabolized, any absorbed sugar is fully excreted in the urine within 6 hours. The urine is collected, and concentrations of the two sugars are measured. Percent absorptions are calculated using the formula below.

$$\% \text{ Absorption} = \frac{\text{Compound Concentration (mg/mL)} \times \text{Urine volume (mL)}}{\text{Amount of Compound Administered}} \times 100$$

Mannitol, a monosaccharide, is passively absorbed through the intestinal mucosa. In contrast, lactulose, a disaccharide, is normally not absorbed unless the mucosal barrier is compromised. In the healthy intestine, the mean absorption of mannitol is 14% of the administered dose, whereas the mean absorption of lactulose is less than 1%. The normal ratio of lactulose-mannitol recovered in urine is < 0.03. An elevated ratio means that excessive lactulose was absorbed, indicating leaky gut syndrome. It is best to have the patient perform the lactulose-mannitol test twice—first in the fasting state, then again after ingestion of a test meal.[90] The lactulose-mannitol ratio was found to be an accurate predictor of relapse when measured in patients with Crohn's disease who were clinically in remission.[91] Recent intake of high carbohydrate meals and prolonged administration of the challenge solutions diminish responses to this test.

Lactulose-Mannitol Testing Protocol

- Swallow a solution of 5 g mannitol and 5 g lactulose
- Collect urine for 6 hours
- Assay for total lactulose and mannitol
- Calculate recoveries

  < 14% Mannitol = Carbohydrate malabsorption

  > 1% Lactulose = Disaccharide hyperpermeability

Markers of Mucosal Inflammation

Given knowledge of the presence of astronomical numbers of potentially pathogenic bacteria separated from the blood by only a single cell's width, one may wonder how chronic disease is avoided. The complexity

Notes:

of host-microbe interactions are starting to be revealed. Resident flora interact with antigen-presenting cells in the lamina propria or cross the epithelial barrier without causing inflammation. The antigen-presenting dendritic cells in the lamina propria extend processes between epithelial cells and into the gut lumen as illustrated in Figure 7.4. By this periscope-like activity, they take up bacteria or antigens associated with bacteria in the microbial flora.[92, 93] Commensal organisms introduced into the gut lumen of a germ-free mouse can gain entry into the gut-associated lymphoid tissue (GALT),[94] and some survive in dendritic cells for a limited time, where they are processed for stimulation of B lymphocytes. The B cells, in turn, produce IgA that can inhibit binding of microorganisms to epithelial cells. The IgA responses also promote bacterial uptake across specialized M cells that overlie Peyer's patches, where various immune cellular activities are at a high pitch. In addition, when introduced into the lumen of mice, probiotic organisms activate regulatory T lymphocytes that have the ability to inhibit subsequent induction of inflammatory responses.[95] These microorganisms must gain access to mucosal dendritic cells to make the induction and expansion of the regulatory immune cells possible. It is when these mechanisms of bacterial recognition and limitation of penetration fail that the powerful immune responses of the GALT can produce pathological inflammation such as that found in celiac disease.

Enteropathies Induced by Gliadin — Inherited factors make some individuals sensitive to a protein called gliadin, present in some cereal grains. Gliadin is a part of the total protein, or gluten, in the grains. When undigested gluten reaches the small intestine, gliadin peptides activate autoimmune reactions in susceptible individuals. As many as 1 in 133 Americans with no previous symptoms or family history of celiac disease may be affected.[96]

Serum IgA should be measured to confirm IgA competence and to assure the validity of the tissue- and antigen-specific tests for gluten-sensitive enteropathies. For total IgA > 10, the other markers are reliable indicators. For total IgA < 10, testing of IgG antibodies to transglutaminase and gliadin may be used. Elevated or moderately depressed IgA can indicate gut-associated lymphoid tissue hyper- or hypofunction, respectively.

Elevated tissue transglutaminase indicates the presence of celiac disease with high sensitivity and specificity for the presence of significant villous atrophy. Antibodies to transglutaminase form only when enterocytes are undergoing rapid degeneration.[97] The antecedent factor of gluten-activated immune response is revealed by elevated antigliadin IgA II. This test may be positive in patients with limited villous atrophy during early stages of the condition.[98, 99] Those with celiac disease should consume a gluten-free diet, eliminating products containing wheat, rye and barley.

Neutrophil-Derived Inflammatory Proteins — Bovine lactoferrin (Lf) is a milk protein that exhibits broad-spectrum antimicrobial action, largely due to its affinity for iron that deprives bacteria of iron critical for their growth.[100] This type of antibacterial mechanism is sometimes called nutritional immunity.[101] Human lactoferrin, an iron-binding glycoprotein secreted by mucosal membranes, is a major granular component of polymorphonuclear neutrophils. When these cells respond to inflammatory signals, lactoferrin is released as part of the defense mechanism. Fecal Lf is a marker of intestinal inflammation in which leukocytes infiltrate the mucosa, increasing the release of neutrophil lactoferrin. Fecal Lf has been extensively used to differentiate inflammatory bowel disease from irritable bowel syndrome (IBS) and non-inflammatory bacterial infections, and it is useful for monitoring IBD treatment efficacy.[89, 102-109]

Various other neutrophil-derived proteins, such as calprotectin (Cal), polymorphonuclear neutrophil-elastase (PMN-e), α_1-antitrypsin and lysozyme (Lys) have been shown to be reliable indicators of intestinal inflammation and can aid in the differentiation of organic intestinal disorders (i.e., inflammatory bowel diseases (IBD), ulcerative colitis (UC), Crohn's disease, infectious gastroenteritis, etc.) from functional intestinal disorders (i.e., IBS).[103-106, 110-117] Fecal levels of these proteins rapidly increase with the influx of leukocytes into the intestinal lumen during inflammation. Among the neutrophil-derived proteins in feces, PMN-e, Cal and Lf represent the most accurate markers of disease activity and severity in patients with ulcerative colitis, with lysozyme being somewhat less useful.[112, 113] Fecal Cal is increased in over 95% of patients with IBD and correlates well with clinical disease activity. PMN-e and Cal also reliably differentiate between patients with IBD and IBS.[115, 116] Thus, there are several markers that provide reliable differentiation of Crohn's disease from IBS.

Fecal Histamine — The detection of fecal histamine is gaining utility as a diagnostic marker. Histaminosis provides direct evidence of intestinal mucosal inflammation and can be used in addition to other less direct markers of inflammation such as fecal Lf, Cal, PMN-e and lysozyme.[118] Amon and colleagues have stated, "There is increasing evidence that enteral histaminosis is a major cause of food intolerance resulting from dysfunctional metabolism of endogenous histamine in certain food stuffs. However, this phenomenon has been poorly characterized, and, due to lack of epidemiologic data, the existence of this condition has probably been underestimated, resulting in improper diagnosis."[119] Fecal histamine levels can have contributions from foods that contain significant amounts of histamine, including various cheeses, sausage, vinegar, some species of fish, red wine, beer and vegetables, including spinach, tomatoes and sauerkraut. Food additives, including taste enhancers such as glutamate; preservatives such as sorbic acid, benzoate, BHT and BHA; and many colorants also act as liberators of histamine. There is also evidence that eosinophils and mast cells accumulate and become activated in inflammatory bowel disorders and also contribute to the presence of histaminosis in a wide spectrum of inflammatory bowel disorders.[120, 121] Bacterial origins of histaminosis also play a significant role, including histamine-producing bacteria (HPB) found in various fishes. *Klebsiella pneumoniae* and *K. oxytoca* are the best-known HPB in fish. However, 22 strains of HPB from fish first identified as *K. pneumoniae* or *K. oxytoca* by commercialized systems were later correctly identified as *Raoultella planticola* (formerly *K. planticola*) by additional tests.

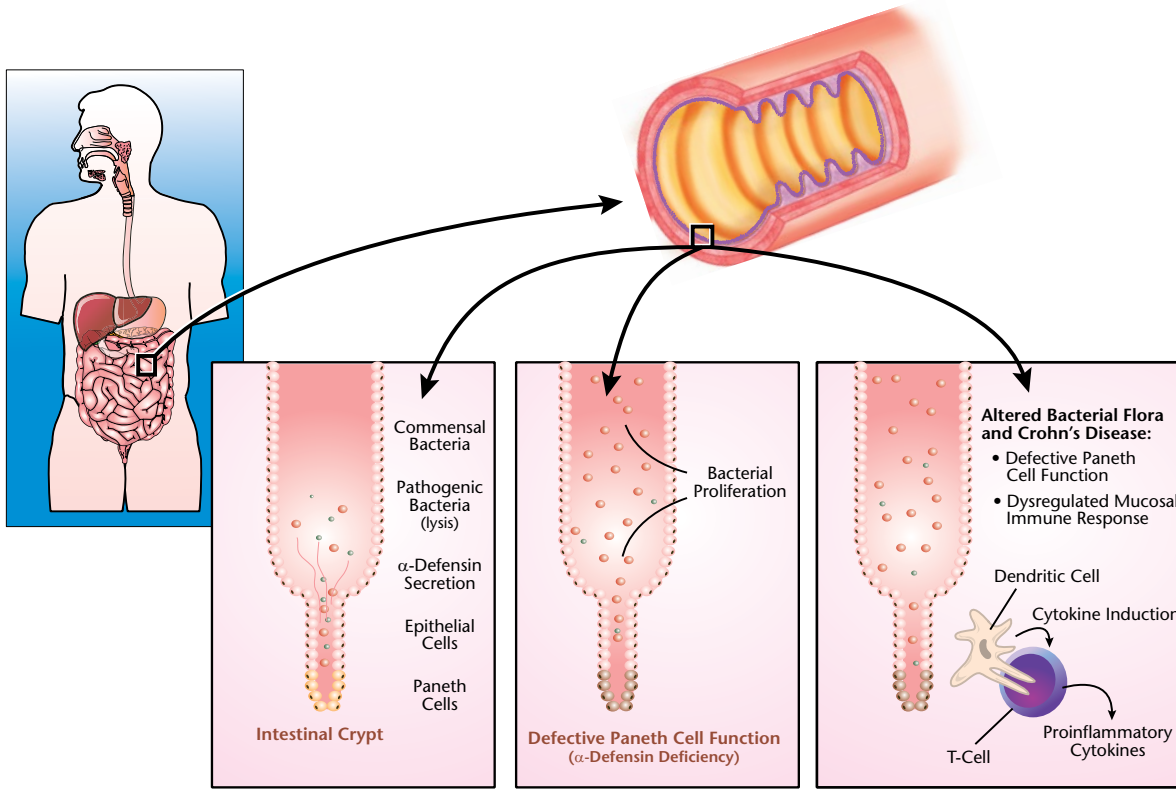

FIGURE 7.6 — **Antibacterial Factors in Mucosal Homeostasis**

The three insets show a progression from health to Crohn's disease. Paneth cells have functions similar to neutrophils. From their position at the base of crypts, they provide host defense against microbes in the small intestine. One important mechanism is the production and secretion of the microbiocidal, α-defensin. Defective Paneth cells have low α-defensin output, allowing opportunistic bacterial overgrowth. Bacterial antigens are detected by dendritic cells that stimulate T-cell cytokine release. The inflammatory cascade leads to further intestinal degradation in a feed-forward cycle. Improvements may come from reducing small intestinal bacterial populations, reducing the load of major antigen-producing foods and improving omega-3 fatty acid body composition to help reduce inflammatory signaling.

Similarly, 5 strains of *R. ornithinolytica* (formerly *K. ornithinolytica*) were isolated from fish as new HPB.[122] These strains, often simply identified as *Klebsiella* spp., may be detected on stool analysis, and this variant of dysbiosis should be considered as a potential reason for the presence of histaminosis and gastrointestinal inflammation.[122, 123] New trends in the pharmacologic treatment of bloody diarrhea, usually related to hemorrhagic gastritis, and diarrhea-predominant IBS often now feature the use of histamine-2 (H_2) receptor antagonist medications, further demonstrating the importance and clinical efficacy of reducing the effects of histamine in the management of inflammatory gastrointestinal disease.[124]

Mucosal Defensins — Defensins are endogenous mucosal antibiotic peptides with microbicidal activity against gram-negative and gram-positive bacteria, fungi, viruses and protozoa.[125] Routine laboratory measurements of fecal defensins have recently become available.[126] Defensins play pivotal roles in innate immunity and the maintenance of the delicate balance between immune tolerance and immune response by regulating rates of microbial colonization and slowing pathogen growth.[127, 128]

Crohn's disease (CD), an idiopathic inflammatory bowel disease, has been linked to reductions in intestinal antibacterial activity in mucosal secretions and increased intestinal bacteria, which may result in chronic inflammation in genetically susceptible individuals.[129] Paneth cells (PCs) are the main source of antimicrobial peptides in the small intestine, including the α-defensins HD5 and HD6 (see Figure 7.6). Deficiency of PC defensins may perpetuate ileal CD by compromising the innate immune defense.[130-132] Ileal CD has also been linked to a mutation in the *NOD2* gene.[133] The Paneth cells that produce the α-defensins express *NOD2*, and mutations of this gene have been found to result in diminished expression of ileal PC defensins.[129, 132, 134-136] Crohn's disease of the colon (versus the small intestine), on the other hand, is characterized not by reduction in PC α-defensins, but a decrease in β-defensins in enterocytes. β-Defensin production is generally stimulated by cytokines and bacterial endotoxins in epithelial tissue and mononuclear phagocytes and may be deficient in subjects with CD. Therefore, the regional subtypes of CD can be associated with different defensin profiles. In both variants, it appears that decreased mucosal defensin levels result in weakened intestinal barrier immune function, increased intestinal microbe levels and chronic inflammation, which may all be crucial in the pathophysiology of CD, ulcerative colitis and possibly other inflammatory bowel disorders.[128, 137, 138] Defensins also appear to play an important role in the suppression of potentially pathogenic bacteria, such as *Klebsiella* and *Yersinia* species, which play a role in molecular mimicry responses and the pathogenesis of a host of autoimmune disorders, including inflammatory arthritis and immunogenic thyroiditis.[139, 140] (For more information on this topic, see sections on intestinal dysbiosis and disease, and bacterial and yeast assessment under "The Microbial Mass" later in this chapter.)

The Immune Barrier

The immune barrier aspect of gastrointestinal health is important in evaluating adverse immune reactions to food and predicting overall intestinal health. Assessments of food reactions offer practical information regarding food choices that enhance health restoration in the patient with compromised GI function.

It can be difficult to grasp the magnitude of the challenge presented to the immune system in the gut. The enzymes of human tissues are designed to handle a flow of substances presented one molecule at a time. Each day, the act of eating introduces an astronomical number of molecules in the form of polymers unsuitable for cell processing, along with toxins and microbes. Whenever the digestive process fails to work in a timely and efficient manner, undigested or partially digested food molecules that exit the stomach become part of an antigen-rich slurry. The mixture passes into a microbial growth chamber—the lumen of the small and large intestines—where constant, warm temperature, neutral pH, and steady flow encourage rapid bacterial growth and reproduction. Bacterial cell turnover yields another host of antigenic molecules from macromolecules that they produce. Cells of the immune system must constantly survey every region of the gut for entry of foreign molecules or microbes, monitor signals for increasing

Notes:

defensive barriers, and, when necessary, mount aggressive removal strategies while keeping destructive forces in check so that normal tissues can continue the never-ending process of nutrient assimilation, repair and maintenance.

The field of immunology is still in a state of controversy over just how adverse reactions to foods should be classified and how best to evaluate the immune function of the GI system. The following is a brief overview of certain details to allow the reader to see the intricacies of this system. Excellent reviews of the cellular and molecular aspects of immunology are available.[141] The principal laboratory tests currently available for evaluation of the state of the gastrointestinal immune barrier function are the measurements of serum levels of antibodies to specific foods and salivary or fecal secretory antibodies.

Throughout the entire length of the small intestine are patches of lymphoid tissue called Peyer's patches (see Figure 7.7), collectively called gut-associated lymphoid tissue (GALT). Because these cells are in the mucosal layer, the term mucosal-associated lymphoid tissue (MALT) is sometimes used as well. Of the total cell mass of the immune system, the gut is by far the largest immune organ. Intraepithelial lymphocytes (IELs) are interspersed among the epithelial cells (enterocytes) lining the intestine. The IELs collectively represent a pool of cells comparable in size to that of all peripheral lymphocytes in the spleen. There has been an explosion of data from research laboratories about the intercellular communication that regulates the activities of this part of the immune system. Cytokines, nuclear factors, interferons and integrins regulate CD4 and CD8 T lymphocytes.[142] The constant challenge of food and microbial antigen presentation frequently leads to abnormal growth. Neoplastic colorectal lesions, for example, contain cytotoxic IELs.[143] One of the results of hyperstimulation of intestinal cellular immune responses is an increase in the permeability of the cellular lining of the small intestine. Tall columnar epithelial cells that form the physical boundary are constantly replaced in the normal small intestine. High rates of cell division at the base of villi results in a constant migration toward the villous tip where old cells are sloughed off into the lumen (Figure 7.5). The combination of high rates of cell replication over the large area of the small intestine and extensive immunoglobulin biosynthesis in the GALT accounts for the consumption of a large percentage of normal caloric intakes to sustain the structure and function of the GI tract. In addition to the energetic demands, large percentages of daily requirements for vitamins, elements, essential amino acids and fatty acids are consumed by the cells of the GI tract.[144]

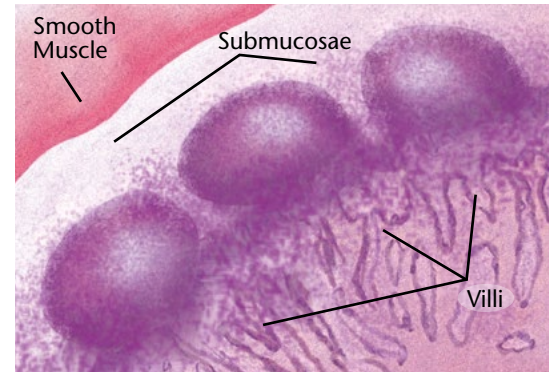

FIGURE 7.7 — **Peyer's Patches**

In this image of canine ileum, three lymphoid follicles of a Peyer's patch can be seen. Peyer's patches that are similar in many ways to lymph nodes are found in the small intestine, especially the ileum. In adults, B lymphocytes predominate in Peyer's patches where they produce the various classes of immunoglobulins except for secretory IgA that is produced by B cells residing in the lamina propria. The muscular layer is at the top left, so the flow of fluid toward the lumen of the gut is down toward the bottom right.

The barrier function depends on tight junctions between the cells. Any breach in cell-to-cell junctions presents an opening for antigen flow and further immune response. Antibody responses to food antigens can result in clinical food allergy symptoms.[11] Children with regressive autism show strikingly increased deposition of IgG immune complexes, along with increases in mucosal lymphocyte density and crypt cell proliferation.[145] Similar deposits are found in gastric antral biopsies from autistic children where they are associated with a novel type of CD8 lymphocyte gastritis.[145]

Interactions between food macromolecules or the fragments released from them by partial digestion and the GALT produce symptoms in distant tissues, especially the skin.[146] Food-GALT reactions can also cause lesions in brain white matter associated with inflammatory bowel disease.[147] There is growing evidence that food or microbe-initiated inflammatory products from the GALT can trigger reactions that are part of the initiation of degenerative diseases, including arthritis, atherosclerosis and dementia.[148] Autoimmune thyroid disease is seen in

much higher prevalence in subjects with celiac disease (i.e., gluten intolerance) than in normal controls.[149] Reduced production of such inflammatory signals may be achieved by normalizing function throughout the gastrointestinal tract.

There is a complex relationship between intestinal hyperpermeability and adverse food reactions. Children and adults with eczema, urticaria or asthma triggered by atopic food allergy exhibit elevated intestinal permeability. These individuals can develop a self-perpetuating cycle because increase in intestinal permeability can lead to the development of food sensitivity, and it may be a result of food allergy.[86, 150, 151] The ultimate solution usually involves relieving the antigenic load by identifying and removing offending foods and restoring the physical barrier to reduce permeability.

In order to understand the differences between abnormalities of antibody classes, it is helpful to establish some definitions and their origins. Antibodies, discovered by the first Nobel prize winner, von Behring, in 1901, came to be called immunoglobulins (Ig), when they were found to migrate with the group called γ-globulins during electrophoresis of serum proteins.[152] Produced in bone by B lymphocytes that differentiate into plasma cells, immunoglobulins constitute humoral immunity, distinct from cellular immunity (see Figure 7.8). Various physical, chemical and antigenic Ig differences led to classification of, first, the higher concentration members, IgG, IgM and IgA, and then the much lower concentration IgD and IgE classes. Characteristics of the classes are summarized in Table 7.2, where the rows are sorted by normal serum concentration. Note that concentrations are directly and linearly related to half-lives. In general, classes with greater impact on immune system responses have shorter half-lives to prevent adverse effects of overstimulation.

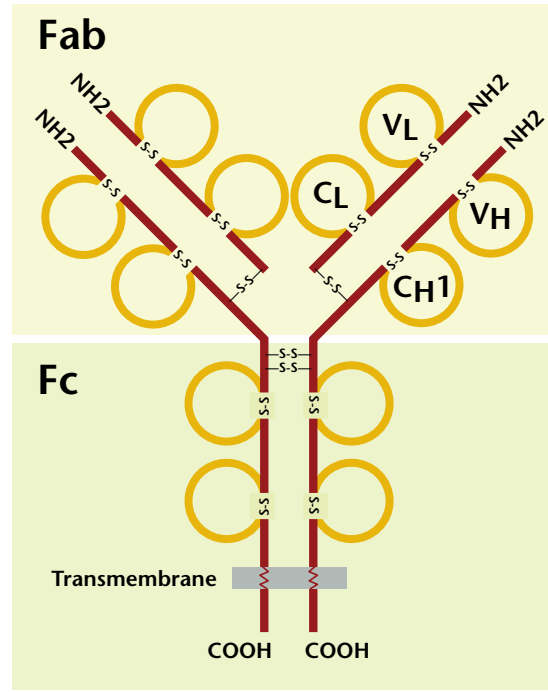

Figure 7.8 — Immunoglobulin Structure

This schematic drawing of an immunoglobulin molecule shows the two heavy chains with their transmembrane (Fc) regions and two light chains that join to form the Fab region with its variable (VL) and constant (CL) light chain domains and similar (VH and CH1) heavy chain domains. In addition to the intrachain disulfide bonds (-S-S-), covalent linkages also form between heavy and light chains in all classes of immunoglobulins and between the heavy chains in all except the IgG_4 class. The immunoglobulin binds at the light chain domains to surface regions (epitopes) of macromolecular antigens from food or microbes that initiate humoral immune response.

Secretory IgA

The lymphoid tissue of the intestinal mucosa secretes immunoglobulin A into the lumen of the GI tract as a first line of defense against microbes and antigens. Produced by plasma cells at the basement membrane of the GI tract, two molecules of IgA are connected by protein chains connecting the heavy chains, forming dimeric sIgA that is transported to the luminal surface of the gut. The term sIgA denotes this difference from monomeric IgA measured in serum. Secretory IgA (sIgA) forms immune complexes with pathogens and allergens, which prevents them from binding to and penetrating the intestinal mucosa.[153] A high antigenic load can result in depressed sIgA, even in healthy, asymptomatic individuals. With time, this decrease in resistance can lead to dysbiosis and an increased risk of infection and allergy.[154, 155]

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TABLE 7.2 — IMMUNOGLOBULIN CLASS PROPERTIES*[152]

Class	Half-Life (days)	Serum Concentration	% of Total Serum Ig	Subclasses	Form	Complement Fixation	Placental Transfer	Biological Properties
IgG	23	700–1,690	75–85	1 (50–70%) 2 (15–25%) 3 (5–15%) 4 (1–5%)	Monovalent Monomer	+ + + –	+++	Secondary antibody
IgA	6	70–380	5–15	1 2	Divalent monomer or dimer	–	–	Mucosal antibody
IgM	5	65–275	5–10	–	Pentamer	+++	–	Primary antibody
IgD	2.5	0.5–3	< 1	–	Divalent monomer	–	–	B-cell Ag† binding
IgE	2	0.1–0.05	< 1	–	Divalent Monomer	–	–	Allergic antibody

** Classes are ordered in decreasing half-life or serum concentration. Percentages detailed for IgG subclasses are relative to total IgG. † Ag = antigen.*

Individuals with selective sIgA deficiency have high titers of antibodies against various food antigens.[156]

The secretory IgA function may be evaluated by measurement of salivary or fecal sIgA levels.[157] Salivary sIgA is a predictor of the release of sIgA at intestinal surfaces.[158] A compromised immune barrier can lead to elevated serum IgA and increased intestinal permeability to toxic gluten lectins. A gluten-free diet decreased intestinal permeability in 64% of sIgA nephropathy patients.[159] Fecal sIgA correlates with salivary sIgA, and both may be predictors of partial mucosal sIgA deficiency as defined by serum IgA > 0.05 g/L.[160] Detection of specific fecal sIgAs directed toward *Rotavirus* and hepatitis A is useful in diagnosis of early stages of infections by these agents.[161, 162]

Suppression of sIgA has been associated with the stress response. The level of sIgA measured in saliva is down-regulated during periods of chronic stress, whereas acute stress induces mobilization and results in a transient increase in sIgA.[163-165] Many studies on the effects of extreme physical and/or emotional stress in test populations, such as military personnel in basic training and competitive endurance athletes, have demonstrated that levels of sIgA become depressed following such levels of stress, whereas cortisol levels increase.[166–170] Changes in levels of sIgA and cortisol in subjects under pre- and post-examination stress have been somewhat contradictory.[171, 172] The high-stress occupation of air traffic controllers did not produce low sIgA, but rather a transient elevation in sIgA and cortisol. These results have been attributed to the acute nature of the stresses experienced by the controllers or the counteracting effects of positive emotional engagement among controllers in the workplace.[173] In other situations, psychological stress may alter antibody response to immunization.[174] Reduced physical activity and excess body fat in children have also been demonstrated to result in decreased levels of sIgA and increased incidence of upper respiratory infection.[175] Relaxing activities, such as choir singing, have been demonstrated to increase positive effect and sIgA, while reducing negative effect and sIgA suppression.[176] Perhaps it is summarized best by Alshuler when she states, "Cortisol suppresses secretory immunoglobulin A (sIgA) in the gastrointestinal tract, which leads to impaired gut antigen sampling. Furthermore, cortisol alters the consistency

TABLE 7.3 — DEFINITION OF COMMON TERMS

Term	Definition	Examples
Food allergy	An abnormal response to a specific food triggered by the mast cell histamine release.	Asthma, hives, lightheadedness, weakness, anaphylaxis.
Food sensitivity	An abnormal response to foods habitually eaten that form large, stable antibody-antigen complexes.	Serum sickness or stress-induced hyperpermeability, leading to long-term chronic inflammatory conditions of the kidney, skin, arthroses and lungs.
Food intolerance	An undesirable reaction to food that does not involve the immune system.	Lactose intolerance, where milk ingestion causes loose stools, gas and nausea.

of the gastrointestinal mucosal barrier. The combined result of these effects is an enhanced immune response to gut-derived antigens and increased translocation of antigenic material to systemic circulation."[177] Stress plays an important role in the compromise of the gastrointestinal mucosal immune response and the development of pan-allergy to foods and potentially the development of autoimmune phenomena via antigen-antibody complex cross-reactivity and molecular mimicry. Combining salivary sIgA with evaluation of cortisol and DHEA (see Chapter 10, "Hormones") may be beneficial in the overall assessment of the stress response and the management of leaky gut, food allergy, inflammatory arthritis, immunogenic thyroiditis, autoimmunity and other chronic diseases.[149, 178-187]

Food-Directed Antibody Testing

Food antibody tests measure levels of specific immunoglobulins in serum directed against food antigens. Elevated levels of food-specific IgE can cause allergic reactions to food involving the anaphylactic response, whereas high food-specific IgG can lead to sensitivity reactions due to food antigen-antibody complex deposition (Table 7.3 and 7.4). Such reactions can lead to a wide variety of symptoms, which can be as diverse as ill-defined malaise and fatigue to digestive disorders, skin problems, aching joints or back pain. One type of reaction can proceed to a different type as the immune response alters to chronic challenge. For example, cow's milk allergy in infancy, even when properly treated, can lead years later to respiratory atopy and persistent atopic dermatitis and recurrent ear infections.[188, 189] These observations have led others to speculate that it is the allergic condition (or sensitivity) rather than a specific "allergy" to milk that increases otitis media risk.[190] A mechanism to explain such phenomena must include initial IgE-mediated atopic reactions that give way to later chronic Ig-Ag reactions of the type described below in the section, "Food-Specific IgG Antibody Testing."

In many people, undetected food reactions can be a significant factor in overall health. The challenge is to clearly define which foods may be causing adverse reactions. The ultimate proof of an adverse food reaction is via an elimination-provocation process, where the suspected food is removed from the diet and then later reintroduced. If symptoms subside when the food is avoided and return upon reintroduction of the food, food intolerance is confirmed. This approach is time consuming, but may be used for immediate reactions where the symptoms may be assessed relatively easily. For example, if watery eyes and headache occur whenever shrimps are eaten, then elimination of shrimps from the diet may prevent the symptoms. If they occur again when a trial of consuming a few shrimps is done, then some antigen in shrimps is causing the problem. In the case of delayed reaction, this process becomes much more challenging since the pairing of food-related reactions is less obvious.

The obviously tedious process of elimination-provocation can be expedited with the use of in vitro food-specific antibody tests. These tests measure the amounts of antibodies that cause food reactions. Although there are several ways foods can cause adverse reactions, the antibody-mediated types are the most common, and these tests provide the clinician and patient useful data to design appropriate diets that avoid the major offending foods.

Table 7.4 —Characteristics of IgE and IgG-Mediated Reactions to Food

IgE Mediated	IgG Mediated
Incidence is relatively low	Incidence is relatively high
Result from infrequent exposure	Result from frequent exposure
Very predictable short term symptoms	Chronic, variable symptoms
Offending food is usually obvious	Offending food frequently not suspected
Basophil/Mast Cell triggered anaphylaxis	Immune complex trigger
Histamine/Leukotriene release	Inflammatory response
Patient aware of offending food	Patient rarely aware of offending food
Antibody persistent for years	Antibody declines within one month
In vitro testing for serum IgE for confirmation	In vitro testing for serum IgG4 shows food offenders and extent of gut permeability
Treatment: Permanent food avoidance and immunotherapy	**Treatment:** Eliminate then rotate food(s), heal gut, improve digestion

Food-Specific IgG Antibody Testing

Abnormal reactions to foods we eat are quite common. Some estimates are as high as 50% for the US population.[191] Clinical presentations range from chronic, mild reactions to serious conditions, such as celiac disease, where gluten, a protein in cereal grains, causes inflammatory reactions leading to degradation of intestinal villus stuctures. Celiac disease can easily be undetected or misdiagnosed because of the highly variable type and severity of symptoms. Abnormal food reactions can cause many different types of symptoms. Common ones include the following:

- **GI**: gas, bloating, pain, diarrhea, constipation
- **Neurological**: migraines, headaches, brain fog, hyperactivity, depression
- **Somatic**: unexplained fatigue, myalgias, joint pain
- **Immune**: recurrent infections (ear, sinuses)
- **Respiratory**: chronic mucus, stuffy nose
- **Dermatological**: rash, urticaria, allergic shiners

With adverse food reactions so prevalent, the challenge is to clearly define which foods may be causing a problem in each patient.

Types of Adverse Food Reactions — Although there is some disagreement underlying mechanisms, most experts agree that adverse reactions to food should be classified as food allergy, food sensitivity, or food intolerance.

(1) Food allergy: This term will be used to designate a response mediated by food-triggered basophil or mast cell histamine release. This can be caused by either IgG or IgE food-specific antibodies. These reactions are immediate in nature and can be severe. This is the Type I allergic reaction.

(2) Food sensitivity: This term relates to a purely immune system-mediated response involving various classes of food-specific immunoglobulin molecules that can form food immune complexes. These complexes can stimulate the complement cascade and localized inflammation. These reactions tend to be delayed—hours up to 7 days after food consumption—in some cases. This is a Type III allergic reaction.

(3) Food intolerance: This term refers to a non-immunological mechanism of adverse food response. Examples would include lactose intolerance and MSG sensitivity.

The IgE-mediated food allergy is the most commonly known reaction where contact with food causes an immediate response. In some cases, this can cause anaphylaxis. This reaction involves basophil or mast cell degranulation and histamine release. Skin scratch testing can often reliably identify offending foods. In vitro tests for the food-specific serum antibodies are also an effective means of identifying reactive foods. This is the method of choice if there is a severe reaction initiated by the food. Since the IgE molecule can persist for years, the most reliable treatment is long-term avoidance of the offending food.

Although the IgE response is the most widely recognized form of an adverse food reaction, the most common type is the IgG-mediated form. This type is less understood because of the intricacies of the human immune system itself. The IgE system is designed to detect and react to relatively tiny amounts of antigen. Witness the common inhalant allergic response to small amounts of unseen pollen in the air. Consumed food, on the other hand, represents a huge amount of potential antigen presentation to the immune system. This situation will elicit primarily an IgG response, not an IgE response. Circulating levels of food-specific IgG can combine with food antigens in the blood, which can then form large food immune complexes that ordinarily are removed from the circulation by macrophages. When macrophages become overloaded with complexes, these accumulate in the blood and can deposit in various tissues, stimulating complement fixation, which leads to a localized inflammatory response. This process can take hours to days to produce a perceived reaction. This delay between food consumption and symptom makes it difficult for a person to recognize that the foods being consumed are actually causing symptoms. This is why this type of adverse food reaction is sometimes referred to as "hidden food allergy." In most cases, the offending foods are those most commonly eaten. These will present large amounts of antigen for the development of the IgG antibody production. Table 7.4 summarizes characteristics of IgE- and IgG-mediated immune reactions to foods.

Interestingly, the ultimate cause of this situation is not necessarily the characteristic of the food itself, rather, the condition of the digestive tract that allows the process to develop. One major theory as to how

delayed food sensitivities develop revolves around the concept of a "leaky gut." Ordinarily the digestive tract will efficiently break down and absorb consumed food as small molecules, amino acids, simple carbohydrates, etc., which are, in general, non-antigenic. If, for various reasons not uncommon today, digestion becomes less efficient and/or the intestinal lining becomes more permeable to large molecules, the conditions are set for the development of an IgG response to eaten food. It is assumed that this "leaky gut" condition allows macromolecular food fragments into the circulation, where they can stimulate a typical immune response where IgG is the primary antibody produced to defend the body against perceived non-self-invaders.

There are four subclasses of IgG, 1 through 4. IgG1 antibodies are the initial IgG class responders to a new food antigen. IgG2 and IgG3 are generally not produced to food antigens. They react to cell surface oligosaccharides of viruses and protozoa, respectively. Once IgG1 binds to the antigen, the antibody-antigen complex is quickly destroyed by the Kupffer cells in the liver and other macrophages. The IgG1-antigen complex can also stimulate the complement cascade and attendant inflammation. This cascade of events is associated with the general malaise experienced from the inflammatory response.

Upon continued exposure to the antigen, IgG1 antibody production will "class switch" to IgG4. Interestingly, IgG4 antigen complex does not activate the complement cascade. IgG4 is monovalent (can only bind to one epitope of an antigen at a time) and can be bi-specific. The Fab regions of the antibody are specific for two different antigens (see Figure 7.9). All other IgG subclasses are divalent and monospecific.[192] IgG4 acts as a "blocking" agent against the actions of IgE and can form small complexes as antigen exposure increases. These IgG4 food immune complexes have a relatively long half-life and are subject to alterations that would affect the structure enough to present as a "new" antigen. It is thought that IgG1 is then produced to attack this complex. Thus begins a whole new cycle: IgG1 →IgG4 →complex →modification →IgG1 →IgG4. Consequently, the complexes can get larger and larger. These larger complexes can activate the complement cascade, initiating inflammatory responses in the body. It is this inflammatory response to a food that is thought to be the root cause of symptoms in this type of adverse food reaction. The symptoms resulting from food sensitivities, therefore, can come from the activation of complement via IgG1/IgG4 food immune complex. Deposition of these complexes can also occur in tissue or organs, leading to damage. This sequence of events is thought to be the most common way individuals develop adverse reactions to foods they eat on a regular basis.

IgG1 antibodies tend to be more "sticky" and can bind more non-selectively to antigens, leading to a greater chance of cross-reactivity and false-positives; for example, watermelon and ragweed are cross-reactive. Measuring both IgG1 and IgG4 together can cause many unnecessary food eliminations. The IgG4 antibody is, therefore, a more clinically relevant marker of chronic food-immune reactions and possible intestinal permeability. IgG4 measurements are less likely to produce false-positives on in vitro tests. In a similar fashion, measurement of total IgG tends to produce a high rate of false-positive reactions.

Although the lack of mechanistic explanations has delayed its recognition by mainstream medical education, the phenomenon of "hidden food allergies" has long been recognized by most practitioners of integrative and functional medicine. Previous presumptions about antibody actions are modified by recent research findings that provide a more complete picture of the diversity of antibody and complement system reactions to food antigens. Food-specific IgG4 antibody testing was developed to meet the need for a reliable assay that would help the clinician to assess delayed food sensitivities that produce patient symptoms.

Notes:

Two studies have been published that show significant clinical improvement through food elimination based on IgG test results. One study examined 114 subjects with a variety of symptoms. Serum IgG antibodies were measured for most commonly consumed foods. Patients eliminated those foods with the highest levels of food-specific IgG, and symptoms were correlated before and after diet changes. In this study, 71% of all symptoms achieved at least a 75% improvement following elimination of these foods.[193] Another study looked at 150 patients with inflammatory bowel disease. Food elimination was based on elevated serum IgG levels to specific foods. Results were compared with patients eating a sham diet. After 12 weeks, patients eliminating foods that showed elevated IgG showed significantly better symptom improvement compared with patients eating a sham elimination diet.[194]

Test Report Interpretation — Many laboratories perform food-specific antibody tests in large panels of up to 100+ foods. This may be somewhat excessive given the fact that most people eat no more than about 20 different foods. Test interpretation can vary depending on various circumstances described below.

Many IgG4 Reactions: If multiple foods have strong elevations of IgG4, an intestinal permeability problem is indicated. This "leaky gut" phenomenon causes numerous food antigens to permeate into the bloodstream and present to the immune system's lymphocytes, which then respond by generating IgG4 antibodies. High levels may or may not reflect adverse symptoms. In general, consuming these foods puts the patient under stress. Avoiding the reactive foods may not completely solve the health problem since the leaky gut problem will still exist, and the person will simply make new antibodies to the new foods leaking into the blood from the gut. A better approach is to have the patient eliminate the 3 to 6 most reactive foods and rotate consumption of the others on a 4-day rotation plan; that is, don't eat a food any more frequently than once every 4 days. By avoiding the most offending foods, the diminished IgG response can provide an interval of relative calm from inflammatory propagation of the gut pathology.[195]

Nutrients that support the development of intestinal integrity include 5 to 10 g daily of glutamine, 1 to 3 g of pantothenic acid daily, and 25 to 50 mg of zinc daily. The tall columnar epithelial cells of the small intestine derive most of their energy from oxidation of glutamine,

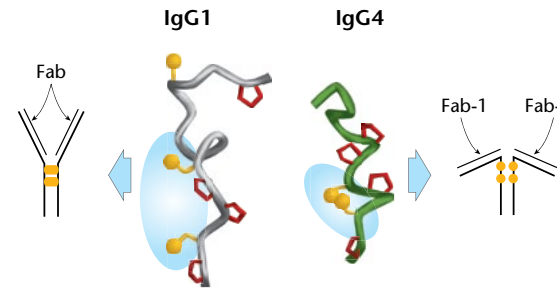

FIGURE 7.9 — Structural Basis for Unique IgG4 Properties

The yellow atoms within the colored ellipses represent cysteine side chains. They are not juxtaposed in the IgG1 heavy chains, allowing disulfide bridge formation in the multi-chain complex that produces the stable "Y" configuration. The corresponding residues in the IgG4 heavy chains form intrachain disulfide bonds, changing the conformation of the final IgG4 complex. The lack of cross-chain disulfide bonds allows hybrid complex formation where the two light chains are different, so they bind only one antigen (monovalent).[192]

and the pantothenic acid and zinc provide assurance of support for increased cell mass. It should not be necessary to maintain this level of support for more than 60 days. The amount of glutamine required may be reduced to 1.5 g doses by adding 10 mg of pyridoxine and 10 mg of α-ketoglutaric acid with each dose. This combination appears to aid the efficiency of epithelial cell utilization of glutamine.[196] Various botanical substances, such as aloe, chamomile, slippery elm, deglycyrrhizinated licorice (DGL), okra and marshmallow, have a long traditional history of use for improving gastrointestinal health, and they generally possess mucilaginous properties that allow for a protective coating of the mucosal lining, reducing irritation and facilitating the healing process. Other herbs and plant bioflavonoids may contribute to intestinal healing by providing an antioxidant and/or anti-inflammatory effect (e.g., ginger, curcumin, boswellia, quercitin, rutin, hesperidin, etc.).[197-208]

Moderate Number of IgG4 Reactions: IgG elevations to less than half of the foods routinely consumed may indicate a less severe intestinal permeability problem, and steps should be taken as indicated above. In many cases, total elimination of all of the reacting foods for 1 month coupled with nutritional support will ameliorate this condition. Food rotation can also be

beneficial as symptoms improve. If symptoms return after a resumption of normal eating patterns, the rotation diet and nutritional support should be continued.

Few IgG4 Reactions: If there are only a few IgG reactions to foods, these should again be eliminated from the diet to test for involvement in patient symptoms. In many cases, there will be significant improvement. Other etiological factors may also be involved in patient health problems.

No IgG4 Reactions: The absence of reactions in this test may be due to several possibilities. First, the gut may be intact and there is no intestinal permeability. Second, non-steroidal anti-inflammatory drugs are known to suppress the immune response and IgG4 production. Next, some individuals do not produce IgG4 antibodies to foods as most people do, and they may compensate for this by producing other IgG subclass antibodies. Finally, since this test measures the immune response to exposure of the system to food antigens, the test will not show a positive reaction if the patient is not consuming a food for reasons of known intolerance. Without antigenic stimulation, IgG will not be produced.[209] This could give a false-negative result for those foods not being consumed. The patient should be counseled that a negative result on this test for the food does not necessarily mean they can eat the food now without causing a reaction if they are already on an elimination diet due to known food reactions. Reintroduction of known food allergens should be done with caution.

The test can be very useful in screening for potential foods the person is eating on a regular basis that may be causing adverse physical reactions. Treatment involves removal of offending foods; digestive aid supplements of betaine HCl and pancreatic enzymes; oral probiotics and prebiotics for establishing favorable bacterial populations; oral glutamine, the primary energy source for intestinal epithelial cells; and supportive nutrients as described above for attenuation of gut permeability.[210] Patients can usually start to reintroduce foods that had been positive after 6 to 9 months, when other acute health threats are absent.

IgE Food Antibodies

Individuals prone to developing strong immediate food allergic responses are called "atopic" and are said to suffer from allergies. The IgE antibody is the cause of allergic reactions most frequently seen as inhalant allergies (e.g., hay fever). Foods can also cause a buildup of IgE in the blood during the early stages of allergic food reactions.[211] The sequence of events in the GALT involves antigen presentation that leads to activation of a class of lymphocytes known as Th2 helper T cells. These cells secrete cytokines that instruct B lymphocytes to produce antigen-specific IgE. On antigen recognition, the presence of IgE in their cell membrane causes stimulation of mast cells, basophils and eosinophils.[141] Mast cell histamine release produces symptoms typical of the allergic response. This mechanism is most often considered to be associated with inhalant allergies.

In most cases, people will easily recognize an IgE-mediated food reaction because the allergic response is immediate—a person eats a food and has a noticeable, immediate reaction. One then learns to avoid these foods in the future. A small percentage of patients can have reactions to as little as 1 mg of offending food. However, for most patients, antigen consumption in the range of 30 to 80 mg is required to produce significant IgE-mediated response to a food.[212] Chronic low exposures can also cause reactions that are not as noticeable and can contribute to chronic illnesses not necessarily associated with the allergic food response. Such low-level IgE responses may contribute to "hidden food allergy," causing chronic symptoms unless the person identifies the foods and eliminates them from the diet.[213-215]

A cardinal feature of the atopic individual is that the offending food causes characteristic symptoms of sneezing, runny nose and itching eyes. Other responses may be present, but the appearance of the characteristic symptoms within minutes of exposure to a food is evidence of IgE-mediated allergy. When allergy first developed as a medical specialty, allergists thought the story ended here. Recent scientific insight acknowledges that there are other mechanisms of "allergy" that produce symptoms related to gut-immune responses such as IgG class of antibody directed at food antigens.

Notes:

The Microbial Mass

After the partially digested food mass (chyme) leaves the stomach, it moves through the small intestine past the ileocecal valve and into the colon. It carries nutrient-rich solutions at neutral pH and at a constant, warm temperature—conditions perfect for the growth of microbial populations. Oxygen, another growth factor for many microbes, is present at very low concentrations. Thus, the populations that predominate are anaerobic. Normal microbial population densities should be viewed as a continuum, rising from about 10^4 per mL in the upper jejunum to over 10^{12} per mL in the colon.[216] Stomach acid and digestive enzymes are major factors controlling species selection and growth rates in chyme. The approximate 3 pounds of bacteria resident in a normal healthy colon constitute a mass that exceeds all organs in the body except skeletal muscle.

The dynamically perpetuated mass of microbes can have many beneficial effects. Their presence stimulates the maintenance of the well-developed villus and its supporting vasculature and musculature.[217] The feces normally are about three-quarters water and one-fourth dry solid matter composed of about 30% bacteria, 10 to 20% fat, 10 to 20% inorganic matter, 2 to 3% protein, and about 30% undigested food roughage.[218] This composition varies as a function of the amount and type of fiber in the diet. The fat in feces is mostly fat synthesized by bacteria or fat from sloughed off epithelial cells, though some can come from unabsorbed dietary fat. The brown color of feces is primarily due to bilirubin derivatives. Products of bacterial action that include indole, skatole, mercaptans and hydrogen sulfide cause the odor; these can vary from person to person depending on their diet and colonic bacterial flora.[219]

Notes:

Although colonic bacteria are capable of digesting small amounts of cellulose, the amount of energy derived from this digested fiber is negligible. Other substances formed as by-products of bacterial activity are short-chain fatty acids, vitamin K, biotin, vitamin B_{12}, thiamin and riboflavin.[216] Bacterial synthesis of vitamin B_{12} is of little significance because it is poorly absorbed in the colon. On the other hand, bacterial-formed vitamin K is nutritionally relevant. In the absence of vitamin K-forming bacteria, vitamin K status declines because dietary vitamin K is normally insufficient.[220]

Many of the biotransformation products coming from bacterial metabolism are toxic. Gram-negative anaerobic bacteria are the principal origin of ammonia produced from peptides and amino acids. Bacteria may be classified as high, moderate or low rates of in vivo ammonia production as shown in Table 7.5. The reduction of their ammonia output when lactulose is added to the medium accounts for the beneficial effects of lactulose in treatment of hepatic encephalopathy.[221] Bacterial

Table 7.5 — Ammonia Producing Classes of Microbes[221]

Microbial Type	Ammonia Production
Clostridium, Enterobacter and *Bacillus* spp.	High
Streptococcus, Micrococcus, Bacteroides, Proteus and *Klebsiella* spp.	Moderate
Lactobacillus, yeasts	Low

production of ammonia and phenols is suspected of contributing to colon cancer. The normal bacteria in healthy humans produce markedly increased fecal ammonia and urinary phenols when humans consume a high-protein diet.[222] The colon has limited ability to detoxify these bacterial metabolites before passing them to the blood.[223] Colonocytes, as well as hepatocytes in the liver, contain cytochrome P450[224] and are capable of reduction and hydrolysis (Phase I reactions). They can also transform toxic compounds by conjugation with donor substrates (Phase II reactions). Failure of the colon to properly detoxify xenobiotics and drugs results in epithelial cell damage, which has major implications for diseases of the colon, including cancer.[79] Once absorbed, toxins present general systemic challenges. Microbial metabolic release, intestinal absorption and systemic distribution of microbial products are illustrated in Figure 7.10

The integration of immunologic and microbiologic concepts can lead to new insight about the origins of many disorders related to the gut. Reactions that have been referred to as food allergies may be immune reactions to antigens that arise from bacterial action on dietary proteins and are better described as enterometabolic disorders.[225]

Microbial Population Assessment

The intestinal flora is a complex ecosystem consisting of over 400 bacterial species that greatly outnumber the total number of cells making up the entire human body.[226] These metabolically active bacteria reside close to the absorptive mucosal surface and are capable of a remarkable repertoire of transforming chemical reactions. Any orally taken compound or a compound entering the intestine through the biliary tract or by secretion directly into the lumen is a potential substrate for bacterial transformation.

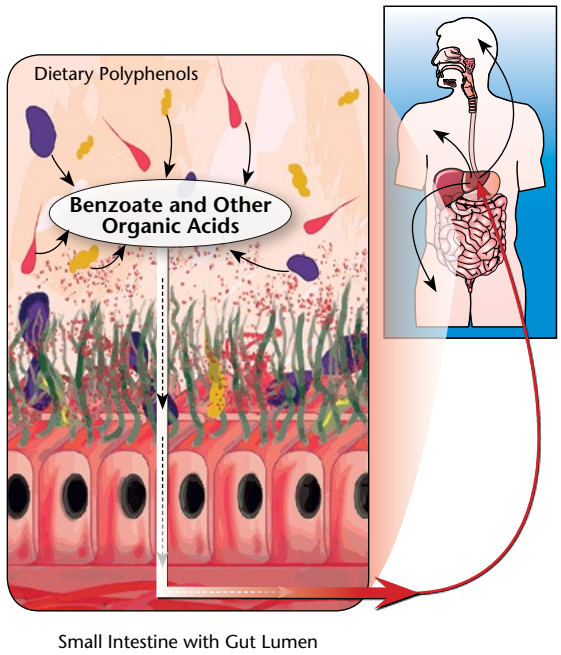

Figure 7.10 — Production, Absorption and Distribution of Microbial Metabolites

The production of microbial metabolic products is greatest in the transitional gut, where their growth rate is greatest. As populations reach their upper densities in the transverse colon, the rate of metabolite production slows and efficiencies of absorption are lower. Absorbed compounds are distributed by the circulatory and lymphatic systems to all tissues of the body where they may have toxic effects. Detoxification reactions convert some products into new compounds, while others appear unchanged in urine.

Anaerobic bacteria are the predominant microorganisms in the human GI tract, outnumbering aerobes by a factor of 10,000 to 1. The most abundant and beneficial or benign anaerobes are *Bifidobacterium, Bacteroides, Fusobacterium, Clostridium, Eubacterium, Peptococcus* and *Peptostreptococcus*. *Bifidobacterium* can comprise up to 25% of the total flora in a healthy adult. A great many other species are present, but in lesser numbers.[227] An imbalance in proportion and numbers of these species can be induced by broad-spectrum antibiotic use. This leads to the dominance of other bacterial species, including *Pseudomonas, Enterobacter, Serratia, Klebsiella, Citrobacter, Proteus, Providencia* and fungi, especially yeasts such as *Candida*. In health, the upper GI tract is sparsely populated with microorganisms. The vast majority of bacteria washed along with saliva from the oral cavity are destroyed in the stomach by gastric juice. Those that survive (if sufficiently resistant to gastric secretions and bile acids) tend to be gram-positive, facultative forms such as *Streptococcus, Staphylococcus* and *Lactobacillus*.[228] The small intestine constitutes a zone of transition between the sparsely populated stomach and the luxuriant bacterial flora of the colon. In the distal ileum, the concentrations of bacteria increase to 10^6 to 10^7 colony-forming units per milliliter.[216] Here, gram-negative bacteria outnumber the gram-positive species. Coliforms predominate and anaerobic bacteria, such as *Bacteroides, Bifidobacterium, Fusobacterium* and *Clostridium*, are found in large numbers. Beyond the ileocecal valve, the bacterial concentration increases steeply. Colonic bacteria number between 10^{11} and 10^{12} colony-forming units per milliliter of fecal material. Multiple dramatic shifts in populations of species occur between the ileocecal valve and the rectum. By the time they are passed from the body in stools, the large majority of the bacteria are no longer viable.

Intestinal Pathogens, Symbioses and Parasites

The spectrum of identifiable diseases of intestinal microbial overgrowth ranges from those causing mild, transient diarrhea to those causing pandemic diarrhea resulting in thousands of deaths. Diarrheal episodes resulted in 2 to 3.5 million physician visits and more than 200,000 hospitalizations per year in the United States

alone in 1996.[229] The treated cases are the acute cases that are easily recognized. The difficult cases are those with mild, chronic GI symptoms associated with excess microbial production of toxins in the intestinal lumen. The incidence of bacterial overgrowth of the small intestine increases steeply after age 70.[73] Elderly patients develop malabsorption syndrome secondary to bacterial overgrowth, often secondary to decreased endogenous acid production or the use of acid-reducing medications, placing them at risk of clinically significant nutritional deficiencies.[230]

The clinical perspective regarding gut ecology has shifted with modern cultural changes. In the early 1900s, the Nobel laureate E. E. Metchnikoff put forth his theory of orthobiosis as a state of health dependent on the correct balance of life forms inhabiting the gastrointestinal tract.[231] It was a commonly held belief that eating up to 3 pounds of meat per day was good practice. Such dietary habits lead to a condition that he termed "putrefactive dysbiosis." Diets high in meat and low in fiber can result in long transit times with undigested protein reaching the colon.

Modern studies give credence to Metchnikoff's hypothesis, showing that numerous toxic chemicals are generated by the action of colonic bacteria on undigested protein.[232] The enzyme urease induced in *Bacteroides, Proteus* and *Klebsiella* species by a high-meat diet hydrolyzes urea to ammonia. This raises stool pH, which is associated with an increased risk of colon cancer.[233] Decarboxylation of amino acids by gut bacteria yields vasoactive and neurotoxic amines, particularly histamine, octopamine, tyramine and tryptamine, which are absorbed and transported to the liver to be deaminated. In severe cirrhosis, these amines enter systemic circulation and contribute to encephalopathy and hypotension or hepatic failure.[234] The bacterial enzyme tryptophanase, also induced by a high-meat diet, degrades tryptophan to carcinogenic phenols.[235] Bacterial β-glucuronidase deconjugates estrogens, increases enterohepatic recirculation of these steroids and decreases their rate of clearance from the body, effectively raising blood estrogen levels and the risk of breast cancer.[236] β-Glucuronidase and other hydrolytic bacterial enzymes also deconjugate bile acids. Deconjugated bile acids are toxic to the colonic epithelium and cause diarrhea; they have been implicated as etiological agents in the development of colon cancer[237] and ulcerative colitis.[238]

Notes:

Opportunistic Overgrowth and Disease

Overgrowth of any one of the more than 400 microbial species in the healthy human gut can produce adverse clinical effects. Excessive colonization of the gut by undesirable microorganisms alters the metabolic or immunologic status of the host.[239, 240] When this state leads to disease or dysfunction, it has been termed "dysbiosis" to distinguish it from the correct balance denoted as orthobiosis.[241] The line between benign opportunistic overgrowth and infectious diseases is difficult to define because apparently benign, small numbers of colony-forming units may be detected for pathogens such as enterohemorrhagic *Escherichia coli*.

Dysbiosis and Inflammatory and Autoimmune Disease

The provocation of joint inflammation by bowel infection with salmonella, shigella and yersinia suggests an etiologic link between colonic bacteria and arthritis.[232] *Klebsiella* and *Citrobacter* species overgrowth has also been etiologically linked to inflammatory arthritities such as rheumatoid arthritis and ankylosing spondylitis, suggesting strongly that clinical evaluation of the intestinal environment should play a role in the assessment and even treatment of such disorders rather than reliance solely on anti-inflammatory agents and other symptomatic palliating medications.[178, 179, 181, 183, 184, 186] Linkage between *Yersinia* overgrowth and autoimmune thyroiditis has also been firmly demonstrated due to cross-reactivity (i.e., molecular mimicry) between *Yersinia* surface binding sites and TSH thyroid receptors, also suggesting gastrointestinal evaluation and optimization may be required for comprehensive management of autoimmune thyroid disorders such as Graves' disease and Hashimoto's thyroiditis.[182, 185, 187] Continued research into the role of molecular mimicry and immune

complex tissue cross-reactivity may indeed suggest gastrointestinal involvement in a large range of autoimmune phenomena in the years to come. Healthy immune barrier functions, including mucosal defensins are emerging as factors of prime importance in the maintenance of proper intestinal flora tolerance and the suppression of potentially pathogenic bacterial strains with autoimmune-stimulating potential.[139, 140]

Dysbiosis and SIDS

Some sulfate-reducing bacteria in the colon produce hydrogen sulfide (H_2S), one of the gases responsible for the unpleasant odor in flatus, and implicated by some preliminary studies as a contributing factor in sudden infant death syndrome (SIDS).[242-244] SIDS is the third leading cause of death among infants in the United States.[245] Colonocyte mechanisms for detoxifying hydrogen sulfide are often not mature in the infant, resulting in absorption of this and, potentially, other microbial toxins that have toxicity comparable to cyanide in rodent studies.[245-249] Peak incidence of SIDS is during the 2- to 4-month age group, which often corresponds to the introduction of bottle feeding and soy-based formulas. These dietary changes facilitate changes in the colonic microflora, which include the colonization of bacteria acting on sulfate.[250] Hydrogen sulfide elevations in the circulation can cause injury to the respiratory center in the brain and pulmonary edema and potentially sudden death.

Evidence of shifts in microbial populations from symbiotes to pathogens may be found in urinary markers, exhaled gases or fecal markers. All such evidence is indirectly related to the growth of organisms in the gut. Direct evidence requires sampling of intestinal content without perturbation of the local environment and performing strictly anaerobic culture growth on a fresh specimen. Such techniques are not used in routine clinical practice.

Due to the predominance of anaerobic bacteria in the colon, isolating colony-forming anaerobes from stool more than a few hours old is difficult in the laboratory and often yields misleading results. The new tools of molecular biology described below will help to overcome these limitations regarding lower colonic assessment. The detection of bacteria and yeast overgrowth in regions of the gut prior to the descending colon is best done by measuring the levels of specific by-products of microbial activity in urine, which serves as a type of "fingerprint" for intestinal overgrowth in the transitional gut.

Urinary analysis for compounds produced by microbes in the gut provides indirect evidence of overgrowth. Accurate identification of species from compounds found in urine is generally not possible. By knowing the relative toxicities of the compounds found elevated, however, the additional information of metabolic impact from the overgrowth can be weighed, as explained in the section below titled, "Urinary Markers of Bacterial Overgrowth."

Dysbiosis and Colon Cancer

Bacterial putrefaction is very old concept regarding intestinal health.[251] The initial observations of odors from patients consuming high-protein and low-vegetable diets led to vague presumptions about various maladies associated with the activities of intestinal bacteria. More recent studies have focused on the well-known association of diet with cancer, especially large bowel cancer.[252-254] The production of phenolic compounds by intestinal bacterial action on dietary protein has been proposed as a potential mechanism connecting low-vegetable diet with increased colon cancer incidence.[255] Pure strains of bacteria grown in culture produce either phenol or p-cresol as shown in Table 7.6. Note that only *Staphylococcus albus* shows significant production of both compounds. All other organisms studied had high specificity for either phenol or p-cresol under those conditions.[255] Early studies had also indicated the origin of the phenols to be unabsorbed tyrosine from dietary protein, as urinary levels showed systematic increases when

Table 7.6 — Phenol and p-Cresol Production by Human Fecal Bacteria

Organism	No. Strains	Volatile Phenol Produced	
		Phenol	p-Cresol
Escherichia coli	6	+	–
Proteus sp.	5	+	–
Streptococcus faecalis	3	+	–
Pseudomonas sp.	5	–	–
Staphylococcus albas	1	–	+
Bacteroides fragilis	3	–	+
Fusobacterium sp.	2	–	+
Bifidobacterium sp.	3	–	+
Clostridium paraputrificum	1	–	+
C. butyricum	1	–	+
C. sporogenes	2	–	+
C. septicum	2	–	+

dietary protein was increased for patients under treatment for "pneumonia, pleurisy and chorea."[256] These researchers also demonstrated the lack of human tissue conversion of benzene into phenol by a trial, amazing from the modern viewpoint, in which 1.6 g of benzene was administered to a patient with myelogenous leukemia. These instances of phenolic compound production from dietary amino acids stand in contrast to the apparently favorable production of similar compounds from dietary polyphenols as discussed above when plant-based diets are consumed. Such differences may account for part of the increased degenerative disease incidence associated with animal protein intake.[257]

Colon Cancer Marker (Fecal M2-PK):

The emergence of the fecal M2-PK marker has improved the sensitivity of colorectal cancer diagnosis.[258, 259] The key regulator of the metabolic alterations found in tumor cells is the glycolytic isoenzyme pyruvate kinase type M2. This enzyme is expressed in all proliferating cells and is universally overexpressed in tumor cells. During carcinogenesis, a shift in pyruvate kinase isoenzymes takes place, with less expression of M1-PK in muscle and brain, L-PK in liver and kidney, and R-PK in red blood cells, and an overexpression of M2-PK in all affected tissues. Therefore, increased M2-PK is associated with abnormal metabolic states specific for tumor cells from various tissues, such as gastrointestinal, lung, renal and pancreatic.[260-268] The % sensitivities for M2-PK and other cancer markers in diagnosing colorectal, gastric, oesophageal and pancreatic cancer are shown in Table 7.7 and compared in Figure 7.11.

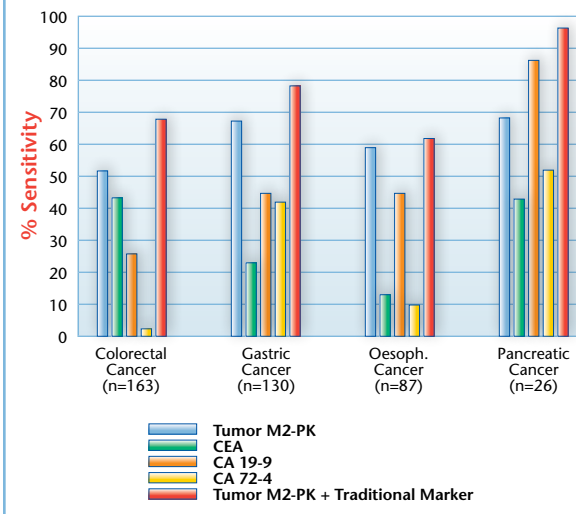

FIGURE 7.11 — Sensitivity of M2-PK in Diagnosis of Gastrointestinal Cancer

The diagnostic sensitivity for plasma M2-PK was evaluated in 413 cancer patients and 141 patients with non-malignant diseases. Highly significant discrimination of tumor patients and controls was found (p = 0.001) for M2-PK, especially when combined with traditional markers CEA or CA-72.[383]

The combination of fecal M2-PK with carcinoembryonic antigen (CEA) and/or CA19-9 in the diagnosis of colorectal and pancreatic cancer strongly increases the sensitivity with only a modest loss of specificity.[50, 258, 259] Tumor M2-PK is now commercially available and is emerging as a valuable part of the diagnostic routine for GI cancer.

TABLE 7.7 — COMPARISON OF THE SENSITIVITIES OF TUMOR M2-PK, CEA, CA 19-9 AND CA 72-4 IN DIFFERENT GASTROINTESTINAL TUMORS

			Sensitivity of:				
		N	Tumor M2-PK	CEA	CA 19-9	CA 72-4	
Colorectal Cancer	Non-metastasized	117	48%	34%	18%	ND	Tumor M2-PK + CEA: 67%
	Metastasized	46	54%	72%	50%	ND	
	Total	163	50%	42%	27%	ND	
Gastric Cancer	Non-metastasized	65	63%	12%	31%	25%	Tumor M2-PK + CA 72-4: 82%
	Metastasized	72	71%	39%	54%	57%	
	Total	130	67%	26%	45%	41%	
Oesoph. Cancer	Non-metastasized	77	57%	9%	16%	10%	Tumor M2-PK + CA 19-9: 65%
	Metastasized	10	60%	20%	70%	14%	
	Total	87	59%	15%	43%	12%	
Pancreatic Cancer	Total	26	73%	42%	85%	43%	96%

The Transitional Gut

The microbial mass increases from levels around 1×10^5 to counts as high as 1×10^{11} in the region roughly encompassing the mid-ileum to the transverse colon. This 6 orders of magnitude increase represents a fantastically high rate of new cell formation with associated intense metabolic activity where metabolic products are formed. Thus, it is from this mid- or transitional gut that most of the microbial products found in breath or urine are chiefly derived.

Breath Hydrogen and Methane

This test offers reasonable sensitivity, and it is very convenient to administer, although it will sometimes give false-negative results. For greater predictive value, it is best to also obtain a baseline breath sample from the patient before consumption of the challenge solution. The fasting patient drinks a challenge dose of lactulose (10 g) or glucose (75 g) solution. Breath samples should be collected every 15 minutes for up to 2 hours. If bacteria exist in the small intestine, they will ferment the sugar and release hydrogen and methane, which can be detected in the breath. The typical fasting breath sample contains less than 10 ppm hydrogen or methane; levels higher than 20 ppm indicate a high probability of bacterial overgrowth. Following a lactulose or glucose challenge, a two-phase response may be seen. The first rise in breath hydrogen occurs within 30 to 60 minutes when lactulose contacts the small intestine; a second, more pronounced rise occurs about 2 hours later when the sugar enters the large intestine. A rapid and prolonged first-phase response is frequently due to small intestine bacterial overgrowth.[269] Interpretation of results is complicated by the large number of false-positive findings as compared with results obtained from bacterial cultures of intestinal lumen aspirates.[270, 271] Combining the findings of elevated fasting breath hydrogen (> 20 ppm) and raising the limit for the post-lactulose challenge increase in breath hydrogen and methane to greater than 15 ppm reduce the chance of false-positive responses.[272]

[^{14}C]Xylose Breath Test

For this test, the patient drinks a solution containing [^{14}C]xylose, which is normally absorbed and excreted in the urine. In the presence of excess bacteria in the small intestine, [^{14}C]xylose is metabolized by bacteria to form $^{14}CO_2$, which is absorbed and eliminated through the lungs. Excess breath $^{14}CO_2$ is diagnostic for bacterial overgrowth of the small intestine. This test has limited usefulness for diagnosing overgrowth in the small intestine because it is prone to giving excessive numbers of false-positive and false-negative results, especially in patients with intestinal motility problems.[273, 274]

Breath Ethanol

Carbohydrate intolerance may be the only symptom of bacterial overgrowth, making it indistinguishable from intestinal candidiasis; in either case, dietary sugars can be fermented to produce endogenous ethanol.[275] The production of ethanol following an oral glucose load was found in 61% of chronically ill patients. The symptoms include abdominal distension, carbohydrate intolerance, fatigue and impaired cognitive function. The majority of cases are thought to be due to yeast overgrowth.[276]

Urinary Markers of Bacterial Overgrowth

The intestinal flora can be thought of as a chemical factory with massive levels of active enzymes. All rapidly growing bacterial species in the small intestine produce metabolic by-products. Many of those products that are not used by competing microbes can be absorbed and appear in urine either unaltered or as conjugates produced in hepatic detoxification reactions. These by-products may be helpful, toxic or benign. The absorbed products may be utilized for energy immediately in the epithelial cells of the gut, acted on by the detoxification systems in the liver or passed into urine unchanged. The patterns of specific compounds that are detected in urine allow some deductions about the nature of the originating organisms.

By the indirect procedure of examining microbial products in urine, the in vitro growth conditions are completely unaltered by the assay. Multiple products may be measured, providing the opportunity to examine patterns reflective of specific types of overgrowth. Putrefactive dysbiosis may be the result of excessive protein

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intake, the overgrowth or imbalance of various bacterial species or exposure to antibiotics. Bacteria much more actively metabolize amino acids than yeast, and not all bacteria carry out the same reactions. Grown under anaerobic conditions, fungi produce unique products that allow their detection. Many of the microbial products that appear in the urine are organic acids that have been further discussed in Chapter 6, "Organic Acids." Direct assay of the microbial mass by stool culture and sensitivity testing is discussed below in the section, "The Colon: Assessing Microbes in Stool."

Urinary Indican — Bacteria in the upper bowel produce the enzymes that catalyze the conversion of tryptophan to indole (Figure 7.12). Absorbed indole is converted in the liver to indoxyl, which is then sulfated to allow urinary excretion as indoxyl sulfate (indican). An elevated level of urinary indican was reported for patients with adult celiac disease[277] and was demonstrated in 8 of 12 patients following jejuno-ileal bypass surgery.[278]

Oral, unabsorbed antibiotics reduce indican excretion. Indican excretion is also reduced when the gut is populated with strains of *Lactobacillus* at levels above 10^5 organisms/g.[279] Probiotics have been shown to decrease indican levels. *L. salivarius*, *L. plantarum* and *L. casei* were more effective in achieving reduced indican than were two strains of *L. acidophilus*.[280]

Indican testing can differentiate pancreatic insufficiency from biliary stasis as the cause of steatorrhea (fatty stools). Patients with steatorrhea due to pancreatic insufficiency show a rise of indican from low values to above normal when they are treated with pancreatic enzyme extract.[281] When steatorrhea is not due to pancreatic insufficiency, addition of oral pancreatic extract did not produce higher indican levels.[281] This scenario demonstrates how bacterial populations respond to increased luminal concentrations of undigested amino acids. Large shifts in bacterial populations induced by the artificial sweetener saccharin have also been demonstrated by changes in indican excretion. This effect is again thought to be related to changes in metabolism of amino acids by gut flora.[282]

No age adjustment for reference limits is necessary, since excretion is constant for young and elderly control subjects.[283] The test may be performed after oral loading of 5 g tryptophan.[284] Reference limits may also be set from data taken under non-loading conditions. Tryptophan loading, which can be monitored by measuring periodic levels of urinary indican, results in neuropsychiatric manifestations due to products of intestinal bacterial conversion of the amino acid.[280] When elevations of phenol and p-cresol are included with that of indican as criteria of abnormal bacterial colonization of the small intestine, the number of false-positives is reduced.[285]

The use of indican results are complicated by impaired protein digestion, which may increase the tryptophan available for bacterial action. The relationship between increased indican and incomplete digestion might be utilized as a measure of protein digestive adequacy. Increased urinary indican has been shown to correlate with enteric protein loss.[286] Indican elevation has revealed that impaired protein digestion and increased bacterial conversion of tryptophan is a complication of cirrhosis of the liver.[287] Some degree of malabsorption was found in 30% of an elderly population by combinations of indican with the Schilling test, as well as other tests described below.[288]

Urinary Phenolic Compounds — Dietary polyphenolics are the principal substrates from which products of transitional gut bacterial metabolism are formed. Further discussion of polyphenol substrates may be found in Chapter 6, "Organic Acids." In addition to the

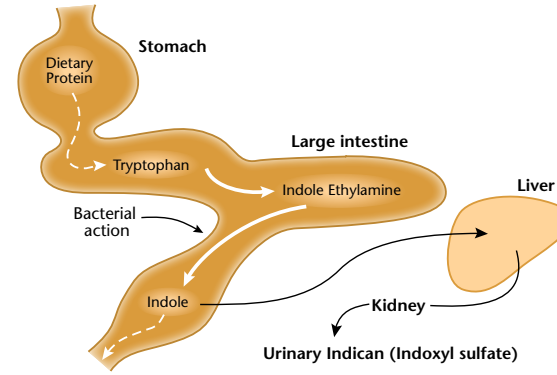

FIGURE 7.12 — Intestinal Tryptophan Conversion to Urinary Indican

Urinary indican arises from unabsorbed dietary tryptophan (Trp). Intestinal bacteria degrade Trp to indole by successive reactions. Indole is efficiently absorbed and quickly oxidized to indoxyl in the liver. The final step of sulfation is carried out in the liver and kidney, producing urinary indoxyl sulfate (indican).

use of polyphenol compounds, intestinal bacteria that contain L-amino acid decarboxylase enzymes degrade tyrosine to tyramine. The tyramine is then deaminated and oxidized to p-hydroxyphenylacetate. This product is excreted unchanged and unconjugated in urine. It was used to identify small bowel disease and bacterial overgrowth syndromes in 360 randomly selected, acutely ill infants and children. In this study, no false-negative and only 2% false-positive results were found.[289] Treatment with metronidazole or mepacrine has been shown to eliminate the p-hydroxyphenylacetic aciduria. Although p-hydroxyphenylacetate can be produced in the liver, abnormally high levels in urine are of bacterial origin.

Urinary p-cresol increases along with an increase in fecal ammonia and fecal volatile sulfur substances. The urinary output of p-cresol is probably due to the action of colonic bacteria on protein; this is based on observed urinary p-cresol increases following increases in fecal short-chain fatty acids and ammonia in people on a high-protein diet.[290]

Phenylacetate is one of the compounds that accumulate in the genetic disorder phenylketonuria (PKU). The neurotoxicity of phenylacetate is probably due to very strong inhibition of synaptic choline acetyltransferase.[291] Phenylacetate elevation due to dysbiosis has the same metabolic effects as elevation due to PKU, where it is linked to behavior and learning disabilities.[292] High urinary benzoate and hippurate have been associated with intestinal bacterial overgrowth[293].

Urinary Tricarballylate — Extensive studies in animals have shown that tricarballylate in urine comes from such bacteria in the intestines.[294] Species that produce this compound frequently infect the foregut of ruminant animals such as cows and may also be present in humans with dysbiosis. Tricarballylate binds magnesium very tightly and prevents absorption, leading to magnesium deficiency in rat studies.[295] Although further studies in humans are needed to determine the origins and dispositions of tricarballylate, the appearance of the compound in upper population quintile amounts is suggestive of interference with magnesium absorption.

Urinary 3,4-Dihydroxyphenylpropionate — *Clostridium* species constitute a major portion of the bacteria of the lower ileum and colon. Being strictly anaerobic, they are difficult to culture from stool and are not reported on most stool cultures. *Clostridium difficile* is associated with enterocolonopathies. Cases of confirmed *Clostridium* overgrowth show elevated levels of dihydroxyphenylpropionate, which fall to baseline with Flagyl treatment, but are unaffected by nystatin.[296] Although other organisms may produce 3,4-dihydroxyphenylpropionate, clostridia are the most commonly encountered genera among those susceptible to Flagyl. Compounds closely related to 3,4-dihydroxyphenylpropionate are produced by the genus *Clostridium*.[297]

Urinary D-Lactate — Another product of bacterial fermentation of sugar is D-lactic acid. Although D-lactic acidosis is usually a complication of short-bowel syndrome or of jejuno-ileal bypass surgery (colonic bacteria being the source of acidosis), elevated levels of D-lactate were found in blood samples of 13 of 470 randomly selected hospitalized patients.[298] Small intestinal fermentation was a likely cause of D-lactic acidosis in these 13 patients, 60% of whom had a history of gastrointestinal surgery or disease. One case has been reported to have D-lactic acidosis 23 years after a jejunal-ileal bypass procedure. The patient presented at that interval from the surgery with signs of D-lactate encephalopathy, including dizziness, ataxia, confusion, headache, memory loss and aggressive behavior.[299] Elevated D-lactic acid can be found in cases of overpopulation of the small intestine with *L. acidophilus*, as a result of low endogenous stomach acid production or the chronic use of acid-reducing medications accompanied by ingestion of large quantities of dietary carbohydrate.[300]

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Urinary Markers of Yeast Overgrowth

As elaborated above, Metchnikoff's ideas of putrefactive dysbiosis have been recognized for several decades. More recently, the prevalence of a new type of dysbiosis has gained recognition. In contrast to putrefactive dysbiosis in the colon, fermentative dysbiosis can be present in the small intestine. Although colonic fermentation is desirable, the proliferation of yeast, fungi and fermentative bacteria in the small intestine has detrimental effects. The production of toxic by-products can overload the detoxification pathways in the liver, and fungal enzymes can damage the mucosal epithelium.

Opportunities for fermentative dysbiosis are most apparent following jejunal or ileal resection or jejunoileal bypass surgery, where aerobic and/or anaerobic colonic organisms were found in jejunal aspirates from 8 of 12 patients.[278] Abnormal overgrowth of microbes occurs in surgical blind loop, intestinal stasis, gastric hypochlorhydria, and immune and nutritional deficiencies. Bacterial consumption of cobalamin lowers blood levels of vitamin B_{12}. Bacterial dehydroxylation of bile salts causes impairment of micelle formation that can lead to steatorrhea.[238] Some of the damage resulting from small intestinal overgrowth is attributable to the action of bacterial proteases that degrade pancreatic and intestinal brush-border enzymes. This causes pancreatic insufficiency and mucosal damage leading to malabsorption. *Candida* colonization of the GI tract impairs mucosal barrier defense against gram-negative bacteria.[301] Endotoxemia resulting from bacterial overgrowth contributes to hepatic damage.[302]

Regular use of antacids reduces the killing of yeast and bacteria in the stomach, and repeated courses of antibiotics cause sudden, dramatic reductions in populations of beneficial bacteria. These conditions set the stage for increased growth of microbes that produce toxins instead of nutrients. What was once healthy fermentation can become unfavorable dysbiotic overgrowth in the upper bowel.[70] Clinicians should be watchful for antacid abuse, which (in conjunction with antibiotic treatment), can lead to the development of disparate symptomatology secondary to intestinal dysbiosis (Table 7.8). This is particularly true for such patients consuming a diet rich in simple sugars. Responses vary widely depending on factors of mucosal integrity, immune competence and metabolic function.

TABLE 7.8 — FACTORS, SYMPTOMS AND DISEASES ASSOCIATED WITH DYSBIOSIS AND INTESTINAL HYPERPERMEABILITY[303, 304]

Contributing Factors	Diseases
Alcohol abuse	Inflammatory bowel disease
Corticosteroid use	Irritable bowel syndrome
NSAIDs use	Celiac disease
Excessive stress	Infectious enterocolitis
Nutrient insufficiencies	Cystic fibrosis
Gastrointestinal infections	Chronic fatigue immune deficiency syndrome
Food reactions	Acne
Improper fasting	Eczema
Symptoms	Psoriasis
Abdominal distention	Urticaria
Diarrhea	Dermatitis herpetiformis
Constipation	Autism
Abdominal pain	Childhood hyperactivity
Food intolerances	Spondyloarthropathies
Skin rashes	Pancreatic insufficiency
Poor exercise tolerance	HIV infection
Shortness of breath	Neoplasia treated with cytotoxic drugs
Cognitive deficits	Hepatic dysfunction
Fatigue and malaise	Alcoholism
Arthralgia	Environmental illness
Myalgia	
Fevers of unknown origin	

D-Arabinitol — D-Arabinitol (DA) is a metabolite of most pathogenic *Candida* species, in vitro as well as in vivo. DA is a five-carbon sugar alcohol that can be assayed by enzymatic analysis. Immunocompromised patients with invasive candidiasis have elevated D-arabinitol-creatine ratios in urine. Positive DA results have been obtained several days to weeks before positive blood cultures, and the normalization of DA levels has been correlated with therapeutic response in both humans and animals.[305, 306]

Measuring serum DA allows prompt diagnosis of invasive candidiasis.[306, 307] The somewhat more discriminating elevated urine D-arabinitol-L-arabinitol (DA/LA) ratio has been found to be a sensitive diagnostic marker for invasive candidiasis in infants treated in neonatal intensive care units. Eight infants with mucocutaneous candidiasis were given empiric antifungal treatment but had negative cultures; 5 of these had repeatedly elevated DA/LA ratios. Three infants with suspected and 4 with confirmed invasive candidiasis had ratios to become normalized during antifungal treatment.[308]

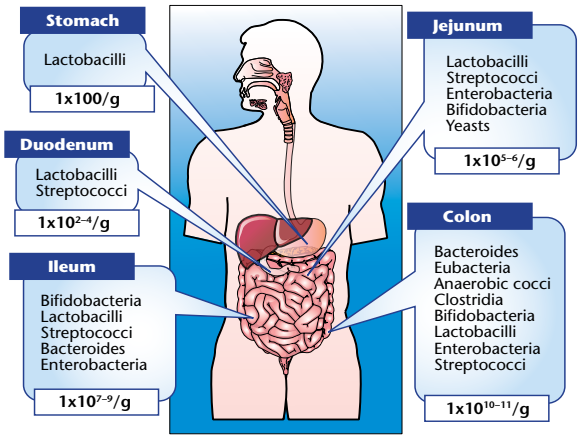

Figure 7.13 — Type and Amount of Bacteria in Regions of The Gut

For a typical healthy individual, bacterial populations change greatly moving from stomach to stool. The genus or class of predominant organism is shown inside each box and the total number of microbes per gram of intestinal content is shown at the bottom of the box.

The Colon: Assessing Microbes in Stool

The population of the microbiota of the human gastrointestinal (GI) tract is widely diverse and complex, with a high population density. All major groups of microorganisms are represented. Although they are predominately bacteria, a variety of protozoa are also present. In the colon there are over 10^{11} bacterial cells per gram and over 400 different species. These bacterial cells outnumber host cells by at least a factor of 10.[310] This microbial population has important influences on host physiological, nutritional and immunological processes. In fact, this biomass should more rightly be considered a rapidly adapting, renewable organ with considerable metabolic activity and significant influence on human health. Consequently, there is renewed and growing interest in identifying the types and activities of these gut microbes.[311]

The normal, healthy balance in microbiota provides colonization resistance to pathogens. Since anaerobes comprise over 95% of these organisms, their analysis is of prime importance. Gut microbes might also stimulate immune responses to prevent conditions such as intestinal dysbiosis. Intestinal dysbiosis may be defined as a state of disordered microbial ecology that causes disease.[312] Specifically, the concept of dysbiosis rests on the assumption that patterns of intestinal flora, specifically overgrowth of some microorganisms found commonly in intestinal flora, have an impact on human health. Symptoms and conditions thought to be caused or complicated by dysbiosis include inflammatory bowel diseases, inflammatory or autoimmune disorders, food allergy, atopic eczema, unexplained fatigue, arthritis, mental/emotional disorders in children and adults, malnutrition, and breast and colon cancer.[241, 313]

Difficulties in Assessing Intestinal Microbiota

Most studies of microbiota in the GI tract have used fecal samples. These do not necessarily represent the populations along the entire GI tract from stomach to rectum. Conditions and species can alter greatly along this tract and generally run from lower to higher population densities. The stomach and proximal small intestine with highly acid conditions and rapid flow contain 10^3 to 10^5 bacteria per gram or milliliter of content. These are predominated by acid-tolerant lactobacilli and streptococci bacteria. The distal small intestine to the ileocecal valve usually reaches to 10^8 bacteria per gram or milliliter of content. The large intestine generates

the highest growth due to longer residence time and ranges from 10^{10} to 10^{11} bacteria per gram or milliliter of content. This region generates a low redox potential and high amount of short-chain fatty acids.

Not only does the microbiota content change throughout the length of the GI tract, but there are also different microenvironments where these organisms can grow. At least four microhabitats exist: the intestinal lumen, the unstirred mucus layer that covers the epithelium, the deeper mucus layer in the crypts between villi, and the glycocalyx of the epithelial cells.[314] Given this diverse ecological community, the question arises as to how to sample the various environments to identify populations of microbes and ultimately understand the host-microbe interactions. This problem is an extremely difficult one since any intervention to obtain a sample potentially disrupts the population. Fecal sampling has been used for years in microbiota assessment. But it should be understood that this sample most appropriately represents organisms growing in the lumen of the colon. In addition, > 98% of fecal bacteria will not grow in oxygen.[314] Therefore, standard culture techniques miss the majority of organisms present.

Conventional Techniques versus New Technologies

Conventional bacteriological methods such as microscopy, culture and identification are used for the analysis and/or quantification of the intestinal microbiota.[315-317] Limitations of conventional methods are their low sensitivities,[318] their inability to detect non-cultivatable bacteria and unknown species, their time-consuming aspects, and their low levels of reproducibility due to the multitude of species to be identified and quantified. In addition, the large differences in growth rates and growth requirements of the different species present in the human gut indicate that quantification by culture is bound to be inaccurate. To overcome the problems of culture, techniques based on 16S ribosomal DNA (rDNA) genes were developed.[319, 320] These include fluorescent in situ hybridization,[321-325] denaturing gradient gel electrophoresis[326, 327] and temperature gradient gel electrophoresis.[328] These techniques have high sensitivities but they are laborious and technically demanding.

Another problematic issue with present stool analysis procedures is that of transport. Since analysis is culture dependent, sample collection must be done using nutrient broth containers to maintain microbial viability. This allows continued growth of species during transport and until the sample is actually plated out for culture. This growth allows for a significant change in the balance of microbes present, since some species will more actively grow at the expense of others, especially in the presence of oxygen. DNA analysis eliminates this problem by placing the specimen in formalin vials for transport. This immediately kills all organisms, freezing the exact balance present at the time of collection. Since DNA hybridization techniques detect only the genes of the microbiota, living specimens are not necessary. This allows the clinician to develop the most appropriate therapy based on the patient's true gut microbiota, resulting in better clinical results.

Microbial Detection with DNA Probes and PCR

One of the most important contributions to molecular biology is the advent of the polymerase chain reaction (PCR). PCR has led to the development of DNA and RNA-based technologies, enabling the detection of a single genome of an infectious agent in any body fluid with improved accuracy and sensitivity. Many infectious agents that are missed by routine cultures, serological assays, DNA probes and Southern blot hybridizations can be detected by PCR. Therefore, PCR-based tests are best suited for the clinical and epidemiological investigation of pathogenic bacteria and viruses. The introduction of PCR in the late 1980s dominated microbial research because it was superior to all previously used culture techniques and the more recently developed DNA probes and kits. PCR-based tests are several orders of magnitude more sensitive than those based on direct hybridization with the DNA probe. PCR does not depend on the ability of an organism to grow in culture. Furthermore, PCR is fast, sensitive and capable of copying a single DNA sequence of a viable or non-viable cell over a billion times within 3 to 5 hours. The sensitivity of the PCR test is also based on the fact that PCR methodology requires only 1 to 5 cells for detection, whereas a positive culture requires an inoculum equivalent to about 1,000 to 5,000

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cells, making PCR the most sensitive detection method available.[329]

Some advantages of PCR amplifications of target microbial DNA for organism detection over traditional culture techniques are:

- Ability to detect non-viable organisms that are not retrievable by culture-based methods
- Ability to detect and identify organisms that cannot be cultured or are extremely difficult to grow (e.g., anaerobes)
- More rapid detection and identification of organisms that grow slowly (e.g., mycobacteria and fungi)
- Ability to detect previously unknown organisms directly in clinical specimens by using broad-range primers
- Ability to quantitate infectious organism burden in patient specimens for better clinical responsiveness

Laboratories that make the transition to molecular diagnostics will become a more integral part of hospital operations, as they can prove the value of their improved services. The clinical microbiology laboratory is transitioning into the molecular age. From pathogen and antibiotic resistance identification to screening tests, rapid molecular diagnostics are playing an increasingly important role in diagnosing and preventing infections and improving overall hospital operations. As physicians, pharmacists and even hospital administrators demand rapid microbiology results, many laboratories are focusing on being part of cross-functional implementation teams that assure not only that the new tests are implemented efficiently, but also that the results affect real change for patient management, hospital operations and laboratory efficacy.

The combination of stool microbial profiling and urinary microbial metabolic product profiles can provide an even more powerful routine evaluation of intestinal microbial status. Populations in the upper regions dominate the metabolic markers in urine while stool specimens reflect the high-density populations of the colon. Case Illustration 7.1 provides an example of the dual application of both approaches in clinical practice.

Refer to Case Illustration 7.1

Parasitology

Parasitology is yet another field of microbiology to be greatly improved with molecular technologies. Parasite infections are a major cause of non-viral diarrhea, even in developed countries. Classically, parasites have been identified by microscopy and enzyme immunoassays.[330] In recent studies, molecular techniques have proven to be more sensitive and specific than classic laboratory methods.[330-332] Because *Giardia* cysts are shed sporadically and the number may vary from day to day, laboratories have adopted multiple stool collections to help increase identification rates for all parasite examinations.[331] And, even with the advent of antigen detection systems, there has long been uncertainty in diagnosis when no ova or parasites are found. Due to the nearly 100% sensitivity and specificity of DNA analysis combined with the need for very low amounts of genomic DNA (as low as 2.5 cells per gram),[331] the previously long specimen collection process, laborious and technically challenging microscopy, and resulting delays in reporting have been alleviated. With PCR technology, only one fecal sample is all that is needed for high sensitivity and specificity in parasitology examinations.

Antibiotic-Resistant Genes

The development of bacterial resistance to antibiotic drugs involves an active change or mutation in the microbial genome, which changes the microbe's metabolic or structural responsiveness to the mechanism of the drug's action. This genetic change is passed in the population as cells replicate. This genetic material can also be passed on to other strains of bacteria through plasmid sharing. The development of antibiotic resistance is becoming a serious public health issue as overuse of antibiotics continually selects for mutated strains that have developed resistance.

The human intestinal microbiota represents over 400 species. All antibiotic resistance strategies that bacteria develop are encoded in one or more genes. These genes are readily shared among and across species and genera and even among distantly related bacteria. These genes confer resistance to different classes of drugs, and their sequences are known. Therefore, using PCR techniques, they can be readily detected in large populations such as those found in fecal material.

The knowledge of the presence of a drug resistance gene may be quite significant for the clinician when considering treatment of a patient for a pathogen infection.

For example, suppose a pathogen is detected in a stool analysis. An analysis of the presence of antibiotic resistance genes is also performed on the sample. Subsequent drug sensitivities are then run on the pathogen and it is found to be sensitive to two antibiotics. But suppose there is also a drug resistant gene present in the sample to one of the drugs (a very possible scenario). It would be imperative, then, that this drug is not used in treating the patient. Otherwise, even though the pathogen is killed the other organisms that have the gene conferring resistance to the drug would thrive relative to other microbes present. This would set up a potentially dangerous situation, where antibiotic resistance is maintained in the population because that gene can be readily spread to other organisms present in the individual as well as the environment.[333, 334] Knowledge of the presence of antibiotic-resistant genes in fecal specimens, therefore, represents a significant advance in the treatment of patients and maintenance of health.

Intestinal Microbiota Associated with Obesity

There are two predominant bacterial groups in the human GI tract, Bacteroidetes and Firmicutes. Recent research has discovered a relationship between the balance of these groups and obesity. Gastrointestinal bacteria have evolved, in a sense, independently from the host organism, in some cases performing functions that the host has consequently not had to evolve itself. One of these functions is the breakdown of dietary polysaccharides for conversion into energy.

Firmicutes bacteria, which include *Bacillus, Clostridium* and *Lactobacillus* species, are very efficient at metabolizing plant polysaccharides into monosaccharides and short-chain fatty acids. These can then be absorbed by the gut and converted to more complex lipids in the liver. In addition, this group secretes a compound that results in increased activity of lipoprotein lipase in adipocytes, resulting in enhanced storage of these lipids. The Bacteroidetes group, which includes *Bacteroides* and *Prevotella* species, are not as efficient in this function. Consequently, the balance of these two groups, it has been found, can significantly affect the accumulation of fat stores in the body. Although obesity ultimately is caused by excess caloric intake, differences in gut microbial ecology may be an important component of energy homeostasis. In effect, obese individuals may have populations of microbiota that force a more efficient extraction and storage of energy than lean individuals possessing a different balance of microbiota.

A Bacteroidetes decrease relative to Firmicutes in the gut has been associated with significant accumulation of body fat both in humans and experimental animals. When germ-free mice are inoculated with this imbalance of microbiota, they have significantly greater accumulations of total body fat and increased insulin resistance. Similar animals inoculated with the better balance remain lean, even though they are eating diets that are exactly the same. Obese versus lean humans also show similar makeup in bacterial groups. Lean individuals have a higher percentage of Bacteroidetes relative to Firmicutes than obese individuals. Interestingly, if obese humans are put on low-carbohydrate or low-fat diets and lose weight, their microbial balance also improves.[335-338]

This concept of microbiota being linked to obesity raises some interesting possibilities. Studies are ongoing to explore this relationship. The use of specific diets or pre- and probiotic therapies may be able to significantly affect microbial balances that affect fat storage. The ability to assess the balance of these "fat bugs" in humans will potentially be an important advance in contributing to the resolution of a significant public health issue, namely obesity.

MICROBIAL METABOLIC MARKERS FROM STOOL TESTING

Fecal β-Glucuronidase

Bacterial β-glucuronidase is an enzyme that can effectively reverse detoxification that has taken place in the liver during the Phase II conjugation reactions (see Chapter 8, "Toxicants and Detoxification"). Bacterial flora may express large amounts of glycosidase enzyme activity, the principal glycosidase being β-glucuronidase. A report showing high levels of β-glucuronidase calls attention to the need to restore beneficial bacterial populations and to the potential for greater enterohepatic circulation that can affect metabolites such as estrogen (see Chapter 10, "Hormones").

Glycosides are compounds containing a non-sugar molecule (aglycone) attached to a sugar derivative such as glucuronic acid by α- or β-glycoside linkage. Glycosides enter the GI tract through dietary intake or from the liver through bile secretions. Most dietary glycosides, predominately flavonoids, come from vegetables and fruits. Glycosides coming from the liver include toxic compounds that are inactivated by

β-glucuronide formation and subsequently secreted into the bowel by way of the bile. The intestinal flora can hydrolyze the β-glucuronide bond, leading to release of the toxic compound, which may be carcinogenic. Therefore, excess β-glucuronidase activity in the small bowel, often due to elevations in certain bacterial populations, is associated with increased risk of cancer, including estrogen-related cancers. The cleavage of glucuronide from estrogen metabolites leads to their increased enterohepatic recirculation.[339-343]

Fecal pH

The colonic microbial mass converts food components into organic acids and amines according to the nature of the substrate passing into the colon and the type of organisms that predominate. A high rate of organic acid production gives acidic stools, whereas low acid formation with increased amines results in higher pH of stool. One of the most important colonic health practices is regular intake of high fiber foods so that beneficial organisms in the colon receive substrate for the production of favorable products such as butyric acid. Direct measurement of fecal pH provides an overall indicator of acid and base balance.

Abnormally acidic or alkaline pH of the stool may be an indicator of poor digestive health. There is increasing evidence that fecal pH can serve as a marker for colon cancer.[233, 344-346] High fecal pH, however, is only indirectly associated with the development of colon cancer and, therefore, is a secondary, rather than a primary, measure of cancer risk.[344] High stool pH appears to correlate with low levels of short-chain fatty acids (especially butyric acid).[347-350] Alkalinity and low butyric acid levels in the stool appear to signal inadequate intake or digestion of fiber, and possibly low levels of beneficial colonic flora.

Various patterns of simultaneous elevation of multiple bacterial and protozoal by-products in urine are found in putrefactive dysbiosis that is also characterized by lowered ratio of *Bifidobacteria* to *Bacteroides*, the major genera of the anaerobic organisms. Fecal pH may be elevated, and β–glucuronidase increases. This scenario has been linked to increased occurrence of colon and breast cancer (possibly due to deconjugation of estrogen-glucuronide complexes) and hepatic encephalopathy.[310]

Fecal Short-Chain Fatty Acids (SCFAs)

Frequently, disorders of the GI tract are associated with intestinal malabsorption. Many tests are useful in the diagnosis of GI disorders but do not effectively differentiate abnormalities of absorption (mucosal function) from digestion (pancreatic function). The fecal short-chain fatty acid test can be helpful in making a differential diagnosis. Short-chain fatty acids (SCFAs) are formed from bacterial fermentation of dietary carbohydrates and amino acids that escape absorption in the small bowel. An alteration in the proportion of various SCFAs, which stay constant in healthy colons, signifies an impaired state of colonic health.[351]

Short-chain fatty acids are well absorbed by the colon and are a significant source of energy for colonic cells. The short-chain fatty acid *n*-butyrate plays a notable role for maintaining colonic health—a decrease of *n*-butyrate oxidation by colonic epithelial cells may be a key factor in the pathogenesis of ulcerative colitis.[352, 353] High acetic acid and low butyric acid in relation to total SCFAs in the feces are found in patients with large bowel adenomas and cancer.[354] The fecal content of *n*-butyrate—formed by the bacterial fermentation of fiber—is particularly critical for colonic health since it is such an important source of energy for the epithelial cells of the colon.[217] Research suggests that inadequate amounts of colonic *n*-butyrate could be a primary factor in the etiology of inflammatory bowel disease, ulcerative colitis and colon cancer.[355]

Notes:

The SCFA products of bacterial fermentation (or putrefaction), isobutyric, valeric and isovaleric acids, come principally from undigested protein.[356, 357] These SCFAs are normally present at low concentrations in the healthy colon. However, maldigestion of protein due to pancreatic enzyme insufficiency can result in excess protein entering the colon. In these cases, fecal isobutyric acid, valeric acid and isovaleric acid will be elevated. Their presence is more likely due to a pancreatic dysfunction rather than an inadequacy of mucosal absorption.

Fecal Phenolics

In Chapter 6, "Organic Acids," it was pointed out that most of the phenolic products are derived from microbial action on dietary polyphenols. The interpretation of an abnormal value for urinary phenols, therefore, must deal with the possibility that the elevation is simply a manifestation of very high dietary polyphenol intake. One way to correct or normalize data for polyphenol intake is to measure the level of intake. Dietary surveys may be employed, but they are notoriously inaccurate and laborious. Another approach is to measure compounds in fecal water that are markers of intake. Dietary phenolic intakes corresponded closely to fecal water polyphenol concentrations in 5 healthy human subjects, and 7 of the polyphenols measured constituted over 90% of total fecal polyphenols. When monophenols were measured, 10 of the many compounds present represented the great majority of monophenols.[358] Phenylacetate was the dominant component in fecal water. Measuring fecal phenylacetate may allow estimation of dietary polyphenol intake while simultaneously providing a marker for the cancer-protective effects. This method and the alternative of assessing simultaneously measured bacterial products that are not derived from dietary polyphenols are discussed in Chapter 6, "Organic Acids."

Fecapentaenes

Fecapentaenes are polyunsaturated ether lipids that are derived from human intestinal bacterial plasmalogens that have similar ether linkages.[359] (See Chapter 5, "Fatty Acids," for a discussion of plasmalogens.) Fecapentaenes cause DNA damage and mutations in human cells,[360, 361] and they are found in human feces where they are thought to play a role in the initiation of colorectal cancer.[362, 363] The structures of highly mutagenic fecapentaenes have been elucidated,[364] and methods for quantification of eight specific fecapentaens in human feces have been published.[365-367] Some researchers have proposed that the widely varying ratios of two principal fecapentaenes can be traced to individual differences in intestinal flora.[368] Bacteroides have been identified as the source of fecapentaenes in a human autopsy study.[369] With the advent of more broad species identification using 16S DNA identification techniques, there is potential for more closely identifying the organisms in the general population with capacity for fecapentaene production.

Ammonia

The indications of ammonia loading of hepatic and renal detoxification pathways by elevation of urinary orotate, citrate, *cis*-aconitate and isocitrate have been discussed in Chapter 6, "Organic Acids." When normal subjects are shifted from low to high animal protein diets, their fecal nitrogen does not increase significantly, indicating that the increased dietary nitrogen is metabolized by intestinal microbes and the ammonia produced is absorbed rather than turned into bacterial protein.[222] Direct evidence of the contribution of intestinal ammonia production is of great concern for patients being treated for hepatic coma. Such patients may be significantly improved by treatment with non-absorbed oral antibiotics such as neomycin or kanamycin. Use of the drugs eliminates intestinal bacterial urease production in most hepatic coma patients.[370] Aerobic and anaerobic cultures from these patients failed to show changes in the number of deaminating bacteria, though urease-producing colonies were virtually eliminated. However, 3 of

TABLE 7.9 — AMMONIA FROM INTESTINAL BACTERIA

Focus	Properties
Microbial Sources	Most bacteria
Tests	Ammonia (fecal), citrate, aconitate, isocitrate and orotate (urine)
Precursor	Dietary protein or endogenous urea
Indication	General upper bowel bacterial overgrowth
Causes	Urea cycle efficiency highly susceptible to substrate (esp. arginine) availability and to genetic polymorphism of urea cycle enzymes
Interventions	Antibacterials, probiotics

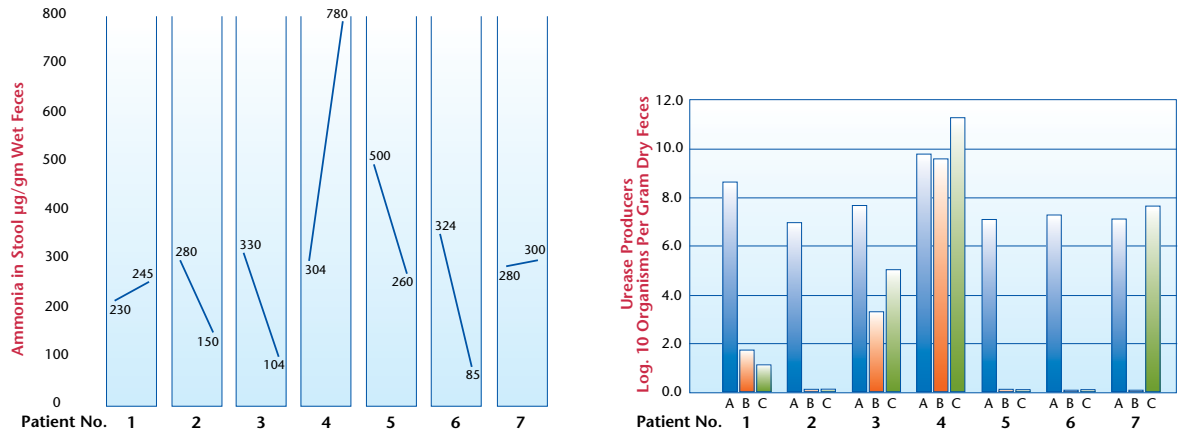


Figure 7.14 — Individual Variability in Response to Neomycin[384]

Colony forming bacteria (right) and stool ammonia (left) are plotted for 7 patients with Laennec's cirrhosis and acute hepatic coma. Bacterial counts were made at baseline (A) and after therapy with 6 g neomycin/day for 3 or 7 days (B and C). Patients 2, 5 and 6 show complete elimination of urease producing organisms and accompanying dramatic drop of fecal ammonia. However, patients 1, 7 and, especially patient 4 had opposite changes showing a high degree of antibacterial resistance.

the 7 patients showed a high degree of antibacterial resistance (Figure 7.14), showing the degree of variability in responses to such therapy. Very active ammonia generation from non-urea sources (presumably amino acids) has been demonstrated in fecal suspension cultures from normal subjects.[371] These data demonstrate the capacity of intestinal bacteria for ammonia production. They also suggest that, although urease-producers persist into the lower colon, deaminating bacteria may inhabit the upper intestinal regions but not be seen in stool cultures. Clinical considerations regarding intestinal bacteria or sources of ammonia are summarized in Table 7.9.

Intestinal Wellness Options

The goal of treatment is to establish intestinal microbial balance (orthobiosis) and healthy mucosal epithelium. Many naturally occurring substances help repair the gut or support the liver when stressed by enteric toxins. For rebuilding the physical and immune barriers and digestive functions, nutritional support includes supplementation with vitamins A, C, E and B_1 through B_{12}; amino acids and peptides such as glutamine and glutathione; and minerals such as zinc, selenium, chromium, molybdenum, manganese and magnesium. Removal of pathogenic overgrowth may be hastened by the use of a wide array of antibiotics, both pharmaceutical and of natural origin (e.g., goldenseal, Oregon grape, barberry and other berberine-containing herbs, garlic, mastic, oil of oregano, olive leaf extract, etc.).[197-199, 204-206, 208] Bactericidal effects should be as specific as possible to avoid the long-term harmful effects of inducing large shifts in microbial populations.

Multiple protocols are available to avoid recurrent overgrowth and to restore mucosal integrity. A protocol known as the "4R program" has been widely applied with success.[372] The general concepts involved in this protocol are outlined in Table 7.10. Generally, an early focus of treatment is restoration of digestive function with betaine HCl, pancreatin and bile acids as needed to replace the normal output of stomach acid, pancreatic fluid and bile, respectively. There is growing evidence

Notes:

TABLE 7.10 — THE FOUR "R" PROGRAM FOR INTESTINAL HEALTH

"R"	Object	Amplification
Remove	Microbial overgrowth	Use bacteriostatic or mycostatic agents of sufficient strength to reduce excessive growth rates
Replace	Digestive factors	Support insufficient digestive secretory factors with oral replacements
Reinoculate	Favorable microbes	Employ oral dosing of viable organisms known to help control toxin-producing specie
Repair	Tissue and immune integrity	Support the growth of healthy intestinal mucosal cells, goblet cells and immune cell responses with key nutrients

of the efficacy of repopulation with favorable species of lactobacilli,[373] especially with the introduction of antibiotic-resistant strains.[374] The consumption of non-absorbed, fermentable carbohydrates, such as fructo-oligosaccharides (FOS) and inulin, stimulate growth of the genera *Bifidobacterium* and *Lactobacillus*.[375] These forms of carbohydrate are found in onion, garlic, chicory, Jerusalem artichoke and wheat.

Insoluble fiber lowers yeast, *Clostridium, Staphylococcus* and *Proteus* in stool cultures and lowers output of ammonia and phenols.[376] Both butyrate enemas and increased dietary soluble fiber have shown potent anti-inflammatory effects both in vivo and in vitro.[377] Shifting from a conventional diet to an uncooked vegan diet has been shown to reduce urinary p-cresol due to a decrease in bacterial enzymes.[378] Small intestinal mucosal regeneration can be aided by intake of supplemental glutamine, the primary energy source for this tissue. The colon mucosa utilizes butyrate, which can be supplied as an enema. Restoration of immune function is aided by deglycyrrhizinated licorice (*Glycyrrhiza glabra*), a stimulant of goblet cell formation.[379] Essential fatty acid balance is needed to assure adequate prostaglandin formation. Deficiencies of essential fatty acids impair the recovery of intestinal mucosa from injury.[380]

The patient should be counseled to avoid NSAIDs, alcohol and other enterotoxic substances. Stress has observable effects on intestinal microbiota. Release of ACTH from fear and anger leads to increased jejunal *E. coli*, loss of *Bifidobacterium* and *Lactobacillus* from fecal samples, and increased levels of the pathogenic *Bacteroides fragilis*.[381] Eating balanced, nutrient-dense meals that provide appropriate amounts of vegetable fiber should become the mainstay of the patient's dietary habits. Patients with food allergies or sensitivities will need to learn how to choose a diet low in the offending foods. Specialized treatments for dysbiosis and "leaky gut" utilizing herbal and probiotic therapies are listed in Table 7.11.

SUMMARY

Evaluation of gastrointestinal function includes detection of inadequate physical and immune barrier functions and measures of the digestion and absorption of food. Pathogenic overgrowth of intestinal microbes in the upper gastrointestinal tract can be detected by measuring their unique products in urine. The patterns reflect the type of organisms that are present within broad categories of bacteria, protozoa or yeast. The information allows discrimination between putrefactive dysbiosis in the colon versus fermentative dysbiosis in the small intestine or combinations of both. The number of compounds involved, and the degree of elevations found, change in direct proportion to the severity of pathogenic overgrowth and loss of mucosal integrity. Stool profiling yields markers of digestive function and results in direct observation of microbial populations and can suggest specific antimicrobial therapies when necessary.

Table 7.11 — Intestinal Wellness Options

Focus	Category		Agents
Digestive function	Stomach		Betaine hydrochloride, L-Histidine
	Pancreas		Pancreatic enzymes
	Hepaticobiliary		Ox bile, Taurine, Olive oil
	Avoid		Antacids, H2 blockers, Proton pump inhibitors
			Cholecystectomy
			Large, rushed meals high in protein and fat
Mucosal integrity	Nutrients supporting cell growth and protection		Zinc, Vitamins A, C, E, Folic acid, Pantothenic acid, Oligopeptide mixtures, Free-form amino acids
	Energy substrate		Small intestine: Glutamine
			Large intestine: Soluble and insoluble fiber
			Butyrate retention enemas
	Cell membrane		Essential fatty acids
	Avoid		NSAIDs
Intestinal dysbiosis	Prebiotics	Bacteria	*L. acidophilus, L. salivarius, L. plantarum* and *L. casei, Bifidobacterium*
		Yeasts	*Saccharomyces boulardii*
		Probiotics	Soluble and insoluble dietary fiber, Fructo-oligosaccharides, Inulin
	Bacteriostatic agents	Bacteria – Severe	Amoxicillin + clavulinic acid or other, as indicated
		Bacteria – Moderate	Berberine-containing herbals such as goldenseal, Citrus seed extract, Olive leaf extract, Aloe vera, Garlic, Glycyrrhiza (licorice)
		Yeast – Severe	Nystatin
		Yeast – Moderate	Capric and undecylenic acids Avoid simple sugars

Notes:

Case Illustrations

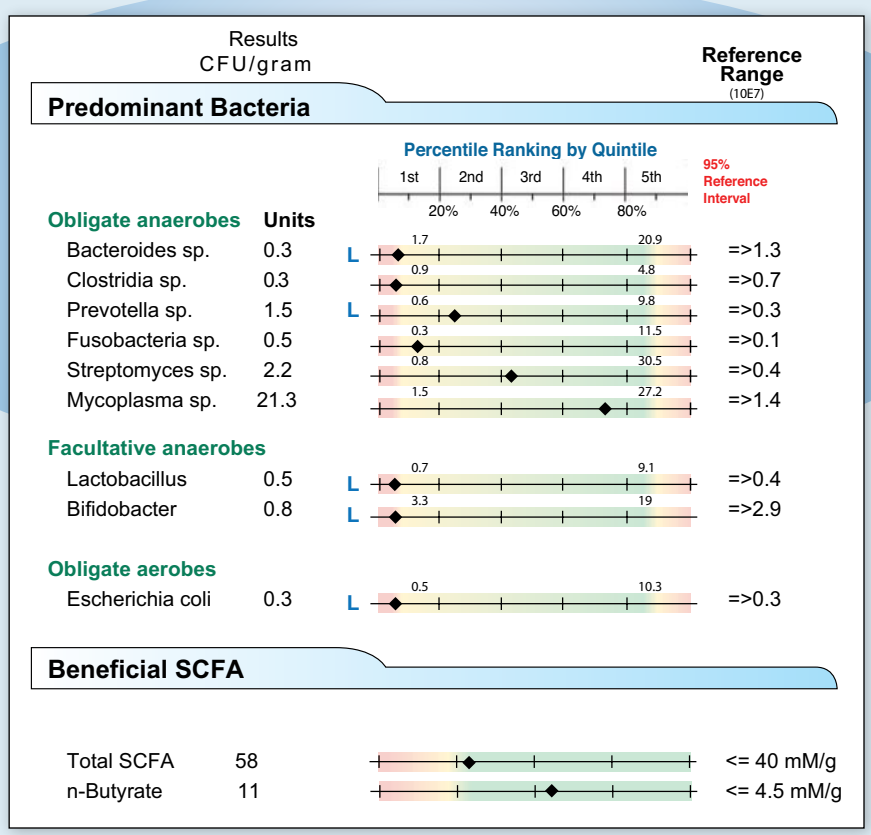
Case Illustration 7.1 — Poor Predominant Flora and Urinary Dysbiosis Products

These results allow comparison of fecal microbial analysis by DNA probe detection (**A**) with urinary organic acid bacterial metabolite testing (**B**). The 50 year-old female has a past history of chronic infections and antibiotic use. She reports that for many months, any attempt to increase the fiber content of her diet produces bloating and flatulence. Her stool microbial profile shows multiple genera of normally predominant bacteria in the first decile (lower first quintile). The colonic population is dominated by *Mycoplasma* species that lack cell walls and, thus, are resistant to most antibiotics such as penicillin that target cell wall synthesis. Low overall bacterial activity is confirmed by the low-normal position of her total short chain fatty acids (SCFA).

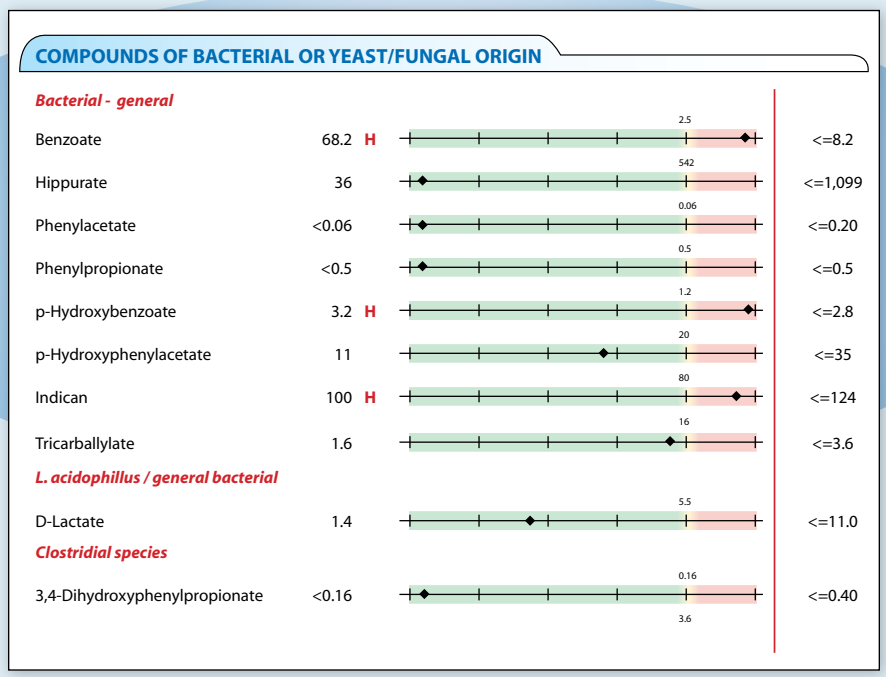
Her urinary profile shows two relevant features. The strongly elevated benzoate reveals her poor capacity for detoxification by glycine conjugation, and disrupted predominant bacteria is further confirmed by the elevated p-hydroxybenzoate and indican along with undetectable phenylacetate, phenylpropionate and 3,4-dihydroxyphenylpropionate. It is possible that the urinary marker pattern reflects *Mycoplasma* dominating in the small intestine. To restore balance, mixed pro-biotic organisms were supplemented along with erythromycin treatment. The low normal hippurate suggests that she may have a glycine conjugase polymorphism that lowers the activity of the enzyme. Even so, her detoxification capacity might be restored by aggressive supplementation of glycine and pantothenic acid. ❖

Notes:

A


Results
CFU/gram
Reference Range
(10E7)
Predominant Bacteria
Percentile Ranking by Quintile
1st
2nd
3rd
4th
5th
20%
40%
60%
80%
95% Reference Interval
Obligate anaerobes
Units
Bacteroides sp. 0.3 L 1.7 20.9 =>1.3
Clostridia sp. 0.3 0.9 4.8 =>0.7
Prevotella sp. 1.5 L 0.6 9.8 =>0.3
Fusobacteria sp. 0.5 0.3 11.5 =>0.1
Streptomyces sp. 2.2 0.8 30.5 =>0.4
Mycoplasma sp. 21.3 1.5 27.2 =>1.4
Facultative anaerobes
Lactobacillus 0.5 L 0.7 9.1 =>0.4
Bifidobacter 0.8 L 3.3 19 =>2.9
Obligate aerobes
Escherichia coli 0.3 L 0.5 10.3 =>0.3
Beneficial SCFA
Total SCFA 58 <= 40 mM/g
n-Butyrate 11 <= 4.5 mM/g

B


COMPOUNDS OF BACTERIAL OR YEAST/FUNGAL ORIGIN
Bacterial - general
Benzoate 68.2 H 2.5 <=8.2
Hippurate 36 542 <=1,099
Phenylacetate <0.06 0.06 <=0.20
Phenylpropionate <0.5 0.5 <=0.5
p-Hydroxybenzoate 3.2 H 1.2 <=2.8
p-Hydroxyphenylacetate 11 20 <=35
Indican 100 H 80 <=124
Tricarballylate 1.6 16 <=3.6
L. acidophillus / general bacterial
D-Lactate 1.4 5.5 <=11.0
Clostridial species
3,4-Dihydroxyphenylpropionate <0.16 0.16 3.6 <=0.40

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Chapter 8

Toxicants and Detoxification

Richard S. Lord and J. Alexander Bralley

CONTENTS

Table 8.1 — Laboratory tests to measure toxin exposure, removal and protection

Assessment of exposure or effects				
Exposure	**Test**	**Result**	**Meaning**	**Interventions**
Heavy metals	Toxic metals – blood, urine or hair	High	Increased body burden	Reduce exposure; oral and I.V. chelation: Protective nutrients
	Oral cavity mercury vapor	High	Dental amalgam mercury release	Replace dental fillings; I.V. chelation: Nutrient support
Environmental organotoxins	Organic toxins – blood	High	Recent exposure	Reduce exposure; detoxification protocols: Hepatic detoxification nutrient support
	Metabolites – urine	High	Recent exposure or recent mobilization	As above plus assess treatment aggressiveness
Intestinal microbial toxins	Microbial products – urine	High	Intestinal microbial overgrowth	Restore microbial balance (see Chapter 7)
Various	Porphyrurias	Specific analytes high	Enzyme inhibitions	Direct testing to verify toxicant levels, body burden reduction and tolerance improvement

Assessment of Detoxification Capacity					
Mechanism	**Test**	**Result**	**Meaning**	**Associations**	**Interventions**
Oxygen radical quenching	Lipid peroxides – serum or urine	High	Antioxidant insufficiency	Low serum antioxidants	Fat-soluble antioxidants
	8-Hydroxy-2′-deoxyguanosine	High	Antioxidant insufficiency	Low serum antioxidants	Water-soluble antioxidants
Ammonia clearance	Orotate – urine	High	Ammonemia	Dysbiosis, urea cycle inefficiency	L-Arginine, α–KG, Mg, Mn
	Citrate – urine	High	Ammonemia	Dysbiosis, renal ammonia clearance	L-Arginine, α–KG, Mg, Mn
Phase I Biotransformation	Glucaric acid – urine	High	Phase I & II induction	Stimulation of hepatic oxidation and conjugation	Reduce toxin load and use support nutrients
	Caffeine clearance – saliva	Low	Low Phase I, slow P450 pathway	Amphetamines, cimetidine, isoniazid, oral contraceptives	Lipoic acid and other hepatic-protective nutrients
	Caffeine clearance – saliva	High	P450 induction	Toxin exposure	Antioxidant protection
Phase II Biotransformation	Salicyluric acid or acetaminophen mercapturate, sulfate, and glucuronide – urine	Low	Low Phase II oxidation	Low cysteine, glutathione, and related nutrients	N-Acetylcysteine, taurine, $NaSO_4$
– Sulfoxidation	Cys/Sulfate ratio	High	Impaired sulfoxidation	Neurological disorders	Mo, Cu, Fe, and $MgSO_4$ (IV)
	Cysteine – plasma	High	Impaired sulfoxidation	Excessive Cys	Mo, Cu, Fe, and $MgSO_4$ (IV)
– Sulfation	Sulfate – plasma or urine	Low	Low Phase II sulfation	Glutathione and sulfate deficiency	N-Acetylcysteine, $MgSO_4$ (IV) Use Cys with care
– Glucuronidation	Bilirubin (indirect) – serum	High	Low glucuronidation	Slow drug clearance	Lower drug dosing
– Glycine conjugation	Benzoate – urine	High	Low glycine conjugation	Glycine depletion	Glycine, Vitamin B_5
Phase I/Phase II	Hepatic Phase I/ Phase II ratio	High	Increased risk of carcinogenesis	Deficiencies of conjugation cofactors	N-Acetylcysteine, glycine, Vitamin B_5, antioxidants

Table 8.1 continued on following page...

INTRODUCTION

The human body is constantly in the presence of potentially harmful agents. It could be argued that any disease process can be caused or complicated by toxic load. Indeed, the health effects of xenobiotic chemicals is drawing increased governmental attention as indicated by the CDC expanding its monitoring of over 200 foreign chemicals and elements humans have in their systems. Environmental compounds can gain entry through the skin, the gastrointestinal tract and the lungs. The field of toxicology primarily deals with environmental toxin exposures and detoxification mechanisms. However, when considering the total toxic load, one must include endogenous products of digestion, energetic metabolism, tissue regeneration, neuronal activity and various cellular control mechanisms that produce compounds that can be injurious unless removed from the body. For example, endogenous ammonia production can easily become the predominant source of toxicants. The total toxic load is composed of human metabolic products and exogenous sources such as microbial metabolites, food-borne components and man-made environmental chemicals. Detoxification is the process of transforming and removing potentially harmful products from the body, and has its own energy, nutrient and regulatory requirements. Diseases result when detoxification is inadequate to handle the load and the toxicants interfere with normal cellular function. This chapter addresses assessment of overall risk, individual biotransformation capacity, and monitoring efficacy of detoxification strategies.

Individual responses to toxin exposure are highly variable due to differences in barrier integrity, biotransformation enzyme activities, eliminative functions and general state of organ reserve. The degree to which disease outcomes are influenced by toxicants depends on levels of exposure and how well the protective mechanisms (influenced by genetic factors) keep tissue-level toxic effects in check. Laboratory assessments can be employed to assess the details of these factors so that specific weaknesses may be addressed.

The last half of the twentieth century was a time of explosive growth of information detailing the extent of industrial toxin release into the environment and the magnitude of human responses to toxins of any origin[1, 2]

Table 8.1 continued from previous page...

Assessment of Major Toxin-Removal Organs

Organ	Test	Result	Meaning	Associations	Interventions
Kidney	Blood urea nitrogen – serum	High	Renal clearance failure	Impaired excretion	Low protein diet
	Creatinine clearance – urine, serum	Low	Renal clearance failure	Impaired excretion	Low protein diet
Liver	Alanine aminotransferase, Aspartate aminotransferase – serum	High	Liver pathology	Impaired hepatic detoxification	Lipoic acid, amino acids, B-vitamins, silymarin, curcumin

Assessment of Protective Nutrients

Nutrient	Test	Result	Meaning	Associations	Interventions
Sulfur amino acids	Met, Cys, Tau, sulfate plasma	Low	Poor sulfur amino acid availability	Glutathione and sulfate depletion	Amino acids, N-Acetylcysteine, Taurine
Glycine	Pyroglutamate – urine	High	Functional glycine insufficiency	Low glycine conjugation, glutathione depletion	Glycine, N-acetylcysteine
	Glycine – plasma or blood spot	Low			
Essential elements	Minerals – erythrocyte (esp. Mg, Zn, Cu, Se, Mn)	Low	Essential element deficiency	Decreased activity of detox enzymes	Mineral supplements and dietary modifications
				Increased heavy metal toxic effects	
Antioxidant vitamins	Vitamins A & E, β-carotene, Coenzyme Q_{10} – serum	Low	Antioxidant insufficiency	Unchecked pro-oxidant effects	Antioxidant-rich foods and supplements
B-Vitamins	Metabolic markers – urine	High	B-complex vitamin insufficiencies	Loss of energy flow and detoxification enzyme activity	B-complex supplements

coupled with our growing understanding of the mechanisms of human detoxification. Clinical effects depend on the combination of toxin load and individual capacities to detoxify. Common clinical clues that point to toxin effects are degeneration in hair, skin, nails, mucous membranes and reproductive function. Beyond these early signs of toxic effects, the list of known toxin-related disorders is very long. Physician associations for discussion of scientific advances and for training in clinical protocols have grown up in response to the growing number of patients who present with syndromes called multiple chemical sensitivity or multisensory sensitivity. This chapter maintains a primary focus on laboratory evaluations for assessing human toxin exposure and capacities for detoxification.

Tests that monitor some of the major hepatic detoxification pathways supply specific insight about potential weaknesses. For example, knowing the status of ammonia challenge and glutathione demand can identify need for supporting urea cycle and sulfur compound utilization to bolster a patient's ability to eliminate toxicants. Measurements of hepatic detoxification function, heavy metal status, and gastrointestinal function can identify potential toxic influences that can then be coupled with nutritional treatments to develop an overall strategy of improving organ function and overall health.

The subject of chronic low-level toxin exposures that may contribute to complex diseases is only beginning to be explored and may have far-reaching impacts on human health. Standard medical training addresses detoxification only in limited areas of patient care, such as drug clearance rates, microbial toxin exposure, and the carcinogenicity of environmental chemicals.

Amongst functional or integrative medical practitioners, however, there is an active recognition of low-level toxin exposures as a cause of undesirable clinical effects.[3, 4] By their nature, low level chronic toxin exposure effects are difficult to reproduce. The weight of favorable anecdotal reports of improvements from interventions focused on removing toxin burden, improving detoxification functions and reducing tissue responses to immune sequelae make it hard for informed clinicians to discount toxicant effects. Detoxification programs can result in an improved sense of well-being, even for "disease-free" individuals. For example, a hypoallergenic diet supplemented with a broad-spectrum macro- and micronutrient drink and intake of at least two quarts of filtered water per day produced significant improvements in objective measures of intestinal permeability and hepatic Phase I and Phase II detoxification capacity.[5] Such results serve as reminders of the omnipresent role of toxins in determining disease and wellness.

Terminology and Scope

Toxicology has been defined as the branch of science dealing with poisons.[9] As commonly used, poison implies a substance not normally encountered that causes harmful effects. Extracts from foxglove, baneberry and hemlock are examples of the many poisons made by plants. To these we must add the toxic metals like arsenic and lead and synthetic chemicals like sulfuric acid and Agent Orange to begin a complete listing of the tens of thousands of substances that might be labeled "poison." Cellular function may be affected by self-generated metabolic stresses as well as environmental toxins. In this chapter the discussion of testing for toxic exposures and the body's detoxification mechanisms will focus on substances not normally found in human tissues.

The terms toxin, toxicant and toxic substance will be used interchangeably to describe potentially harmful agents. The term detoxification, derived from the verb "to detoxify" will be used in preference to the shortened form, detoxication. Acute or chronic exposure can manifest as a poisonous episode or as a contributory factor to a degenerative disease state. Comprehensive lists of toxins and levels of release into the environment are available.[6-8]

Notes:

Dose-Response Curve and the Hormesis Model. The nature of the dose-response has long been considered as that of a threshold above which exposures cause an effect, and below which no effect occurs (Figure 8.1). The presumption of a sigmoidal dose-response curve has led to studies focusing on high-dose toxicology and the development of calculated values such as the lowest observed adverse effect level (LOAEL). A large number of compounds have been studied in order to identify doses that are likely to be at or above the no-observed-adverse-effect level (NOAEL). However, this concept is incomplete when evaluating toxicant effects in biological systems.

The hormesis model is less familiar, but perhaps a more appropriate dose-response curve for understanding toxicant action in the body. It indicates opposite responses or positive benefit at very low and very high exposures. The name "hormesis" first appeared in scientific publication in 1943 to describe biphasic fungal responses to red cedar extracts. A stimulation of fungal growth by red cedar extracts at high concentration was found to revert to an inhibitory effect at very low concentration.[10] Studies in the late 1800s found that chemical disinfectants demonstrated inhibitory effects on yeast metabolism at high doses, whereas stimulatory effects were seen at low doses.[11] A large and rapidly growing number of well-controlled demonstrations of the principle have been reported and alternately have been described as U-shaped, J-shaped, biphasic or bell shaped dose-response curves (Figure 8.2). There have been serious proposals to adopt hormesis as the default dose-response model in risk assessment.[12]

The hormesis concept is a new way of thinking about nutrient and toxicant effects. Toxicants, traditionally thought to have negative effects, and nutrients that are known to have such positive effects actually can have both positive and negative effects, depending on dose. The toxic potential for vitamin A at very high intake is a well-known reversal phenomenon, along with the effects of most pharmaceuticals, as mentioned previously. Other examples of the hormesis concept abound. A compound that is normally quite safe, such as glutamic acid, can become the agent of brain neurotoxic degeneration in Alzheimer's disease,[13] and insulin can be the agent responsible for pregnancy-induced hypertension.[14] On the other hand, the "poison" effects of certain herbal extracts may become beneficial at very low exposures. Finally, intestinal bacterial products that can overload hepatic detoxification pathways under conditions of overgrowth may well become helpful at lower concentrations. This is the case for the products of *Lactobacillus acidophilus* as described in Chapter 6, "Organic Acids."

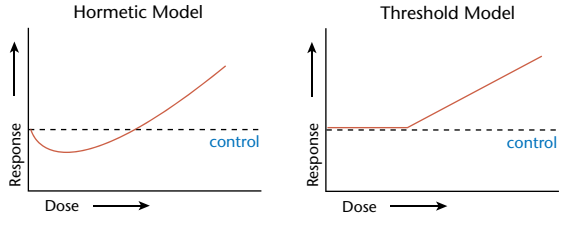


FIGURE 8.1 — Models for Representing Dose-Responses of Toxicants

The threshold model presumes a progressively lower response from high exposures all the way down to the threshold dose (the dose of zero response). The threshold model presumes there is no significant effect below the threshold dose. In the Hormetic model, levels below the threshold generate effects in the opposite direction from those above the threshold. Responses may be measures of carcinogenicity, apoptosis, mitochondrial function or other parameters that reflect cellular or tissue function.

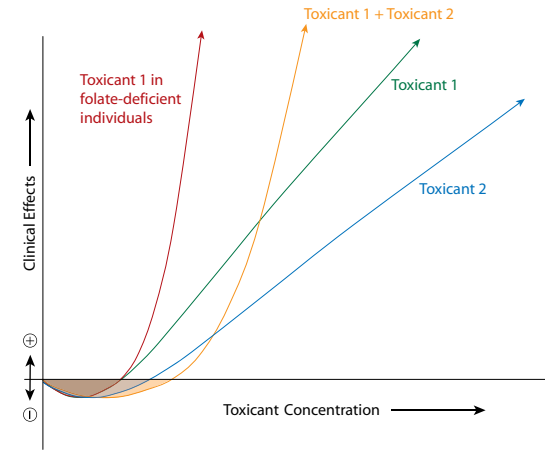


FIGURE 8.2 — The Hormesis Model of Toxicant Effects[12]

The hormesis model may apply to most toxicant effects in biological systems. The effect holds whether the response is measured for individual toxicants or combinations. The threshold and slope of the response versus dose curve varies as shown for Toxicant 1 relative to Toxicant 2. Combined exposure produces a new, frequently more severe response (Toxicant 1 + Toxicant 2). Nutrient status can modify the threshold, and specific nutrient deficiency can significantly worsen the response at a given exposure.

Sources and Effects of Toxins

We live in a world increasingly laden with man-made chemicals and toxic metals released from industrial processes. Most people in "advanced" nations no longer scratch out their living from farming and livestock. We also have the challenge of living in a world where the "normal" detoxification pathways lack "normal" stimulation and where "abnormal" exposures abound. In this context, "normal" refers to those levels of exposure for pre-industrialized human society. There are multiple ways of classifying toxicants. Table 8.2 shows division into inorganic and organic chemicals with subdivision by source.

Table 8.2 — Classes and Examples of Toxicant Sources

Heavy metals
Industrial release (lead, cadmium, mercury, arsenic, aluminum, nickel)
Iatrogenic (mercury, aluminum)
Small organic molecules
Xenobiotic (pesticides, defoliants)
Iatrogenic (pharmaceuticals)
Plant (muscarine, nicotine)
Microbial (cresol, aflatoxin)
Macromolecular
Animal (venom)
Microbial (cholera)
Antigens (food, pharmaceuticals, microbial)

Xenobiotics are compounds that are not produced in biological systems. Their occurrence in significant amounts in human tissues is generally due to activities of civilization. The demands of modern society for plastics, alloys and convenience foods have led to higher levels of exposure and greater variety of toxins. Some important sources of toxins are pesticides, food, food packaging, organotoxins and pharmaceuticals.

Pesticides. Stimulated by public concerns over widespread use of insecticides, especially DDT, the specialty of pesticide toxicology is now providing insight into the range of effects from residues on food.[7] Living with higher toxin loads must be viewed as a side effect of human advancement in pest control, crop production and convenience items. The introduction of chlorinated hydrocarbon pesticides, especially DDT, improved the control of insect-borne diseases in humans. For example, monthly malaria incidence dropped from over 70 per 1,000 inhabitants in some towns of Italy to virtually undetectable levels after DDT was used to reduce mosquitoes.[15] The initial successes have led to an ever-growing number and amount of pesticides used for disease control and improved crop yields. Pentachlorophenol (PCP) is a pesticide used industrially as a wood preservative for utility poles, railroad ties and wharf pilings. Advances such as PCP must be weighed against toxic threats of the agents employed. Extensive reviews of pesticide classifications, toxicities[16] and effects of pesticides on the immune system[17] are available.

Foods. To maintain perspective on the relative contributions of man-made and natural toxicants, we note that foods are quite laden with chemicals that add to the toxic burden of human tissues. When carcinogenicity is used to evaluate toxic potential, strong arguments have been made that, on average, environmental chemicals are of minor importance compared with carcinogens found in foods. Over 1,000 chemicals have been detected in roasted coffee: more than half of those tested (19 of 28) are rodent carcinogens.[18] This finding does not mean that drinking coffee is dangerous, but rather that there are enormous safety factors built into worst-case scenarios of rodent cancer testing. Humans can eat the tremendous variety of natural chemical "rodent carcinogens" because we are extremely well protected by many defense systems. Of course, carcinogenicity is not the only concern, and many circumstances cause exposures far above the average for the general population. Hypospermia and chronic progressive nephropathy are produced in rats by moderate phthalate exposure.[19]

Food Packaging. Phthalate esters, used in the production of plastics are an environmental insult of high concern from food packaging chemical contaminants. Chronic exposure of rats to di(2-ethylhexyl) phthalate (DEHP) causes decreased kidney function and a significantly higher incidence of focal cysts.[21] Patients on hemodialysis are an example of special exposure because they have been regularly exposed to unusually high amounts of di-2-ethylhexyl phthalate used in the manufacture of plastic tubing.[20] Increased hepatocarcinogenesis in rodent liver accompanied by high levels of peroxisome proliferation were found at DEHP

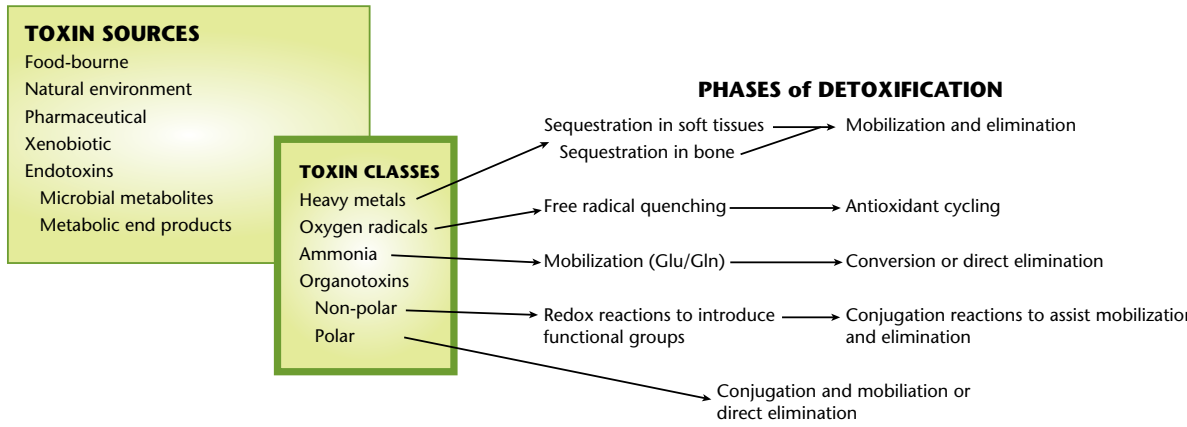


FIGURE 8.3. — **Classes of Toxins and Methods of Detoxification**

Multiple sources may contribute to total exposure for any of the toxin classes shown. The class of toxin determines the phases of detoxification required for removal.

exposures above 500 ppm.[22] These are two of several potential toxic consequences of chronic low-level exposure to phthalates. Human exposure levels to several hundreds of compounds are being actively monitored by geographical region in the United States. The US government[23] and the US Centers for Disease Control and Prevention[24] maintain Web sites rich in information on toxin exposures.

Pharmaceuticals. Because pharmaceuticals are usually designed to produce alterations in normal metabolism, toxic side effects must be evaluated as a routine part of their development. Acetaminophen exerts toxic effects by lowering hepatic glutathione and sulfate reserves.[26] Thiazolidinediones used for diabetes produce highly unpredictable hepatotoxicity.[27] These effects may be long-term, insidious changes that are difficult to associate with the medication. Other exposures produce more rapid effects, such as changes in brain and mitochondrial function. For example, single doses of the Alzheimer's drug mimetidine (a derivative of adamantane) cause neuronal lesions in rats. Body size-adjusted toxic doses in rats are only sixfold below the recommended human dose.[28]

Scientists recognize the political problem of raising issues that might call for costly industrial or governmental action.[29, 30] Widespread concern about such subjects has led to the formation of professional medical organizations such as the American Academy of Environmental Medicine (aaem.com) and the American Environmental Health Foundation (aehf.com). The Federal Clean Water Act currently lists about 300 substances as hazardous industrial discharges into waterways, and it establishes the minimum amount of spillage that must be reported to the National Response Center. The difficulty in controlling small discharges is illustrated by the allowance of companies to release 999 pounds of benzene without reporting.[31] There are many reports of such instances and numerous reviews of health threats from environmental chemicals.[32-35]

Notes:

Physical, Chemical and Immunologic Barriers

Since increased toxicant exposure is a modern fact of life, the clinical impact relies on the body's ability to remove toxins. Toxin management requires adequate nutritional resources. A significant portion of total body energy generation, as well as integrated function of the gastrointestinal, hepaticobiliary, cardiovascular, renal, lung, skin and immune systems are required. The overall functions are to prevent toxin entry, to identify toxins that penetrate the barriers, to modify toxins into more easily managed forms and to provide mechanisms for removal. The immune system is included in this discussion because of the central importance of handling macromolecular and cellular agents that can cause such devastating effects via the combination of atopic reactions and large antigen-antibody complexes as described in Chapter 7, "GI Function." Such reactions may be considered components of detoxification.

Humans are protected from the effects of disease-producing substances by physical, chemical and immunologic barriers. The skin and mucosal surfaces make up the physical barriers. The immunologic barrier is maintained by the secretion of special classes of antibodies, mainly secretory IgA, which bind to large molecules recognized as foreign to the body. The chemical barrier is made up of the gastric secretions of the stomach and the extracellular and intracellular enzyme systems of the tissues.

The gastrointestinal tract is the major source or entry route for toxic substances. The portal vein delivers compounds that are water soluble directly to the liver, whereas lipid-soluble compounds enter systemic circulation via the lymphatic system along with dietary fat. Tissue distribution of toxins depends on the route of entry. The lungs provide a very efficient surface for transfer directly to systemic blood and from there to central nervous tissue or to sites like adipose tissue, where accumulation is favored because of slow turnover. Entry through the skin, likewise, is a route that bypasses the liver and favors toxin accumulation in adipose tissue without being modified by detoxification enzymes.

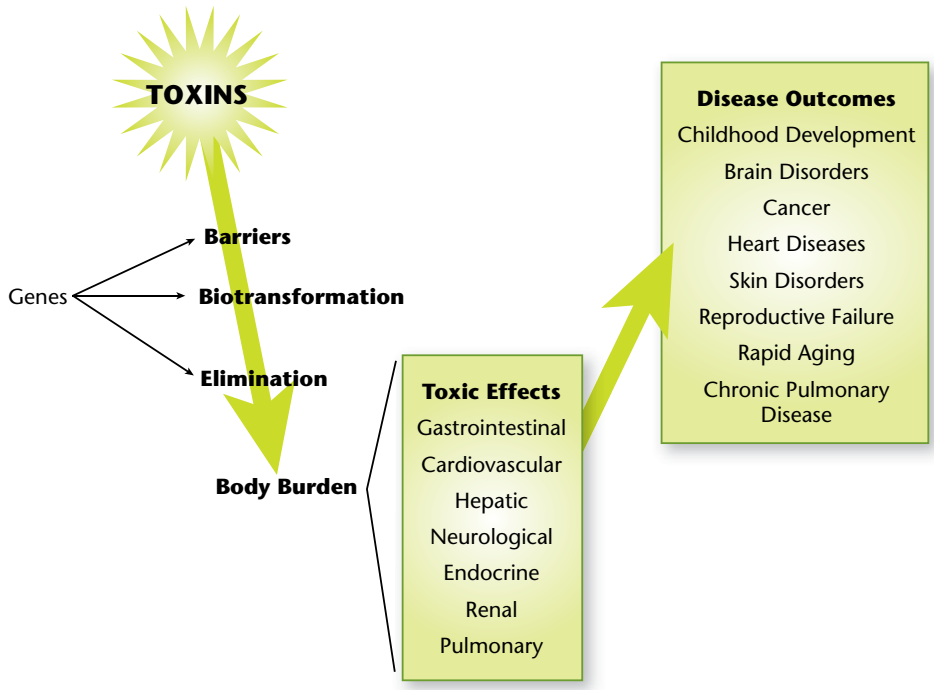


Figure 8.4 — Schematic Representation of Variables Governing Toxic Consequences

Increased exposures or genetically impaired functions of barrier maintenance, biotransformation enzyme activities or elimination mechanisms may overload or cause dysfunctional detoxification systems. These effects manifest as exacerbations of various patient conditions.

Multilayer Toxicant Insults. There is potential for additive or synergistic effects that may not be addressed by assays for exposure to single chemicals.[28] Multiple factors can contribute to a detoxification-compromised individual, such as integrity of their physiological barriers, nutrient sufficiency, use of certain pharmaceuticals, diet, genetics, length and severity of chronic exposure, and the presence of disease such as diabetes. Environmental exposures must be considered as incremental loads on top of potential drug-induced hepato- or nephrotoxicity due to use of pharmaceuticals. For example, polybrominated biphenyls (PBPs) alter the response of the kidney to other toxic chemicals, making their insidious effect more hazardous than some direct-acting nephrotoxicants.[25] A clinician will rightly suspect total toxin load as the cause of disease for the patient with intestinal bacterial overgrowth, long-term use of pharmaceuticals and a diet of foods with high toxicant concentration while living in the inner region of a large city. If that patient happens to have genetic weaknesses in one or more detoxifying pathway enzyme, then the health risks of inadequate detoxification capacity increase dramatically.

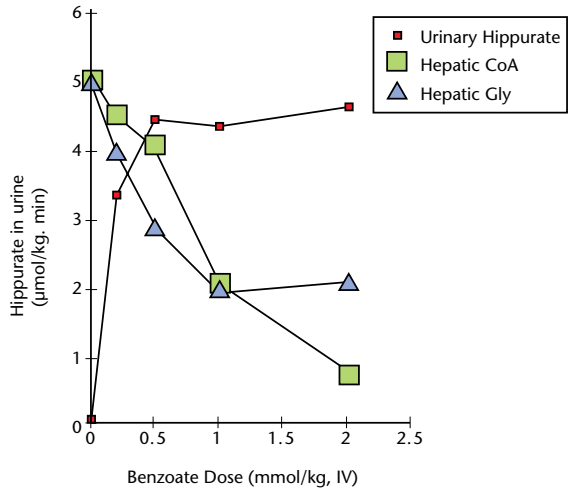


FIGURE 8.5 — Hepatic Glycine and Coenzyme A Response to Benzoic Acid Loading[38]

As benzoic acid dose increases, hippurate formation rises to a plateau, indicating metabolic saturation kinetics. Simultaneously, levels of the associated cofactor, coenzyme A, decline, indicating that the conversion capacity is exceeded because of coenzyme A limitation. Supplementation with glycine improves hepatic glycine availability, and additional pantothenic acid can prevent such dramatic falls in coenzyme A.

Mechanisms of Cell Injury Induced by Toxins

Toxicants may be injurious via interferences with DNA regulatory events, poisoning of enzyme-active sites, binding to regulatory sites of receptors and depleting stores of cofactors. Some toxicants have highly tissue-specific effects. For example, methyl mercury causes selective necrosis to the cerebellum. Evidence has shown that such compounds make the cells more vulnerable to reactive oxygen species because they deplete cerebral glutathione, thereby reducing cellular antioxidant capacity.[36] Chronic toxicant exposure acts to deplete critical cell growth factors. Figure 8.5 shows the effect of increasing doses of benzoic acid on hepatic stores of glycine and coenzyme A. This effect is easily offset by supplementation with glycine and pantothenic acid. Combinations of dietary intake, pharmaceutical use and intestinal microbial production can produce exposures in the range shown in the figure. This effect is just one of many potential nutrient depletion scenarios resulting from chronic toxicant exposure. Table 8.3 summarizes some known mechanisms of toxic consequences. Multiple reviews are available where such mechanisms are discussed in detail.[9, 37] Some reviewers include special sections on immunotoxicology.[6]

Oxygen Radicals and Ammonia

The following chapter presents concepts of oxidative challenge due to various radical oxygen species. In the context of this chapter, it is important to keep in mind that such challenge to cellular function is a part of the constant background upon which other potential toxins play. Thus elevated levels of lipid peroxides or 8-OHdG reveal PUFA and DNA oxidative damage rates that exceed capacities for neutralizing oxygen radicals. Another principal part of that background is ammonia arising from the rhythmic processing of amino acids and from intestinal bacterial metabolism. Urinary orotate, citrate, cis-aconitate and isocitrate are markers of the hepatic and renal capacity to deal with ammonia burdens.

Because these matters are of such critical importance and constant challenge, we might label the assessment of protection from oxidant and ammonia challenge as the first order priority for assessment when detoxification

Table 8.3 —Mechanisms of Toxicant-Induced Health Threats

Mechanism	Example	Reference
DNA regulatory interference	Phthalate inhibition of proliferation and induction of apoptosis in developing bone marrow B cells	39
DNA damage	Amelioration of aflatoxin DNA binding levels by I3C	40
Enzyme cofactor competition	Lead exposure in battery plant workers produces low delta-aminolevulinic acid dehydratase (ALAD) activity	41
Cell receptor binding	Phthalate activation of sex hormone receptors	42
General oxidative stress	Acetaminophen hepatotoxicity	43
Tissue-specific oxidative stress	Cerebellar necrosis from 2-halopropionic acids, thiophene, methylhalides, methylmercury, methylazoxymethanol (MAM) and trichlorfon exposure	36
Immune hypersensitivity	Antibiotic sensitivities in patients with cystic fibrosis	44
Autoimmune diseases	Omega-3 polyunsaturated fatty acid modulation of rheumatoid arthritis	45
Immunosuppression	Pesticide alteration of induced cellular and humoral immune responses	46

is suspected to play a significant clinical role. This high order of priority places examination of urinary 8-OHdG and orotate at the top rank of laboratory evaluations. Correcting abnormalities of these markers may call for more detailed investigations of contributing toxic exposures.

Testing Toxin Load

Both toxic metals and small organic molecules may be measured in body fluids and tissues by various laboratory methods. Because toxic metals can bind avidly to many tissue proteins, they tend to accumulate with repeated exposure. Since the half-life of many metal-binding proteins is relatively slow, testing performed months after the exposure can reveal elevated tissue levels. Non-polar organotoxins accumulate in adipose tissue, so long-term exposure assessment may require a fat biopsy specimen. When blood or urine is used for organotoxin exposure, the test must be done shortly after exposure because of rapid clearance rates. Measurement of exposure to organic toxins is complicated by migration from body fluids to tissue sites and chemical modifications creating multiple forms of most compounds.[47] The extracellular matrix is a site to be considered for accumulation. The proteoglycan structures have a very large number of sites that tend to have low affinity but high binding capacity for molecules with polar character.

Toxic Heavy Metals

A preliminary discussion of toxic heavy metals was included in Chapter 3 because elemental screening profiles of blood, urine or hair frequently include both essential and toxic metals. Some of the more complex details of these assessments will be covered here.

Metallothionein is a metal ion binding protein that is particularly abundant in the kidney where it serves to sequester essential elements, preventing their spillage into urine. The metallothionein binding sites may also be occupied by toxic heavy metals. Because each molecule of metallothionein offers multiple temporary binding sites, one may think of urinary challenge assessments as ways of determining the level of metallothionein loading with toxic elements. Renal metallothionein loading status is revealed more dramatically by using appropriate doses of chelating agents such as EDTA, DMSA or DMPS prior to collecting urine for analysis. These chelating or complexing agents effectively compete for the metallothionein binding sites, causing release of bound toxic elements so that a much larger fraction spills into urine.

Notes:

Long-term accumulation of toxic heavy metals in bone is much more difficult to assess. Because of the differential in mobilization rates from soft tissue and bone, sequential monitoring of body fluid levels frequently reveals one toxic metal rising as another is normalized. This effect is due to the redistribution of toxic heavy metals between soft tissue binding sites, especially metallothionein, and bone that is a long-term accrual site with slow turnover. The toxic potential of heavy metals is exerted because they bind to critical sites on enzymes, DNA, or transport or regulatory proteins. As procedures are used to lower metallothionein content of toxicants, they redistribute from bone or soft tissue interference sites.

Some clinical researchers have attempted to perform direct measurements of metallothionein, reasoning that impaired ability to form the protein will produce heightened toxic metal susceptibility. The difficulty with this evaluation is that metallothionein is a highly inducible protein, making a single-point test result difficult to interpret. A low metallothionein reading may imply few acute toxic or nutrient metal intake challenges in the interval of time just before the specimen was taken rather than a chronic inability to form metallothionein. Recent implementation of dietary supplements containing zinc, for example, can cause increases in metallothionein levels.[48] Other indirect tests are useful, such as plasma methionine and taurine or urinary sulfate. These tests are discussed in the chapters on amino acids and organic acids.

Mercury exposure is particularly difficult to assess because of the tendency of mercury to form covalent bonds with proteins, thus sequestering the mercury from chelating agents. This behavior is one reason that methyl mercury accumulates in brain tissue.[49] Methyl mercury-induced excitotoxicity occurs because it preferentially accumulates in astrocytes.[50] Because of the extreme toxicity of methyl mercury and the fact that measurements of concentrations in blood, urine or hair show total mercury, devices have been developed for the direct measurement of mercury vapors in the oral cavity. Elevated methyl mercury in the mouth is related to the presence of mercury amalgam dental fillings.[51] When significant exposure to heavy metals is known or suspected for pregnant women, testing of amniotic fluid may be done to reveal levels of fetal exposure.[52] Loss of productivity in the United States in 2004 is estimated at $8.7 billion, because of more than 316,588 children

Table 8.4 — Toxin Exposure Testing

Toxic Metals	
Toxicant Exposure	**Test**
Arsenic, cadmium, chromium, cobalt, lead, mercury, nickel	DMSA challenge 24 hr. urine, hair, whole blood
Methyl mercury	Oral vapor
Xenobiotics by Direct Concentration Measurement	
Classes of Toxicants Detected	**Specimen**
Polyaromatic hydrocarbons (PAHs) – Benzopyrene	Whole blood or serum
Chlorinated hydrocarbons – Heptachlor, DDT	Whole blood or serum
Polychlorinated biphenyls (PCBs)	Whole blood or serum
Metabolites Measured in Urine	
Toxicant	**Test**
Ammonia from impaired urea cycle	Orotate
Ammonia appearing as urinary salts	Citrate, *cis*-aconitate, isocitrate
Xylene exposure	2-Methylhippurate
Any toxicant requiring sulfation	Sulfate
Intestinal bacteria, yeast and fungi products from amino acids	p- Hydroxyphenylacetate, p-cresol and other metabolites
Intestinal bacteria and yeasts products from carbohydrates	D-Lactic acid, arabinitol and other metabolites

each year who are born with cord-blood mercury levels > 5.8 µg/L. Such levels of mercury are associated with loss of IQ[53] and have been heavily debated as a contributing agent to disorders of the autism spectrum.[54–59] The controversy is heightened because it is linked to the worldwide preventive health procedure of immunization. Widely used forms of injectable immunization solutions have been preserved with thimerosol (Figure 8.6). Investigative news reports about apparent withholding of adverse information by public organizations have spurred intense public interest, especially from parents of autistic children.[60]

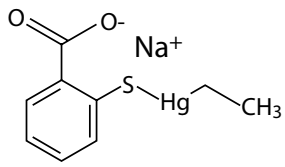


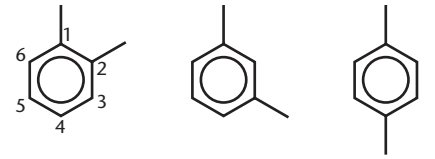
Figure 8.6 — The Chemical Structure of Thimerosal

Thimerosal is used as a preservative in vaccine solutions and antiseptic preparations because it effectively poisons microbial metabolism, preventing bacterial growth. Many studies have sought to determine whether small doses of thimerisol administered in vaccines might exert significant toxic effects in small children.

Organotoxins

Measurement of long-term organotoxin exposure is still challenging, although recent advances in detection may change that. One particularly difficult aspect of measuring human exposure to organotoxins is that these toxins are promptly removed from body fluids, converted to derivatives, and thereafter may be stored in adipose tissue. Measurement of organotoxins in body fluids offers information on the individual's recent acute exposure only. Fortunately, technological advances offer promise such as the technique termed metabonomics, which uses NMR spectroscopy and high-throughput systems to examine "the impact of genetic modifications and toxicological interventions on the network of transcripts, proteins, and metabolites found within a cell, tissue, or organism."[61]

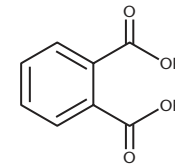
Xylene



1,2-dimethylbenzene *(ortho-xylene)* 1,3-dimethylbenzene *(meta-xylene)* 1,4-dimethylbenzene *(para-xylene)*

Another approach is to measure urinary levels of the products of hepatic detoxification that can reveal the level of sequestered compound still being processed through hepatic pathways long after the initial exposure. Xylene is one of the most common organic solvents released into the environment. It occurs in three isomers that differ in the position of the methyl groups. Xylene exposure is revealed by measuring the oxidation/conjugation product 2-methylhippurate.[62] The 3- and 4-methylhippurate products can also be measured, but, since they virtually always rise in parallel to the 2-methyl form, there is no new information to be gained regarding a patient's exposure to xylene. Levels in urine correlated significantly with the time-weighted average intensity of exposure. Rates of clearance were lowered in exposed workers who regularly smoke and consume alcohol due to lowered rates of conversion, meaning that xylene accumulation in adipose tissue should be increased.[63]

Phthalates



Phthalates
(R and R′ = C_nH_{2n+1}; n = 4-15)

Phthalates are esters of 1,2-benzenedicarboxylic acid, where the alcohols that form the ester bonds have chain lengths from 4 to 15 carbon atoms long. Phthalates are so widely used in the production of plastics, cosmetics and other convenience items that exposure to phthalates is universal for all but the most primitive and remote cultures. The di-(2-ethylhexyl) phthalate ester (DEHP) is added in varying amounts to certain plastics to increase their flexibility. Numerous other uses in consumer products consume the worldwide production of, roughly, a billion pounds of phthalates per year.

Phthalates have been shown to have numerous physiological effects, mainly related to steroid hormone disruptions such as prenatal phthalate exposure, causing impaired male reproductive development,[64] and endometriosis.[65] Although studies on individual xenoestrogens have found them to be of low estrogenic potency, when multiple compounds are present at their no-observed-effect concentrations (NOEC), dramatic enhancement of estrogen receptor alpha response to estradiol was found.[66] Such results demonstrate the additive effects of multiple exposures. Phthalate diesters with –CH2– chains ranging in length from 3 to 6 carbon atoms exhibit human estrogen receptor alpha-mediated estrogenic activity and estrogen receptor beta-mediated antiestrogenic activity in a dose-dependent manner.[67] Obese, insulin-resistant adult males excrete higher amounts of mono-benzyl phthalate (MBzP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-ethyl phthalate (MEP) than controls. Multiple linear regression analysis adjusted for age, race/ethnicity, fat and total calorie consumption, physical activity level, serum cotinine, and urine creatinine excretion of the compounds predicted of waist circumference and insulin resistance with $p < 0.013$.[68] These results from various kinds of experiments demonstrate that disruption of estrogen receptor signaling is one mechanism by which environmental exposure to phthalates can have negative health consequences.

Regarding markers of exposure, measurements of urinary phthalates displayed a reasonable degree of temporal reliability, and the wide concentration range found indicates that they are appropriate biomarkers for use in epidemiologic studies of environmental exposures in relation to health outcomes in children. Table 8.5 provides some reference for mean concentrations and distributions in one urban population.[69] Urinary mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were found to be sensitive biomarkers of DEHP exposure. Their presence in 62 individuals at levels 4-fold higher than the parent xenobiotic indicates that human DEHP metabolism involves hydrolysis of 1 ester bond, giving MEHP, followed by oxidation to MEOHP and MEHHP.[70] The US Environmental Protection Agency publishes regular updates of their surveys of human phthalate exposures and potential chronic health effects and carcinogenicity assessment for lifetime exposure.[71-73] Data from the 1999 and 2001 surveys are shown in Figure 8.7.

TABLE 8.5 — URINARY PHTHALATE METABOLITE CONCENTRATIONS IN NEW YORK CITY CHILDREN[69]

Urinary phthalate metabolites were measured in 195 children living in New York, NY. All of the compounds shown in the table were detected in virtually evey child tested. The 50th and 95th percentiles for the children in New York (in µg/mg creatinine) were routinely higher than the US averages reported by the CDC. Tertile categories for metabolites and class sums (Sum1 or Sum2), based on a single sample were found to be highly representative of the 6-month average of several samples.

Abbreviation	% Detect	Geometric Mean	50th Percentile	CDC 50th Percentile	Min	Max	95th Percentile	CDC 95th Percentile
mEP	100	176	149	81.2	15.3	6410	1378	837
mECPP	100	124	104	NA	16.5	1,806	726	NA
mEHHP	100	84	76	34.2	6.4	1,101	592	211
mEOHP	100	53	50	22.8	4.6	710	350	130
mEHP	97.5	11	11	5.38	0.5	207	90	31.2
Sum1	—	521	481	NA	45.6	6,901	3,587	NA
Sum2	—	940	789	NA	99.1	11,883	5,640	NA
mBP	100	52	52	35.1	9.1	660	165	146
mBzP	100	40	36	37.2	2.8	823	287	195
miBP	98.7	16	15	5.17	2.1	158	50	24.3
mCPP	94.3	6.3	6.1	7.07	0.1	94	53	26.4

mEP = Mono-ethyl phthalate; mECPP = Mono-2-ethyl-5-carboxypentylbutyl phthalate; mEHHP = Mono-2-ethyl-5-hydroxyhexyl phthalate; mEOHP = Mono-2-ethyl-5-oxohexyl phthalate; mEHP = Mono-2-ethylhexyl phthalate; mBP = Mono-n-butyl phthalate; mBzP = Mono-benzyld phthalate; miBP = Mono-iso-butyl phthalate; mCPP = Mono-3-carboxypropyl

Sum1: mEHHP + mEOHP + mEHP; Sum2: mECPP + mEHHP + mEOHP + mEHP; NA: not available

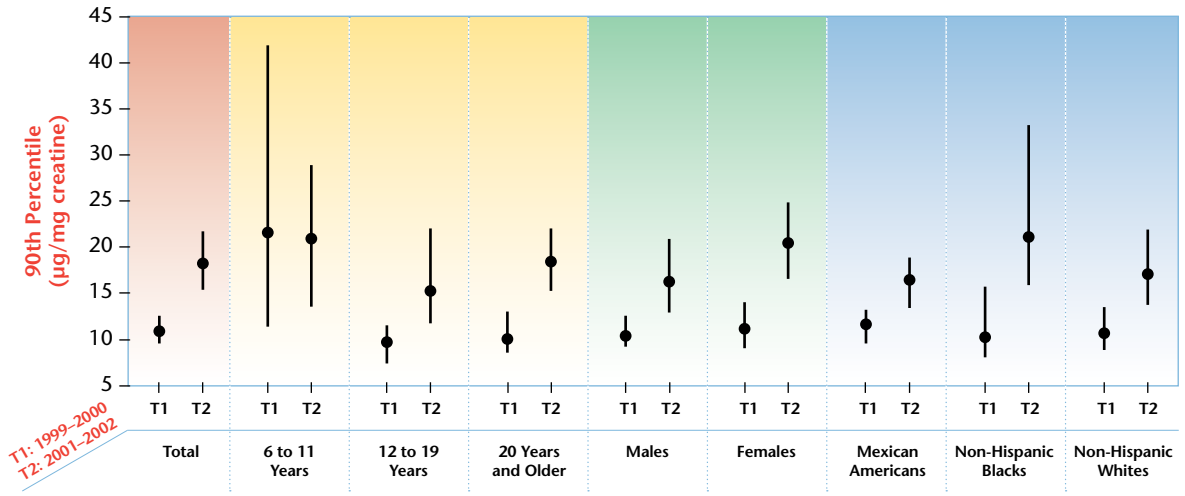


Figure 8.7 — 90th Percentile MEHP Levels

These data from the US population aged 6 years and older were collected in 1999 and 2002 by the US National Health and Nutrition Examination Survey.[71] The 90th percentile values with 95% confidence interval bars show MEHP concentrations by age, gender and ethnic background. Increasing levels of exposure are seen by comparing the 1999 (T1) levels with those in 2002 (T2). The overall total levels rose by about 90% in the 3-year interval. Only children 6 to 11 years old showed no increase, but they had much higher and more highly variable MEHP levels, even in 1999, than any other age group.

Products of Glycation or Lipoxidation

Glucose can react non-enzymatically with amino groups of proteins to yield adducts (known as Maillard reaction products) that spontaneously and irreversibly degrade to form compounds called advanced glycation end products (AGEs). These reactions may take place in food preparation, especially bread baking, and the same reaction is a part of normal biological aging, where tissue proteins are altered by glucose. Rates of formation of AGEs increase in diabetes due to hyperglycemia and in renal failure due to high levels of reactive carbonyl compounds (RCOs). Accumulation of AGEs contributes to uremic toxicity.[74] Through other irreversible reactions starting with lipid oxidation, lipoxidation end products (ALEs) also may be formed. Together, these metabolic end products represent a special class of toxicological challenge that can cause vascular stiffening[75] and contribute to oxidant stress and cytokine stimulation to produce inflammatory responses, especially in diabetic patients.[76]

Some success has been reported for synthetic AGE-breaking compounds.[77, 78] These drugs, however, may present further risk of toxicity.[79] Prevention by normalizing plasma glucose and RCO levels is the obviously preferred patient management approach. The physiological effects of AGEs are mediated through their interaction with cell receptors (RAGE), and serum levels of soluble RAGE (sRAGE) are commercially available to measure AGE exposure.[80]

Testing Toxin Effects

Urinary Porphyrin Profiling

Heme is required at the active sites of oxygen-binding, oxygen-utilizing and oxidizing systems, hemoglobin (and myoglobin), cytochromes, and mitochondrial electron carriers. Heme is a macrocyclic, iron-sequestering molecule that is synthesized in most human tissues (predominantly liver and bone marrow) by a pathway with intermediates called porphyrinogens. The final phase of metal incorporation inserts iron, cobalt or magnesium into the protoporphyrin ring to produce heme, cobalamin and, in plants, chlorophyll, respectively. These complex organometallic structures are sometimes called the

pigments of life.[81] Porphyrins are oxidized by-products that have escaped from the pathway. The spilling of porphyrins into urine generates porphyrinurias. For example, iron deficiency causes a blockage near the end of the pathway where iron is incorporated resulting in elevated levels of erythrocyte protoporphyrin (EP) and serum zinc protoporphyrin (ZnPP or ZP).

The porphyrin pathway involves eight enzymes in a sequence beginning and ending in the mitochondria, with four steps occuring in the cytosol. Glycine and succinyl-CoA are joined in the initial reaction of the sequence, producing delta-aminolevulinic acid (ALA). The porphyrin ring is formed in subsequent steps (Figure 8.8). Restriction of an enzyme activity following uroporphyrinogen creation produces backup of one or more porphyrinogens that leads to a rise of the corresponding porphyrins in urine, called porphyrinuria. The term porphyria may be reserved for primary conditions exhibiting specific clinical symptoms caused by an inherited defect in one or more of the heme biosynthetic enzymes.[82] Porphyrinopathy is an umbrella term for any disorder in porphyrin metabolism. The abbreviations shown in Table 8.6 may be used for brevity.

Table 8.6 — Abbreviations for Porphyrin Pathway Intermediates

Intermediate	Abbreviation
Uroporphyrin	Uro
Heptacarboxyporphyrin	Hepta
Hexacarboxyporphyrin	Hexa
Pentacarboxyporphyrin	Penta
Precoproporphyrin	Precopro
Coproporphyrin I	Copro I
Coproporphyrin III	Copro III

Inherited Enzyme Defects

The utility of urinary porphyrins as a diagnostic tool is not new—its use has been documented in the medical literature since 1934, and review articles summarize the genetic and molecular aspects of the various clinical manifestations that are listed in Table 8.7.[83, 84] Porphyrias, which can be inherited or acquired, are often diagnosed with the aid of information regarding the distribution profile of individual porphyrin intermediates in urine.[85] Porphyrins are particularly well suited as biomarkers for two reasons. First, the pathway is highly active, so any disturbance tends to cause rapid and relatively large accumulations of intermediates. Second, the enzymes of the porphyrin-producing pathway are widely distributed in human tissues and some of them are highly sensitive to the presence of various toxins.

Up-regulation of the heme biosynthetic pathway is another mechanism by which porphyria can be precipitated. Table 8.9 summarizes various conditions that generate porphyria by altering the overall activity of the pathway. Elevations from these factors can be mistaken for genetic or toxicant-mediated porphyria. Calculation of ratios of key intermediates like precoproporphyrin to uroporphyrinogen can identify the presence of specific inhibitions caused by toxicants while minimizing possible effects of creatinine variability when overnight urine is utilized. Increases in the ratio indicate true abnormal intermediate accumulation independent of overall pathway fluctuations. Active porphyria occurs when ALA overproduction coincides with inhibition of one or more of the porphyrin pathway enzymes (Table 8.8). In other words, the blockage is apparent as elevations of specific intermediates relative to others when the pathway is accelerated. It is estimated that among cases of inherited porphyrinogenic enzyme deficiencies, as many as 90% are healthy

Table 8.7 — Symptoms Associated with Porphyrinopathies

Primary Complaints	Associated Symptoms	Condition Exacerbated by
Neurologic presentations: Abdominal pain; nausea; vomiting; constipation; seizures	Headaches; difficulty in concentration; personality changes; weakness; muscle and joint aches; unsteady gait; poor coordination; numbness; tingling of arms and legs; fluid retention; rapid heart rate; high blood pressure; increased sweating; intermittent fever	Low carbohydrate diets (skipped meals); intake of alcoholic beverages; medications, including sulfa-drug antibiotics, barbiturates, estrogen, birth control pills; exposure to toxic chemicals
Cutaneous presentations: Changes in skin pigmentation; changes in facial hair; fragile skin; rashes; blistering	Dark-colored urine (especially after its exposure to sunlight), and above symptoms may be present	Above factors, and skin symptoms made worse by exposure to sunlight. Copper or brass jewelry exacerbates reaction

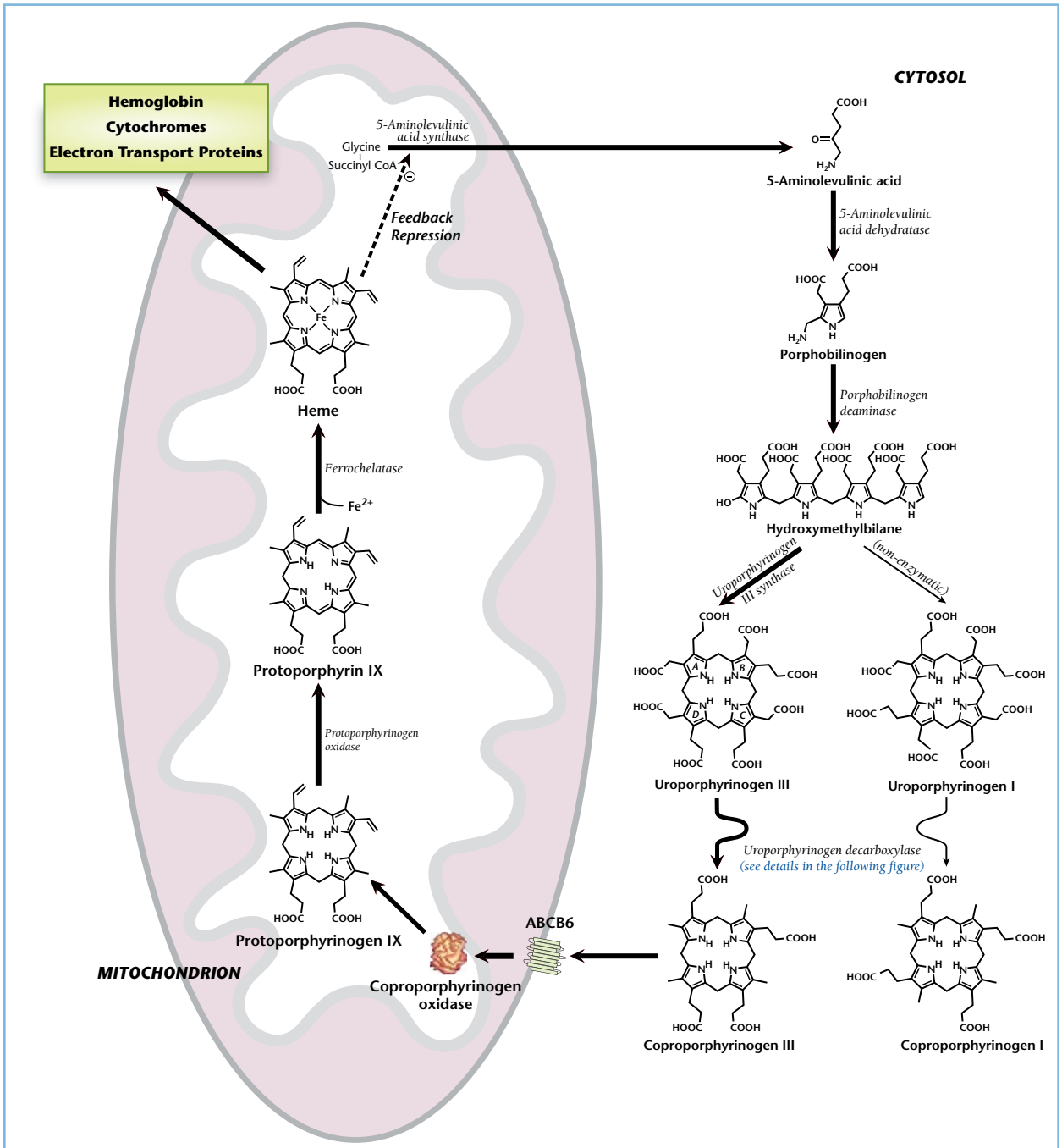


FIGURE 8.8 — **Porphyrin Intermediates in Heme Biosynthesis**

Heme synthesis starts and ends in the mitochondrial matrix, but the ring-forming and decarboxylation steps are performed by cytosolic enzymes. Ring closure of hydroxymethylbilane produces a macrocycle with 20 carbon atoms and 4 nitrogen atoms. Eight carboxylic acid groups extend from the macrocyclic ring of uroporphyrinogens I and III that differ only in the positions of one pair of acetate and propionate groups. Stepwise decarboxylations produce compounds with 7, 6, 5 or 4 acid groups, causing a large change in polarity that prepares the ring for resting in the binding sites of heme proteins. Ultimately, insertion of iron results in a stable, organometallic structure that participates in metabolism as coenzymes and oxygen carriers. The decarboxylase enzymes are points of toxin interference in the pathway. Heme is exported from mitochondria for incorporation into cellular hemoproteins. As free heme levels increase, feedback regulation on 5-aminolevulinic acid synthase occurs, particularly in liver, slowing down the flow of products.

TABLE 8.8 — ENZYMATIC DEFECTS OF SOME INHERITED PORPHYRIAS

Porphyria	Enzymatic Defect	Porphyrinuria
Acute intermittent porphyria	Porphobilinogen deaminase	Uro
Congenital erythropoietic porphyria	Uroporphyrinogen cosynthetase	Uro, Copro
Porphyria cutanea tardia and hepatoerythropoeitic porphyria	Uroporphyrinogen decarboxylase	Uro, Hepta
Hereditary coproporphyria	Coproporphyrinogen	Copro
Variegate porphyria	Prototporphyrinogen	Copro
Protoporphyria	Ferrochelatase	None (Fecal Proto)

TABLE 8.9 — CONDITIONS THAT CAN CAUSE PORPHYRIA

Genetic Disorders	
Hereditary hyperbilirubinemias	– Dubin-Johnson syndrome – Rotor's syndrome
Bronze baby syndrome	
Erythrohepatic protoporphyria	
Hereditary tyrosinemia	
Metabolic Disturbances	
Diabetes mellitus	
Myocardial infarction	
Hematologic diseases	– Hemolytic, sideroachrestic, sideroblastic, aplastic anemias – Ineffective erythropoiesis (intramedullary hemolysis) – Pernicious anemia – Thalassemia – Leukemia – Erythroblastosis
Disturbance of iron metabolism	– Hemosiderosis – Idiopathic and secondary hemochromatosis – Iron-deficiency anemia
Diseases	
Infectious diseases	– Mononucleosis – Acute poliomyelitis
Liver diseases	– Cirrhosis – Active chronic hepatitis – Toxic and infectious hepatitis – Fatty liver – Alcoholic liver syndromes – Drug injury – Cholestasis – Cholangitis – Biliary cirrhosis
Malignancies	– Hepatocellular tumors – Hepatic metastases – Pancreatic carcinoma – Lymphomatosis
Other Conditions	
Pregnancy	
Carbohydrate fasting	

throughout adulthood until their porphyria is triggered midlife by toxic chemicals or drugs, an acute illness or worsening chronic condition, or a major dietary change.[86]

Environmental Toxicant Effects

Porphyrins measured in urine serve as biomarkers of toxin effects. Toxic chemicals can affect human biochemistry at any level of exposure. Fortunately, the body has mechanisms for transforming, eliminating or compartmentalizing the many toxic chemicals encountered over a lifetime. Nonetheless, these "safety" mechanisms may be inadequate in the modern industrialized society, especially for susceptible people such as the elderly, children, individuals with poor nutritional habits, and others who are physiologically stressed.[87, 88] Recognizing and identifying offending chemicals can present a difficult challenge for the clinician. Many chemicals exert their effect at such low concentrations that they escape direct detection except by very sophisticated laboratory methods. Compartmentalization in tissues, especially brain, that are difficult to access makes routine direct concentration measurements impractical. Elevated porphyrins in urine serve as biomarkers to verify the clinical observations of symptomatic effects of toxicity. Current analytical advances make routine profiling of the multiple intermediates a powerful tool for assessing toxic effects of heavy metals and some xenobiotics.

Details of the decarboxylation steps in heme biosynthesis are shown in Figure 8.9. Porphyrinogens (precursors to porphyrins) are easily oxidized to porphyrins by non-enzymatic metal catalysts. However, enzyme-specific effects result from binding of toxins to specific sites on the enzymes that carry out the porphyrin biosynthesis. In rats, chronic low-level exposure to methyl mercury was associated with characteristic urinary porphyrin changes. These included highly

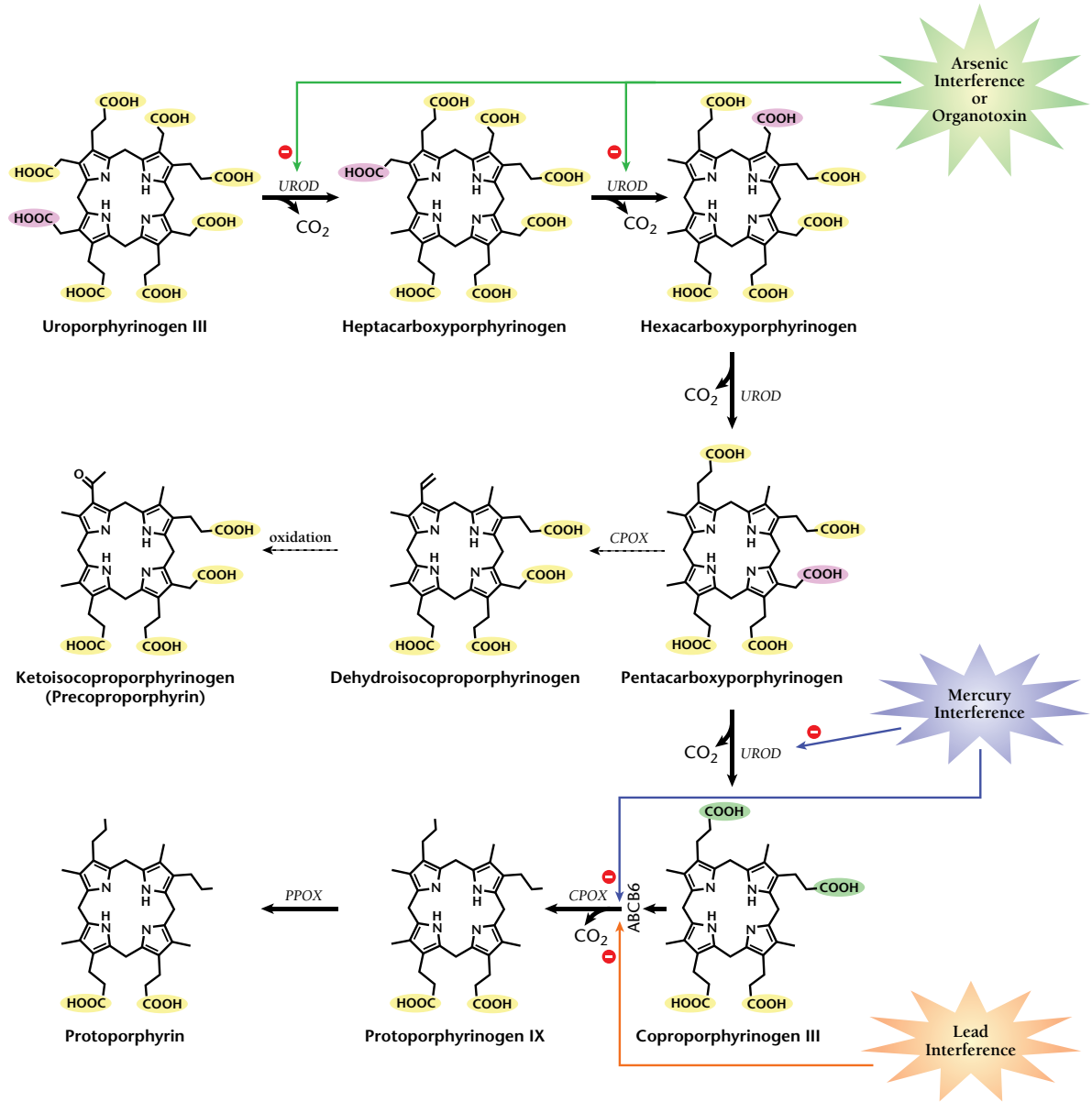


FIGURE 8.9 — **Toxic Metal Interferences in Porphyrin Pathway Decarboxylation Reactions**

The normal heme-forming pathway involves six decarboxylation steps, four of which are carried out by a single enzyme, uroporphyrinogen decarboxylase (UROD). Binding of toxicants causes the creation of an altered binding site that causes slowing of the conversion of uroporphyrinogen III to protoproporphyrin IV. Accumulating pentacarboxyporphyrinogen due to mercury poisoning may be acted on by coproporphyrinogen oxidase (CPOX), yielding the abnormal product ketoisocoproporphyrinogen that is thought to account for the chromatographic peak called precoproporphyrin. Carboxyl groups are shaded yellow and the groups that are cleaved by UROD and CPOX are shaded purple and green, respectively.

Notes:

elevated levels of Copro and Penta intermediates, which were not found in urine of unexposed animals. These distinct changes increased in a dose- and time-related fashion, and reverted to normal levels once exposure was removed.[89] In human studies, a comparable change in the urinary porphyrin profile was also observed among dentists with occupational exposure to mercury.[90] Urinary porphyrin profiles were also shown to correlate significantly with mercury body burden and with specific neurobehavioral deficits associated with low-level (mean levels of 36 µg/L) mercury exposure.[91] Urinary mercury levels greater than 20 µg/mL produced urinary porphyrin elevations (esp. precoproporphyrin) comparable to those found in rats with specific low-level mercury exposure.[90, 92] Ongoing validation studies of dental practitioners with low-level occupational mercury exposure have continued to demonstrate the predicted urinary porphyrin profile change (elevated urinary Penta, Precopro and Copro) among subjects.[92] A final fulfillment of proof that the porphyria is not only characteristic of mercury exposure but, in fact, also caused by the toxic effects of mercury has come by demonstration of sequential induction and removal of porphyrinuria by exposure followed by mobilization with the metal chelator DMPS.[93] The data from this study are reproduced in Figure 8.10. Evidence for the preferential elevation of penta- and precopro by mercury is summarized in Table 8.10. Detecting the pattern unique to mercury requires measurement of precoproporphyrin.

Since the conversion of uroporphyrinogen to coproporphyrinogen III involves four decarboxylation reactions catalyzed by the same enzyme, it is initially curious that toxins affect only the fourth reaction. The explanation seems to lie in the fact that uroporphyrinogen decarboxylase is a complex dimeric protein containing two active sites. The dimeric structure allows for alteration as carboxyl group removal decreases substrate polarity.[94] This allows the rationale for slowing of the final decarboxylation of Penta by toxins without affecting the previous three decarboxylations.[95] Thus the distinctive pattern of mercury poisoning can appear in porphyrin profiles. Laboratory analysis of urine from a mercury-toxic patient by high-performance liquid chromatography shows an abnormal compound that is called precoproporphyrin because it emerges shortly ahead of coproporphyrin. This compound is thought to be the ketoisocoproporphyrin by-product shown as the corresponding porphyrinogen in Figure 8.9, although no

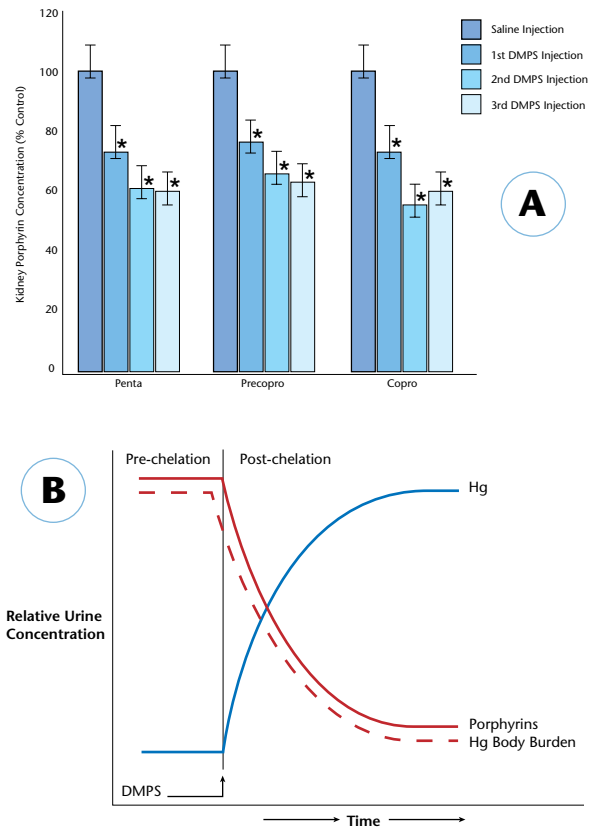


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FIGURE 8.10 — Urinary Porphyrin Changes with Consecutive DMPS Injections[83]

In mercury-exposed rats, urinary levels of penta, precopro and coproporphyrin are elevated. The data shown in (A) reveal how kidney levels of these porphyrins are lowered with removal of mercury by 3 consecutive DMPS injections (100 mg/kg, IP) at 72-hour intervals. The porphyrin levels are displayed as a percentage of pre-chelation saline injection concentrations. *Indicates a significantly different result ($p < 0.05$). Based on such data from animal and human studies, the hypothetical relationship depicted in (B) was proposed. The presence of mercury produces elevated porphyrin levels and its removal is indirectly related to lowering of the characteristic porphyrinuria.

Notes:

Table 8.10 — Reports Showing Porphyria Associated with Mercury Toxicity

Year	Principal Finding	Reference
1991	Dose response increases of penta, precopro and copro in rats (24 hr).	79
1995	Higher levels of penta, precopro and copro in dentists (spot conc. and ng/mg creat.).	81
1996	Higher levels of penta, precopro and copro in dentists correlated with higher urinary mercury and neurological effects.	82
2001	Rats dosed with mercury show declining penta, precopro and copro with sequential dosing with DMPS.	83
2001	Editorial discussion of rationale for extrapolation of rat studies to humans.	92
2006	Higher copro and precopro in autistics (but not Asperger's) and lowering with DMSA treatment.	93
2006	Higher copro (not penta, precopro not measured) in autism and ASD.	94
2007	In rats, total porphyrins (colorimetric) increased with Hg exposure, but not when Se-Met was coadministered.	95

definitive structural confirmation studies are available at the time of writing this book. The enzyme inhibition caused by mercury results in accumulation of pentacarboxyporphyrin that is oxidized by CPOX to form the abnormal porphyrin called precoproporphyrin and thought to be ketoisocoproporhyrin. The mechanism for toxic metal interference is thought to involve active site discrimination on the uroporphyrinogen decarboxylase enzyme as illustrated in Figure 8.11.[89]

A recent finding of heme transporters that regulate movement of Copro III into the mitochondria offers an alternative point of toxic interference to explain the high Copro III associated with mercury exposure.[96] Inhibition of the transporter would tend to raise levels of Copro III and precursors. Evidence for the preferential elevation of Copro I relative to Copro III by arsenic is summarized in Table 8.11. Measuring both Copro III and I rather than total coproporphyrins allows the detection of this perturbation. This perturbation points to involvement of uroporphyrinogen decarboxylase because the elevation of Copro I over III indicates a block in the formation rather than the disposition of Copro III. Such a block would cause more spilling of Uro into the non-productive pathway to Copro I.

Chronic exposure to toxic metals, including lead, mercury, arsenic, aluminum and cadmium often results in organ-specific accumulation that compromises target-organ physiology. Heavy metals damage many aspects of metabolism. Similarly, chronic exposure to organic chemicals such as herbicides, pesticides, and industrial and manufacturing by-products can have deleterious impact on the body's biochemistry, which results in the decline of

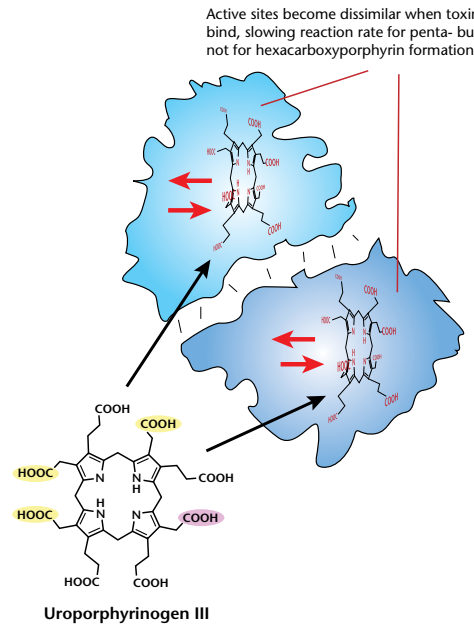

Figure 8.11 — Uroporphyrin Decarboxylase Active Dimer

The blue shaded form represents the silhouette of the enzyme uroporphyrin decarboxylase. The crystallographic three-dimensional structural detail has been determined for this enzyme, showing that the active form is a dimer with the two active sites facing toward the center. The inset shows the structure of uroporphyrinogen III (Uro) with the first three leaving carboxyl groups shaded in yellow and the final one in purple. Two molecules of Uro are shown in oblique view at the active sites. As carboxyl groups are removed, the sequentially created intermediates must shift their positions. Toxicants like heavy metals may bind to the enzyme in ways that alter the affinity for specific intermediates.

Table 8.11 — Reports Showing Porphyria Associated with Arsenic Toxicity

Year	Principal Finding	Reference
1994	Uro, copro I (high I:III ratio)	96
1999	Uro, copro III and high I:III ratio (humans)	97
2001	ALA (As[III] in rats and guinea pigs)	98
2002	Uro, copro I, copro III, proto IV (As[V] in rats)	99
2002	Uro and penta (As[III] in humans)	100
2002	Copro I, III and proto IV (rat and human)	101
2004	Copro I and III (mice)	102
2006	Copro I and, later, III (mice)	103
2006	Copro I and high I:III ratio (in geese)	104

cellular function.[1] A well-studied incident of hexachlorobenzene-induced porphyrinuria occurred due to ingestion of wheat that had been preserved with the chlorinated hydrocarbon, intending that it would be used for crop planting.[97,98] Polychlorinated phenyls (e.g., dioxin, PCBs),[99] and many drugs[100] may induce porphyrinuria.[101] Whatever the cause, when porphyrins accumulate they induce oxidative cellular damage and contribute to lowered hemoglobin and cytochrome P450 enzymes. Chronological listings of reports showing porphyrias associated with mercury and arsenic are provided in Tables 8.10 and 8.11, respectively. Table 8.12 provides a summary of the most common patterns associated with specific environmental toxins.

Table 8.12 — Environmental Toxin-Induced Porphyrinurias

Environmental Toxin	Urinary Porphyrin Elevation (or as noted)*
Arsenic	Uroporphyrins Heptacarboxyporphyrin Hexacarboxyporphyrin Pentacarboxyporphyrin Coprophyrin I High Copro I:III ratio
Mercury	Precoproporphyrin Pentacarboxyporphyrin Coproporphyrin (total)
Lead	Aminolevulinic acid (ALA) Coproporphyrin III Coproporphyrin I (sometimes) Zinc protoporphyrin
Hexachlorobenzene	Uroporphyrins
Methyl chloride	Coproporphyrins
Dioxin	Uroporphyrins
Polyvinylchloride	Coproporphyrins
Polybrominated biphenyl	Coproporphyrins (Uroporphyrins)

** Rule out use of ethanol, estrogens, oral contraceptives, antibiotics, sedatives, analgesics and dietary brewer's yeast, and rule out pregnancy, liver disease, malignancies and pernicious or iron-deficiency anemias.*

Clinical Applications

Attacks of neuropsychiatric symptoms that occur in acute porphyrias may be due to a toxic surplus of 5-aminolevulinic acid or deficiency of vital hemoproteins resulting from impaired synthesis of heme. Accumulation of phototoxic porphyrins produces the solar hypersensitivity found in cutaneous porphyrias.[81] In this condition, the most affected heme precursors are frequently found at levels more than severalfold greater than laboratory upper limits during symptomatic porphyrias. Milder presentations are found in most environmental toxin-induced porphyrinurias. It has been suggested that chronic, mild porphyria may be an etiologic factor in multiple chemical sensitivities, Persian Gulf War syndrome, chronic fatigue and conditions associated with silicone breast implants, although direct evidence for such causation is lacking.[115]

Mounting evidence implicates mercury as a specific risk factor for regressive autism.[55] Arguments for potential interactions such as interference with the function of methionine synthase in the brain have been proposed.[116] However, mercury exposure levels from thimerosal-preserved immunizations or maternal transfer are relatively low, making demonstration of metabolic toxicity difficult. Examination of patterns for urinary excretion of Penta, Precopro and Copro has revealed significantly higher occurrences in autistic children compared with controls.[103] These results have been confirmed in a second study using data from a separate laboratory.[104] Such studies give evidence implicating mercury as a

Table 8.13 — Some Drugs That Cause or Exacerbate Porphyria

Antipyrine	Amidopyrine
Aminoglutethimide	Barbiturates
Carbamazepine	Carbromal
Chloropropramide	Chloral hydrate
Danazol	Dapsone
Diclofenac	Diphenylhydantoin
Ergot preparations	Ethanol (acute)
Ethclorvynol	Ethinamate
Glutethimide	Griseofulvin
Isopropylmeprobamate	Mephenyltoin
Meprobamate	Methylprylon
N-butylscopolammaonium bromide	Nitrous oxide
Novobiocin	Phenylbutazone
Primadone	Pyrazolone preparations
Succinimides	Sulfonamide antibiotics
Sulfonthylmethane	Sulfonmethane
Synthetic estrogens, progestins	Tolazamide
Tolbutamide	Trimethadone
Valproic acid	

contributing factor in regressive autism and related childhood developmental disorders. The finding of porphyrinuria indicative of the mercury effect in an autistic child, especially when verified by direct measurement of mercury, is evidence justifying further clinical action to reduce body burden of mercury.

Changes in the urinary porphyrins (i.e., porphyrinuria) coincident with provocation (e.g., fasting) or therapeutic intervention (e.g., medications, chelation therapy) are suggestive of some type of porphyrinopathy. Latent PCT is frequently activated by such metabolic challenges. If the patient's response upon provocation can be duplicated, then the possibility of a diagnosis of porphyria should be investigated. Urinary porphyrin elevations of three or more times the upper limit of the reference range as found in inherited metabolic acute porphyrins may indicate that organ accumulation of porphyrins is reaching pathological levels.[208, 209] In such cases, a comprehensive genetic porphyria work-up and toxin body burden testing is warranted. For out-of-range results that are lower than three times the upper limit, the rationale for further porphyria testing is predicated on the availability of corroborating clinical and/or biochemical data such as complaints, family and patient medical history. Female sex hormone use can raise levels,[117] and consumption of brewer's yeast has been reported to cause a pseudoporphyria.[118] Drugs that can affect test measurements include aminosalicylic acid, birth control pills, barbiturates, chloral hydrate, chlorpropamide, ethyl alcohol, griseofulvin, morphine, phenazopyridine, procaine, and sulfonamides.[119] A more extensive list is shown in Table 8.13.

Figure 8.12 represents some of the multiple genetic, nutritional and toxicant influences on the porphyrin biosynthesis and degradation pathways. In patients with multiple exposures and compromised nutrient status, the pattern of porphyrinuria may not allow exclusive assignment of single toxicant effects because of overlapping interferences. Use of porphyrin tests as biomarkers of chemical toxicity is useful in combination with other laboratory tests (e.g., blood, urine or hair analysis in cases of suspected metal toxicity). The clinician should realize that there are many conditions unrelated to primary or toxicant-induced porphyria that can cause porphyrinuria. When considering a urinary porphyrin result, the clinician should be mindful that the distribution of normal urinary porphyrin values, representing healthy individuals, overlaps significantly with values representing those who have suffered from porphyria at one time or another. An observed porphyria may be the result of a chemical insult to a pathway enzyme combined with a stressor like iron deficiency that modifies heme pathway activity.[95]

Patients testing mildly positive on the urinary porphyrins test should be followed up with more specific testing such as toxic element testing of chelation-challenged urine for a differential diagnosis. Tests that assay toxic metals directly in biological samples are

Notes:

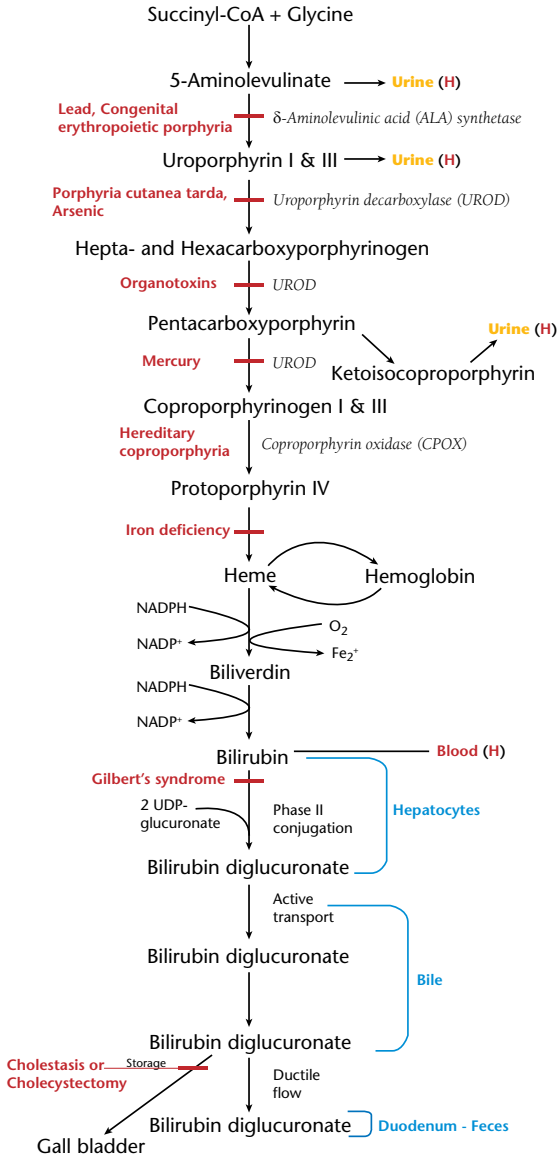

FIGURE 8.12 — Interferences in the Pathway for Heme Formation and Degradation

From the initial reactions of the biosynthetic pathway to the final steps of elimination, the intense activity to form and clear heme presents numerous opportunities for interferences due to toxins, nutrient deficiency, genetic weaknesses or iatrogenic interventions. The intermediates that pass into urine can serve as markers of exposure to environmental toxins, whereas those that accumulate can become endotoxins. When the sum of interferences slows the rate of heme production below that required to maintain total-body oxygen transport, anemia results, and free iron oxidative challenge may occur. When the degradation reactions or secretory systems are blocked, failure of clearance can cause symptoms due to drug overdosing or maldigestion.

essential for confirming whether the toxicity symptoms are caused by a metal. When a significant toxic element metabolic impact is seen in the porphyrin pathway, other functional tests may show abnormalities related to the metabolic perturbations of the toxicant on metabolic pathways governed by enzymes sensitive to toxic effects of the element. For example, oxidative stress markers may show elevations of citric acid cycle enzymes,[120] or electron transport system proteins may be affected.[121, 122]

DETOXIFICATION MECHANISMS

Mechanisms that protect human tissues from toxicity include barriers to penetration, mobilization and excretion. Metabolic biotransformation produces more easily removed chemical derivatives via gastrointestinal tract, kidney, skin and lungs. Metabolism of toxicants can occur in two phases. Phase I is an oxidation step that converts molecules into substrates for the Phase II enzymes.

For many compounds, only one of these two phases is required. For example, normal doses of acetaminophen can be cleared by utilizing the combination of Phase II sulfation and glucuronidation pathways. At sufficiently high intake, these pathways fail to prevent Phase I oxidation reactions that yield reactive acetaminophen metabolites that then require glutathione conjugation (see Figure 8.13). At still higher intake, the accumulation of highly reactive quinone metabolites produces oxidative stress and non-specific reactions with structural proteins and enzymes. Such alterations are a type of metabolic poisoning of enzymes because they are removed from participation in normal metabolism.

From the perspective of evaluating individual total detoxification ability, the classes of enzymes involved may be summarized as shown in Table 8.14. The Phase I and II biotransformation systems that are frequently discussed with regard to clearance of xenobiotic and pharmaceutical compounds are an important part of this set.

Endogenous toxins and exogenous diet-derived or pharmacological compounds are converted to more water-soluble forms by enzymatic action (primarily hepatic) in order to have their toxic potential removed. Although detoxification pathways will act on a very wide variety of chemicals, they do not neutralize all foreign compounds. Highly toxic organic mercury and organic phosphorus compounds owe their great potency to the fact that they

Table 8.14 — Classes of Biotransformation Enzymes

Biotransformation Class	Example Enzyme or Set	
Oxygen Radical Conversion	Superoxide dismutase	
	Catalase	
	Glutathione peroxidase	
Ammonia Removal	Urea cycle enzymes	
	Renal citrate synthetase	
Immunocompetence	Cyclooxygenase	
Mixed Function Oxygenase Systems (Phase I)	Microsomal cytochrome P450	– N-, O- & S-Dealkylations – N-, S- & P-Oxidations – Sulfur, halogen and azo removals
	Microsomal flavin-containing monooxygenase	
	Other oxidation-reduction systems	– Alcohol dehydrogenase
	Aldehyde and ketone oxidases and reductases	– Xanthine oxidase – Glutathione peroxidase – Monoamine oxidase
Conjugation Reactions (Phase II)	Glucuronidation	
	Glutathione transferases (mercaptans)	
	S-Methylation	
	N-Methylation	
	Acetylation	
	Sulfotransferases	
	Thioltransferases	
	Peptide bond formation (glycination)	

are highly reactive and unaffected by the detoxification enzymes of the liver. However, the luminal transport of mercury is greatly attenuated when L-cystine, L-serine, L-histidine and L-tryptophan are present, showing how amino acid therapy might reduce the effects of mercury exposure.[123] Cyanide binds so rapidly and strongly to hemoglobin that it also avoids all mechanisms for neutralization and excretion.

The finding that combination dosing with alpha-ketoglutarate and sodium thiosulfate produces a 70% increase in survival of mice exposed to cyanide is another demonstration of how amino acids (that can generate α–KG and sulfur amino acids) can protect against toxic effects.[124] Hepatic detoxification capacity testing does not evaluate how organic mercury and phosphorus and cyanide are controlled.

Detoxification encompasses mechanisms that enhance tolerance, induce mobilization, increase metabolic conversion rates and induce excretion of toxins. Each of these mechanisms may be addressed by clinical interventions, and each is amenable, in various degrees, to laboratory evaluation. For adverse health consequences from chronic low-level exposures, tolerance is the central issue. Since a toxin-free environment is unrealistic, the enhancement and maintenance of normal detoxification mechanisms through lifestyle modification and use of nutrients is essential. The clinical question is when to use any of the various interventions that are available.

Notes:

Answers to this question are provided by laboratory evaluations of toxin exposure, total-body toxic burden and detoxification capacities (Table 8.1).

For a full picture of a patient's response to toxins, it is important to assess (1) normal exposure tolerance and (2) the ability to handle unusual episodes of high exposure. Some toxins accumulate in the body over the course of weeks (or longer) after environmental exposures and the ingestion and processing of food. Other toxins accrue from short-term acute exposures that can vary over intervals of a few seconds to several hours. Episodes of high exposure occur in some occupations such as the life-threatening topical exposure to methyl mercury solutions, a risk, for example, in some research laboratory environments. Cumulative effects can result from home use of solvents or insecticides.[125] An important example of endogenous toxin production is the random oxidation effects of radical oxygen species (ROS). These damaging compounds are produced as a result of the energy-yielding oxidative pathways in every human cell.[126] Since this is a special form of toxin exposure, the topic of oxidant damage and antioxidant status has been reserved for Chapter 9, "Oxidant Stress."

Another source of chronic exposure requiring detoxification capacity is the flow of compounds from microbial actions on food residues in the gut. This phenomenon is most active in the small intestine as discussed in Chapter 6, "Organic Acids." This source may be considered either normal, chronic background exposure or episodic, acute exposure, depending on the extent of microbial overgrowth and the integrity of the physical barrier. Evaluations of total toxin exposure should always include testing for elevated flow of toxic microbial products formed in the gut.

Notes:

Clinical Laboratory Assessments of Detoxification

Diagnosis of the toxic patient relies on proper use of clinical laboratory data linked with a medical history that can reveal episodes of exogenous toxicant exposure. Some measures show metabolic effects of impaired detoxification systems, whereas others show the presence of the toxicant by direct measurement.

Toxicant Indications from Standard Serum Chemistry Profiles

Urea in serum, commonly known as blood urea nitrogen (BUN), is the normal non-toxic (or one might say "detoxified") form of ammonia, so elevated BUN is a sign of increased ammonia loading and conversion by the urea cycle. A high value is expected to be found in patients where urinary orotate is elevated because that marker appears when ammonia loading exceeds urea cycle capacity for conversion into urea. This relationship may not be found in patients with genetic defects in urea cycle enzymes, preventing normal rates of reaction. See Chapter 4, "Amino Acids," for more detailed description of the urea cycle enzymes.

Ammonia assays may be added to profiles of serum chemistries for a direct assessment of ammonemia. When such results are used in tandem with functional markers found in urine organic acid profiles, it is valuable to keep in mind the relatively transient nature of ammonia due to the multiple mechanisms dynamically linked for ensuring optimal removal following episodes of exposure. Thus lack of correlation may be due to differences in timing of specimen collection. The tests can reveal progressive states of mild, episodic ammonemia to the dangerously severe, chronic state as outlined in Table 8.15. Profound chronic elevations of serum ammonia are a cardinal sign of late-stage liver failure with immediate, life-threatening effects known as hepatic encephalopathy.

Bilirubin elevation due to impaired glucuronidation is discussed in Case Illustration 8.1, where mild Gilbert's syndrome was apparently a part of specific diet-related symptoms. The well-known effects reported for severe Gilbert's cases illustrates the toxicity of the normal hemoglobin product, bilirubin. A patient with even mildly elevated serum bilirubin is immediately among those where therapeutic drug monitoring is important because

Table 8.15 — Markers Associated with Stages of Ammonemia

Laboratory Result	Indication
Elevated urinary citrate, *cis*-aconitate and isocitrate	Increased renal clearance
Elevated urinary orotate with mild serum ammonia elevation	Urea cycle capacity exceeded
Strongly elevated serum ammonia	Encephalopathy

of the likelihood that drug half-lives are significantly extended. For example, acetaminophen glucuronide formation was found to be 31% lower in subjects with Gilbert's syndrome than that in normal controls.[127] In this particular instance, the mild forms of the inherited disorder can confer a favorable metabolic protection. Serum bilirubin can function as an antioxidant and inhibit hydroperoxide formation and alpha-tocopherol consumption, resulting in plasma lipids being protected from peroxidation.[128] Rising bilirubin levels in patients without Gilbert's syndrome can signal liver disease that has obvious ramifications for detoxification functions.

Refer to Case Illustration 8.1

Creatinine measured concurrently in serum and urine can be used to calculate creatinine clearance for assessment of kidney damage. Increased serum creatinine can indicate kidney damage due to autoimmune diseases, congestive heart failure, atherosclerosis or complications of diabetes. In any event the loss of kidney function is a major consideration for evaluating toxicant effects due to the loss of a major removal route.

Urinary Markers of Detoxification

Compounds present in unchallenged overnight urine reveal aspects of xenobiotic exposure, endogenous toxin handling and detoxification functions. Unchallenged urinary markers are summarized in Table 8.1, and they were described in Chapter 6, "Organic Acids." Data from an unchallenged urine specimen cannot show capacities for toxin removal. However, such testing does allow simultaneous examination of the levels of intestinal microbial toxins and markers for hepatic conjugation substrate adequacy. For example, a patient with elevated p-cresol and pyroglutamate has both a high exposure level (elevated p-cresol) and a low detoxification capacity (elevated pyroglutamate). Elevated pyroglutamate indicates specific depletion of glutathione, which takes on special significance when intestinal bacteria are causing increased release of toxic products.

Urinary markers of dysbiosis should be examined carefully in cases of chronic fatigue because signs of small intestinal bacterial overgrowth signal increased transient ammonia loading, which may cause symptoms of lethargy. Intestinal bacteria can contribute significantly to the total load of ammonia,[129] and the level of contribution is affected by dietary composition.[130, 131] Exercise also causes a rise in blood ammonia, especially if glucose availability is impaired.[132] Ammonia and other endotoxins as well as environmental toxin loading are factors that can contribute to chronic fatigue.[133-135]

Ammonia is toxic to the central nervous system, and the amount of ammonia produced each day by a healthy individual exceeds the CNS toxic limit by several orders of magnitude. Since even transient elevations of blood ammonia can lead to lapses in mental ability and sensation of muscle fatigue,[136] evidence of poor ammonia clearance from blood provides valuable insight into clinical presentation and treatment. The largest routine source of ammonia is dietary protein because the majority of the amine groups are converted into ammonia. Because it carries two amine groups, glutamine is particularly known to challenge the ammonia-clearing pathways.[137]

Ammonia is normally cleared by the reactions of the urea cycle. This pathway is highly energy (ATP) demanding and dependent on adequate concentrations of arginine and aspartic and glutamic acids.[138] The most critical of these is arginine. Elevated urinary citrate and orotate are signs of ammonia loading and poor ammonia clearance due to arginine insufficiency, respectively.[139] Supplemental alpha-ketoglutaric acid is also useful for lowering ammonia levels in blood.[140, 141]

Potential toxicants like acetaminophen that require sulfation for clearance cause lowering of serum and urinary sulfate and depletion of total-body sulfur amino acids.[142] Intake of sulfur-rich proteins or sulfur-containing amino acids like methionine or N-acetylcysteine raise available organic sulfur and restore sulfate levels.[143, 144] A patient with both high toxicant exposure and oxidative challenge can quickly have total demand for sulfur amino acids and glutathione reach levels that stress rates of supply from dietary sources. Normal sulfate excretion

is approximately 25 millimoles in 24 hours, so sulfate reserves must be maintained by intake of sulfur-containing amino acids.[145] Sodium sulfate dosing helps by sparing the demand for organic sulfur compounds to enter pathways for conversion to sulfate.

TESTING FOR HEPATIC DETOXIFICATION CAPACITY

The human liver contains several different types of cells that are organized into microscopic arrays called "lobules." Each cell can perform hundreds of different biochemical reactions. In addition, the biochemical activity of each cell depends on its interaction with other cells and with the extracellular matrix. Testing for detoxification capacity can reveal the status of specific functions in these cells.

Any history of hepatic inflammatory disorder is evidence that overall organ reserve is low and that hepatic functions may be globally depressed.[146] Such incidents include infections caused by virus, bacteria or parasites (hepatitis, tuberculosis and amoebic liver disease, respectively); drugs (prescription and abused); toxins (DDT or carbon tetrachloride); and excess alcohol intake. Acute toxic exposures can lead to hepatic necrosis and elevated levels of the serum enzymes, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT) and creatine phosphokinase (CPK). Concurrent rises in GGT, AST and ALT on follow-up testing identifies alcoholic men under treatment who have resumed drinking.[147] Low blood urea nitrogen (BUN) signals hepatic failure and increased risk of critical ammonemic sequelae.[148] Such findings signal general hepatic functional impairment but are not necessarily related to toxin exposure.

Exposure to volatile petrochemical substances in the workplace causes elevated GGT, implying toxic liver degeneration.[149] Aspartic acid may help to protect against toxic liver damage.[150]

For many potentially toxic compounds, the detoxification process involves two phases that operate in a

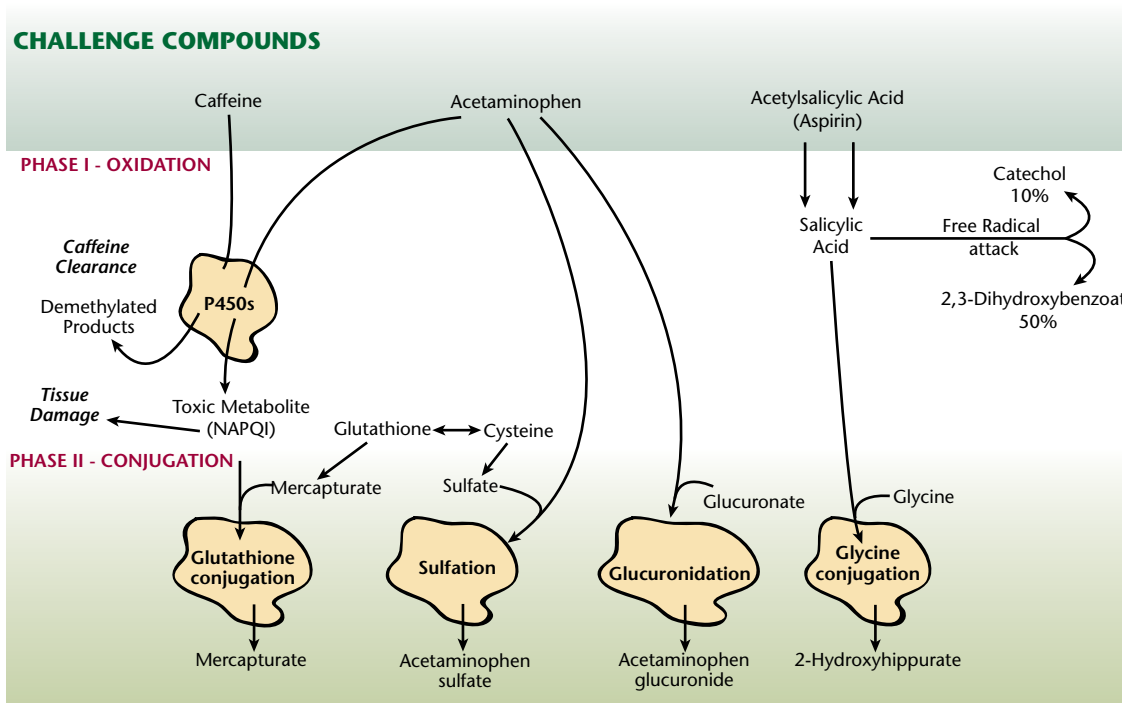

FIGURE 8.13 — Use of Challenge Compounds to Evaluate Phases of Detoxification

Clearance of oral challenge with caffeine, acetaminophen and aspirin clearance may be monitored by submitting timed specimens for measurement of salivary caffeine and urinary acetaminophen mercapturate, acetaminophen sulfate, acetaminophen glucuronide and 2-hydroxyhippurate. Urinary 2,3-dihydroxbenzoate also may be measured for assessment of the product of hydroxyl radical oxidation of salicylic acid.

Table 8.16 — Substrates of Cytochrome CYP450 Enzymes

Type of CYP450	Substrates	
CYP1A1	Estrone, Brassica vegetables containing Diindolylmethane (DIM) or Indole 3 carbinole (I3C)	
CYP1A2	Theophylline, caffeine, phenacetin, acetaminophen	
CYP2C Family	Phenytoin, ibuprofen, naproxen, oxicam drugs, S-warfarin, Diazepam, hexobarbitone, imipramine, omeprazole	
CYP2D6	Cardiology	– Alprenolol, bopindolol, carvedilol, metoprolol, propranolol
	Psychiatry	– Amitriptyline, clomipramine, desipramine, nortriptyline
	Others	– Codeine, dextromethorphan, ethylmorphine, 4-methoxyamphetamin
CYP2E1	Acetaminophen, caffeine, alcohol, chlorzoxazone, enflurane	
CYP3A	Lidocaine, erythromycin, cyclosporin, ketoconazole, testosterone, estradiol, cortisone	

serial fashion so that the product of the first phase becomes the substrate of the second. The difference from the sequence of reactions for the pathways of energy and biosynthetic metabolism is that the enzymes can react with multiple types of compounds, producing overlap in the potential fates of a given toxicant. In Phase I, new chemical groups are introduced to substrates.[151] Although this phase is sometimes described as "oxidation," groups are introduced by oxidation and reduction as well as several other types of reactions as outlined in Table 8.14. In Phase II (sometimes called conjugation), additional groups that confer greater water solubility are introduced so that the products can be transported from enterocytes or hepatocytes. In the liver, the products enter either the biliary system or the blood, from which they may be removed by the kidneys and excreted in urine. Efficient clearance of a given compound may require only Phase I or Phase II type reactions or both. Tables 8.16, 8.17 and 8.18 summarize information about the compounds that stimulate the P450 emzymes system and compounds that are acted on by P450 and conjugating enzymes, respectively.

Comprehensive testing for capacities in all areas of hepatic detoxification is not yet widely available. However, tests that show activities in the major enzyme systems can give valuable clinical insight regarding how patients respond to toxin loads. For investigations of specific toxicant clearance capacities, the challenge compounds must be non-toxic at the levels used and must require the same pathway as the class of toxins under evaluation. The common, non-prescription drugs caffeine, acetaminophen and salicylic acid undergo well-defined hepatic detoxification pathways (Figure 8.13). Detoxification capacity may be evaluated by administering these compounds at a sufficiently high dose to challenge the pathways used for their detoxification. Because caffeine, acetaminophen and acetylsalicylic acid are readily available and well tolerated by most individuals, they are suitable for such use. Quantitative analysis of clearance rates or urinary products of the detoxification reactions provides evidence of detoxification capacities of those enzymes for processing other toxins. Antioxidant protection status may be assessed in the same procedure. For example, free radical attack on salicylic acid is revealed by measuring the percentage of salycilate converted to urinary 2,3-dihydroxybenzoate.[152]

In this detoxification process, products of the first phase of detoxification can be much more toxic than the original compound. This means that any impairment in a subsequent conjugation step can lead to accumulation of the more highly toxic intermediate. The reactive metabolites may form covalent bonds with various macromolecular cell components. Modified cell regulatory sites can lead to cell injury, whereas DNA modification can be carcinogenic. For example, the analgesic acetaminophen is a leading cause of toxic drug overdose because of its toxic quinone metabolite that causes

Table 8.17 — Substances That Can Induce P450 Enzymes

Drugs	Alcohol, barbiturates, sulfonamides
Dietary	High-protein or brassica diets, saturated fats
Hormones	Steroid hormones
Foods	Charcoal-broiled meats, oranges, sassafras, tangerines
Vitamins	Niacin, riboflavin
Xenobiotics	Carbon tetrachloride, dioxin, exhaust fumes, organophosphorus pesticides, paint fumes

TABLE 8.18 — CONJUGATION PATHWAYS USED FOR SPECIFIC COMPOUNDS

Pathway	Xenobiotics		Drugs		Natural Compounds	
Glutathione conjugation	Styrene Acrolein Ethylene oxide Benzopyrenes Methyl parathion Chlorobenzene	Anthracene Toxic metals Petroleum distillates Naphthalene	Acetaminophen Penicillin Ethacrynic acid Tetracycline		Bacterial toxins Aflatoxin Lipid Peroxides Ethyl alcohol Quercitin	N-Acetylcysteine Prostaglandins Baterial toxins Bilirubin Leukotreine A4
Sulfation	Aniline Pentachbrophenol Terpenes Amines Hydroxylamines Phenols		Acetaminophen Methyl dopa Minoxidil Metaraminol Phenylephrine		DHEA Quercitin Bile acids Safrole Tyramine Thyroxine Estrogens Testosterone Cortisol	Catecholamines Melatonin 3-Hydroxy coumarin 25-Hydroxy vitamin D Ethyl alcohol CCK Cerebrosides
Gycine conjugation	Napthylacetic acid Aliphatic amines		Salicylates Nicotinic Acid Chlorpheniramine Brompheniramine		Bile acids Cinnamic acids PABA	Plant Acids Benzoic acid Phenylacetic acid
Taurine conjugation	Propionic acid Caprylic acid				Bile acids Stearic acid Palmitic acid Myristic acid	Lauric acid Decanoic acid Butyric acid
Glucuronidation	Aniline Carbamates Phenols Thiophenol Butanol N-Hydroxy-2-napthylamine		Salicylates Acetaminophen Morphine Meprobamate Benzodiazepines Clofibric acid Naproxen Digoxin	Phenylbutazone Valproic acid Steroids Lorazepam Ciramadol Propranolol Oxazepa	Bilirubin Estrogens Melatonin Bile acids Vitamin E	Vitamin A Vitamin K Vitamin D Steroid hormones
Acetylation	2 Aminofluorene Analine		Clonazepam Dapsone Mescaline Isoniazid Hydralazine	Procainamide Benzidine Sulfonamides Promizole	Serotonin PABA Histamine Tryptamine	Caffeine Choline Tyramine Coenzyme A
Methylation	Paraquat Beta-carbolines Isoquinolines Mercury Lead	Arsenic Thallium Tin Pyridine	Thiouracil Isoetharine Rimiterol Dobutamine Butanephine	Elouphed Morphine Levaphanol Nalorphine	Histamine Epinephrine Dopamine Norepinephrine L-Dopa Apomorphine Hydroxyestradiols	

breaks in DNA.[153] Even inactive modified proteins can lead to pathology by presenting as haptens for antibody production, and this autoimmune response may be injurious.

Phase I Testing by Caffeine Clearance

The ability to clear a challenge dose of caffeine is an indicator of the detoxification capacity of the hepatic Phase I pathway through cytochrome P450 (CYP450). The enzyme system called microsomal P450 mixed-function oxidase is used to oxidize such compounds to prepare them for removal. Its activity toward all similar molecules is reflected by how fast caffeine is removed from the body. After an oral challenge dose of 200 mg caffeine, saliva samples are taken at intervals of two and eight hours. The saliva is analyzed for caffeine, the concentration of which closely parallels that in blood.[154,155] This test evaluates only the isoforms of CYP450 that act on molecules with structure similar to caffeine. Many other CYP450 isoforms are required for oxidation of other classes of toxicants.

It is important to evaluate the CYP450 enzymes because they handle such a wide variety of toxins. They

have a limited capacity, and there is great individual variability in rates of expression of the genes. The CYP450 step tends to be rate limiting for overall toxicant clearance because the rate at which they process substrates is very slow relative to most other enzymes—only about one molecule per enzyme per minute is transformed.[156]

If Caffeine Clearance Is HIGH: A high value signifies that the liver is very actively removing caffeine and similar compounds using the microsomal P450 enzyme. When this activity is elevated, it is especially important to minimize exposure to environmental toxins such as car exhausts, food contaminants and pesticides since the P450 enzyme can convert such substances into more toxic compounds, many of which are pro-carcinogenic. As exposure decreases, enzyme activity should decline. Smokers and those who maintain a high-protein diet will usually demonstrate a higher caffeine clearance due to continuous induction of this enzyme system, although the several subtypes of P450 enzymes are specifically induced by the compounds on which they act. This means that caffeine clearance may be unaffected by induction of the P450-1A1 class by supplementation of I3C as is commonly done to increase the rate of 2-hydroxyestrone formation (see Chapter 10).

Gut microbes are capable of generating many toxins that must be degraded by the P450 enzyme system. If an imbalance exists between the beneficial and pathogenic bacteria, increased levels of toxic compounds can be absorbed through the gut wall. See Chapter 7, "GI Function," for routines to restore intestinal microbial balance.

The P450 enzyme system causes chemical oxidation, wherein dangerous free radical compounds are formed. The level of protection from the destructive effects of such products depends largely on intake of antioxidant nutrients. Therefore, supplementation of the antioxidant nutrients such as vitamins C and E, beta-carotene, zinc, selenium and lipoic acid may be helpful (see Chapter 10) to support hepatic Phase I detoxification.

If Clearance Rate Is LOW: For an individual with average food intake and environmental exposure, a low caffeine clearance rate indicates genetic polymorphism or loss of liver function. Examples of conditions leading to such loss are infectious liver diseases and alcoholic cirrhosis.[157]

A low result indicates that the processing and degradation of foreign compounds is slower than normal. Since most xenobiotic toxicants are non-polar, they will tend to accumulate in adipose tissue because they are not cleared from blood. Effects are typically seen in the central nervous system and in higher rates of neuromuscular function degeneration.

The P450 enzymes are an inducible, on-demand detoxification system. If there has been low exposure to environmental inducers, a low value may simply indicate a low exposure. For most individuals, however, it is important to decrease toxic exposure and increase the enzyme activity. Supplementation with high-quality protein, iron, riboflavin and ascorbic acid may be considered, along with routines to stimulate detoxification through the skin and gastrointestinal tract. Once P450 enzyme production has increased, activity rates depend on the availability of magnesium and niacin (for NADPH synthesis).[156]

Phase II Testing

Benzoic Acid Clearance: Figure 8.5 shows how benzoic acid loading of humans can deplete glycine, the conjugation substrate, and coenzyme A, the cofactor required for glycine conjugation. The very real potential for some patients being in a state of glycine and pantothenic acid insufficiency is shown by these results and others where urinary pyroglutamate is found to rise as glycine insufficiency develops.[158] Because glycine is one of the three amino acids used to form glutathione, insufficiency of glycine may be associated with total-body glutathione depletion. Exposure to pentachlorophenol (PCP), a pesticide used industrially as a wood preservative for utility poles, railroad ties and wharf pilings can induce a 60% reduction in glutathione content in mouse tissue, explaining the cytotoxic and genotoxic properties of PCP.[159]

Notes:

Acetaminophen Conversion: A 650 mg dose of acetaminophen taken in the evening is converted to various conjugation products overnight, and the levels in the overnight urine reflect the activities of conjugation enzymes. Pathways utilizing glutathione, glucuronic acid or sulfate produce the mercapturate, glucuronate or sulfate conjugates, respectively, of acetaminophen (Figure 8.13). The drug is initially oxidized to the reactive metabolite, N-acetyl-p-benzoquinone imine (NABQI), which is highly toxic and must be removed by conjugation with mercapturic acid via glutathione activation.[160] Pathways utilizing sulfur compounds in detoxification are illustrated in Figure 8.14.

The activity of enzymes that carry out sulfation of acetaminophen can be limited because of limited availability of the active sulfate donor, 3'-phosphoadenosine 5'-phosphosulfate (PAPS). The supply of PAPS is, in turn, limited by the availability of its precursor, inorganic sulfate. Administration of sodium sulfate and the sulfate precursor N-acetylcysteine can enhance the formation of acetaminophen sulfate.[161] A test result showing a low percent conversion into acetaminophen sulfate means that the patient has reduced capacity to remove toxic compounds requiring sulfation. Long-term accumulation of the initial toxicants or the intermediate oxidation products can produce widely varying symptoms.

Patients highly susceptible to environmental illness have diminished capacity to form acetaminophen sulfate.[162] A lowered capacity for xenobiotic metabolism, especially of compounds containing sulfur, is a major risk factor for the development of Alzheimer's disease.[163] In ruminants, sulfate metabolism is impaired by excessive dietary molybdenum.[164]

Obese patients may show glucuronide values above normal because obesity is associated with enhanced capacity for biotransformation of drugs via glucuronide conjugation. Conjugating capacity by this mechanism increases in proportion to total body weight and is consistent among drugs.[165] Cigarette smoking is more likely to induce drug oxidation rather than drug conjugation, and thus the habit becomes a factor to consider in the interpretation of results for smokers.[166] Smokers show a high rate of caffeine clearance but low rates of acetaminophen mercapturate formation. Because oleic acid activates the glucuronidation enzyme system,[167] recommendation of olive oil as a dietary fat is one component of intervention when production of acetaminophen glucuronide is low.

When used as an analgesic, recommended doses of acetaminophen should not be exceeded because of hepatotoxic effects due to the hepatic sulfur conjugation pathway having a relatively low capacity. Acetaminophen, on the other hand has a high affinity for the conjugating enzymes, preventing them from processing other toxins and leading to hepatic necrosis in the

Notes:

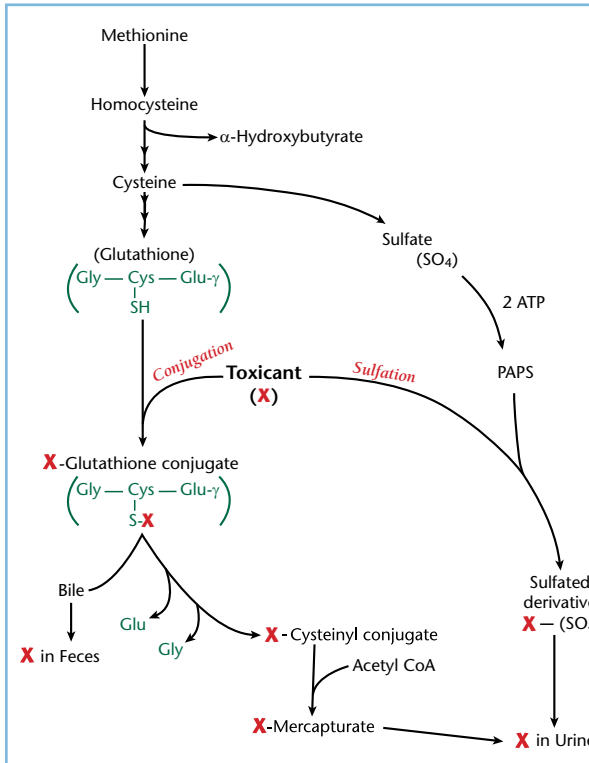

FIGURE 8.14 — Glutathione and Sulfate Conjugate Formation

Xenobiotic compounds (and natural toxicants or pharmaceuticals) may be removed in Phase II of detoxification by attaching highly polar groups to increase water solubility. The sulfur-containing amino acids are used to produce glutathione and sulfate for the conjugation steps. Sulfated products are passed into urine, whereas glutathione conjugates leave via the biliary system or, after glutamyl and glycyl groups are removed to form mercapturates, via urinary excretion.

absence of sufficient sulfur compounds. For prevention of such hepatotoxic effects, both inorganic sulfate (as IV $MgSO_4$) and oral N-acetylcysteine are effective in providing the necessary increase in active sulfate (PAPS).[161]

REFER TO CASE ILLUSTRATION 8.1

Phase II Testing by Salicylic Acid Challenge

Phase II detoxification includes conjugation with glycine, sulfate and glucuronic acid. A 650 mg dose of acetylsalicylic acid (aspirin) taken in the evening is converted to urinary conjugation products that appear in overnight urine (Figure 8.15). A rapid, non-enzymatic reaction produces salicylic acid with release of acetate. The free salicylate may then undergo either of two enzymatic conjugation reactions with glucuronic acid or enzymatic glycination, and the proportions of the conjugated products that appear in urine reflect the ability to transform compounds by these pathways.[168] When glycine is adequate, the glycine conjugate salicyluric acid is formed more easily than the glucuronides, so the relative amounts allow an assessment of these pathways.[169]

The results of this part of the challenge test are interpreted for adequacy of glucuronide and glycine conjugation capacity. For example, the individual described earlier with a mild expression of Gilbert's syndrome will show a low percentage conversion to the glucuronides. Low percentage conversion to salicyluric acid means poor glycine conjugation due to inadequate hepatic glycine or coenzyme A (pantothenic acid) availability. Such individuals will have slow clearance of many drugs and natural toxicants. Oral supplementation with glycine and pantothenic acid can be effective for increasing rates of glycination. The salicylate challenge test may be used to perform an additional assessment of antioxidant status because the high rate of reactivity of salicylate with hydroxyl radicals to produce the two di-hydroxylated products shown in Figure 8.15. Elevated percentages of conversion into catechol or 2,3-dihydroxybenzoic acid indicates inadequate antioxidant status to protect against hydroxyl radical damage to PUFAs, DNA and proteins.[170]

The Phase I/Phase II Ratios

These ratios can warn of early risk of carcinogenic or hepatotoxic compound accumulation. The oxidation reactions of Phase I frequently produce compounds that are of greater (sometimes extremely greater) toxic potential than the initial xenobiotic. The normal progression would lead to efficient removal via Phase II reactions. Patients with abnormally low Phase II or an increased ratio of Phase I to Phase II may display hypersensitivity to xenobiotics, pharmaceuticals and other toxin sources. If either of these conditions is found, detoxification is impaired and the results of toxin exposure are amplified.

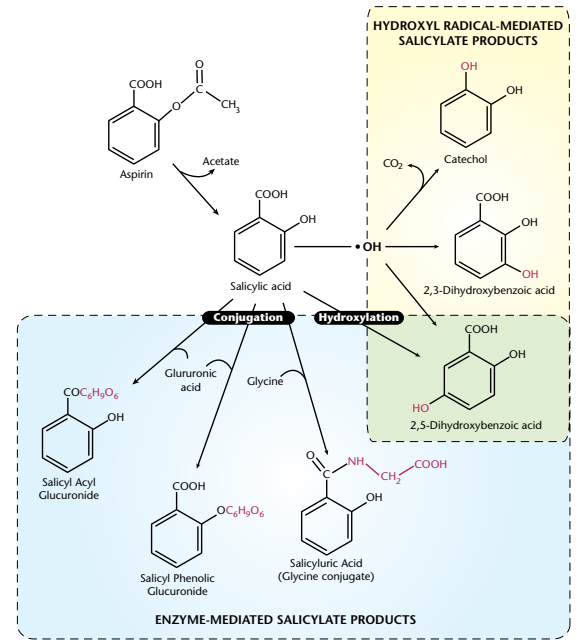

FIGURE 8.15 — Detoxification Products of Aspirin

Measuring detoxification products in urine after challenge with aspirin affords options to the assessment shown in Figure 8.12. After oral challenge with aspirin, urine is collected and analyzed for salicyl glucuronides, salicyluric acid and catechol or 2,3-dihydroxybenzoic acid. Calculated percentages of conversion provide assessments of the relative rates of conversion into products of different enzymatic or free radical oxidation reactions.

Figure 8.16 shows the detoxification challenge profile report from a 59-year-old female who had been diagnosed with a type of bipolar disorder that involved spasmodic contractions of carotid arteries and eye muscles. At the time of the test she was taking both prednisone and imuran and she had been advised to use several herbal extracts because of clinical impressions that she was in a state of toxin accumulation. A urine challenge test had shown elevated lead and she

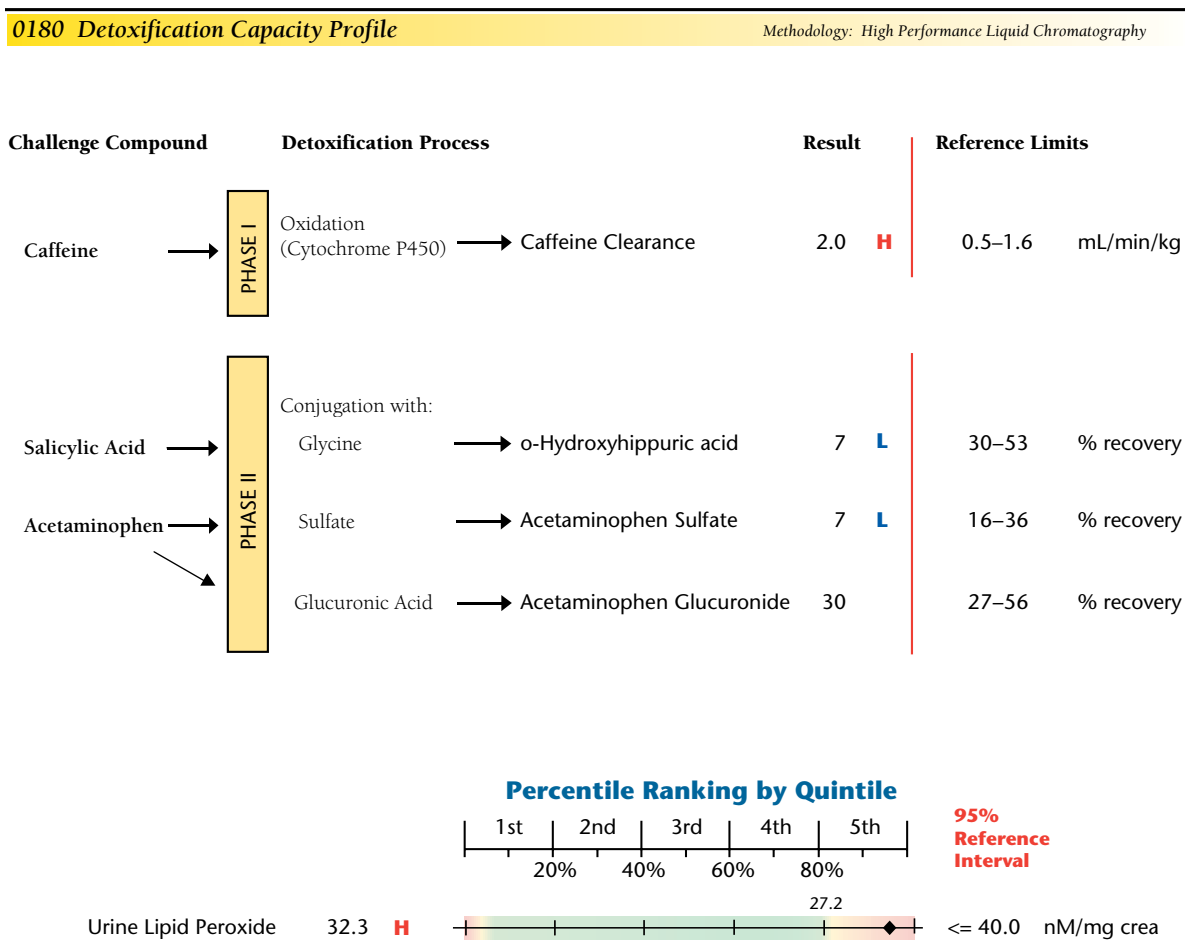

FIGURE 8.16 — Hepatic Detoxification Challenge Report Example

Elevated caffeine clearance indicates a strong Phase I oxidative response of the hepatic P450 enzymes, whereas all three Phase II conjugation steps are at or below their low limits. This pattern leads to the pathological detoxifier state due to accumulation of highly toxic intermediates from the oxidation steps. Her oxidative damage rates are elevated according to the 5th quintile level of her urine lipid peroxide test.

was using supplemental lipoic acid and N-acetylcysteine that may have been intensifying the mobilization of lead from inert deposition sites in bone.

The detoxification challenge profile revealed that she was in danger of increased toxic consequences from the accumulation of oxidized products because the caffeine clearance rate was high and the ratios of caffeine clearance to glycination, sulfation and glucuronidation conversion were all high. These results meant that her P450 enzymes had a high rate of production of products that were only slowly removed by final conversion to conjugated products. The accompanying urinary lipid peroxide result was elevated, confirming her need for decreased stimulation of oxidation and increased conjugation. This could be accomplished by increasing antioxidant supplementation and conjugation precursors such as glycine, N-acetylcysteine and/or glutathione.

Table 8.19 — Summary of Challenge Tests for Detoxification

Test	Result	Meaning	Associations
Caffeine clearance	Low	– Low Phase I – Slow P450 pathway	Amphetamines, cimetidine, isoniazid, oral contraceptives
	High	P450 induction	Toxin exposure
Salicylulric acid or acetaminophen mercapturate, sulfate, or glucuronide	Low	Low Phase II	– Low cysteine, glutathione, and related nutrients – Low stimulation
Phase I/Phase II ratio	High	– Increased risk of carcinogenesis – Chemical sensitivity	Deficiencies of conjugation cofactors
Cys/Sulfate ratio	High	Impaired sulfoxidation	Neurological disorders
Cys (plasma)	High	Impaired sulfoxidation	Excessive Cys
Sulfate (plasma)	Low	Glutathione deficiency	Use Cys with care

The Cysteine/Sulfate Ratio

Cysteine is the precursor for hepatic sulfate formation (Figure 8.14). Disturbances in this pathway have been observed in Alzheimer's disease, which showed early morning plasma cysteine/sulfate ratios that were four to five times higher than controls.[171] This situation leads to concerns about metabolic interference from excess cysteine and reduced xenobiotic detoxification due to sulfate deficiency. The various challenge tests commonly used to asses detoxification capacities are summarized in Table 8.19.

Methylation Status Assessment

Methylation is a term that must be used with care in discussing patient metabolic status assessment because of the multiplicity of implications that require discrimination. The first clarification needed is to expand the definition to the more encompassing term of "single carbon transfer status." As described in Chapter 2, "Vitamins," active methyl groups can be created by direct activation of methionine to SAM and by alpha-carbon extraction from serine or glycine cleavage to form 5, 10-methylene-THF (see Figure 2.11). The single carbon group may then undergo various reversible transformations, ultimately being trapped in the methylfolate form until vitamin B_{12}-catalyzed transfer to homocysteine can occur with reformation of methionine.

The two metabolic processes that dominate single carbon transfer status are biotransformation of compounds for elimination and glutathione formation in response to oxidative stress. Glutathione also takes part in biotransformation via the formation of mercaptan conjugates. Secondary in total flux to these processes are biosynthetic processes whereby methyl groups are inserted in rings such as in DNA methylation or catecholamine biosynthesis.

Analysis of red blood cell activity of histamine N-methyltransferase (HNMT) has been proposed as a way to assess methylation in brain and other tissues.[172-177] HNMT catalyzes the N-tau-methylation of histamine and structurally related compounds. This reaction places a methyl group on the number 2 ring nitrogen of histamine.

Notes:

Ethanol Intoxication

Alcohol-dependent individuals show a 300% variation in their rate of clearance of ethanol from blood.[178] Several clinical laboratory tests are pertinent to nutrient status critical for alcohol detoxification. Amino acids tend to become depleted due to lowered dietary protein intake and impaired digestive function. Amino acid insufficiencies can reduce the efficacy of detoxification programs for alcoholic patients because of the behavioral impact of poor neurotransmitter synthesis. Brain serotonin and dopamine levels are directly impacted by lowered plasma levels of tryptophan and tyrosine, respectively (see Chapter 4, "Amino Acids"). Thus, chronic ethanol consumption has negative effects on many aspects of nutrient intake and assimilation.

Ethanol is metabolized through at least two inducible pathways in the liver. Alcohol and aldehyde dehydrogenase enzymes make up the most well-known pathway, but ethanol-inducible cytochrome P450 enzymes can also play a role. High ethanol consumption causes variations in the activities and synthesis of these enzymes. The ethanol-inducible cytochrome P450 enzymes may be decreased by components in garlic.[179] Simultaneous exposure to other classes of toxicants adds further variability in responses among alcohol-dependent patients.

The ethanol-inducible cytochrome P450 is believed to be predominantly microsomal. High rates of activity lead to oxidative damage and generation of radical species that ultimately contribute to lipid peroxidation. Inhibition of this enzyme by the oxidation product trans-4-hydroxy-2-nonenal seems to be a mechanism of negative regulation that can result in limiting ethanol oxidation capacity.[180] Concurrent ethanol and lead exposure greatly increases hepatic zinc loss,[181] and moderate amounts of ethanol decrease the conversion of toluene to the glycine conjugate hippuric acid by 37%.[181] A simple way to assess the adequacy of glycine conjugation is to check for urinary benzoate elevation in a urinary organic acid profile (Chapter 6, "Organic Acids").

Notes:

Intervention Options

To treat abnormal effects of toxin exposure, one must reduce exposure and facilitate removal of toxic burdens. Although identification of sources of exposure is never simple, cases of chronic exposure generally present further difficulty in locating the multiple toxin sources. Books dealing with management of the chemically sensitive patient are available.[182]

Refer to Case Illustration 8.1

Toxin-Related Nutrient Status

In the preface to his landmark work on chronic, low-level toxic responses, sometimes called "chemical sensitivity," Dr. William Rea describes the relationship of nutrients to pollutants: "The final chapter in this volume is on nutrition, which provides fuel for the endocrine, immunologic, and neurological systems and equips the body to respond to pollutant exposure."[182] He aims to lead physicians to realize the importance of strengthening innate systems to properly handle toxins through nutrient therapy.

Nutrition plays a key role in toxin management, which requires a significant portion of total-body energy generation, as well as integrated function of the gastrointestinal, hepaticobiliary, cardiovascular, renal, lung, skin and immune systems. The overall functions are to prevent toxin entry, to identify toxins that penetrate the barriers, to modify toxins into more easily managed forms and to provide mechanisms for removal. The immune system is included in this discussion because of the central importance of handling macromolecular and cellular agents that can cause such devastating effects via the combination of atopic reactions and large antigen-antibody complexes as described in Chapter 7, "GI Function." Such reactions may be considered as components of detoxification.

Detoxification pathways are metabolically demanding. Nutrients are consumed in numerous toxin-modifying reactions that generally involve the formation of new chemical bonds in energy-requiring steps.[183] Toxic effects of copper-based antineoplastic drugs (Casiopeinas) cause inhibition of mitochondrial functions, decreasing overall energy for detoxification reactions.[184]

Nutrient deficiencies, in turn, cause difficulty with toxicant clearance. One example is the marked reduction in the clearance of acetaminophen due to inhibition

Table 8.20 — Detoxification Functions of Specific Nutrients

Nutrient	Function
Vitamin C	Increased mobilization, toxic metal binding, and antioxidant protection
B-complex vitamins	Hepatic enzyme cofactors
Lipoic acid	Hepatic protection and antioxidant regeneration
N-acetylcysteine (NAC)	Glutathione formation and direct complexation
Cysteine	Sulfur amino acid
Methionine	Methyl donor and sulfur supply
S-Adenosylmethioinine (SAM)	Active form of methionine
Glycine	Hepatic conjugation
Free-form essential amino acid mixture	Mitochondrial energy production
Sulfate	Hepatic conjugation
Calcium	Lead protection
Magnesium	Multiple hepatic and other effects
Selenium	Glutathione regeneration and mercury protection
Manganese	Glutathione regeneration
Copper	Glutathione regeneration
Zinc	Glutathione regeneration and cadmium protection

of energy pathways.[185] Even mild nutrient depletions can alter dose-response characteristics.[186] Thus, a patient's response to drugs and environmental toxins depends on their nutritional status, whereas exposures to toxins exacerbate nutrient depletions. Dietary habits have direct influence on detoxification pathways. Low-protein diets cause increased mortality of laboratory animals from exposure to pesticides, chlorinated hydrocarbons and organophosphates.[156]

Specific nutrient interventions should be guided by test results. Although all essential nutrients are required to maintain normal metabolic function of hepatic cells, those most closely involved with support of detoxification are shown in Table 8.20. N-acetylcysteine has become a favored oral supplement for glutathione synthesis, but it must be used with care. In patients with high heavy metal body burden, cysteine may increase mobilization of heavy metals from sequestered sites. Selenium is a mineral that is directly involved in glutathione maintenance through activation of glutathione peroxidase.

Because of similarities in chemical bonding, heavy metals show reduced toxic effects when specific inhibitory nutrient elements are increased as shown in Table 8.20. A good example of this effect is the protective action of dietary calcium against the toxic accumulation of lead, both of which compete for the same binding sites. The fact that lead deposits in bone where it interacts with the calcium regulatory enzymes[187] further confirms the competition of these two elements for binding sites. Extra calcium during pregnancy even protects against transfer of lead to the fetus from a mother with prior lead exposure.[188] Thus, if a test such as a hair mineral profile reveals an elevated lead level and concurrent negative calcium balance, aggressive calcium supplementation as well as removal of lead exposure is indicated. Recognition of such relationships between nutrients and toxicants leads to better clinical insight regarding the variations in individual responses to toxin exposure. The data presented here on nutrient-toxic element interactions are a simplification of the total picture of such effects, which is quite complex with multiple cross-element relationships.[189]

Amino acids containing sulfur and essential trace elements are high on the list of priority nutrients in virtually every case of clinical toxicology. Beans, eggs, garlic and onions are foods that are rich sources for many of these critical nutrients. The cruciferous family of vegetables (cabbage, broccoli, brussels sprouts) contain substances that stimulate the liver to produce Phase I and II enzymes.

Clinical Management of Detoxification

When toxin exposure is determined to merit clinical intervention, the first order of business is to reduce the total body burden of toxin by avoiding exposure. For xenobiotics, the source must be identified, and avoided. This sometimes means extensive modifications of home and work environments.[182]

For toxins resulting from intestinal microbial overgrowth, methods for controlling microbial populations and improving the integrity of the physical barrier may be implemented (see Chapter 7, "GI Function"). A summary of such interventions is presented in Table 8.21.

Removal of foods that elicit immune responses will ease a major burden from the toxin entry barrier of the gut. The normal opening of channels for the outward flow of secretory IgA is impaired in chronic inflammatory states of the gut. Formation of antigen-IgG complexes and loss of antioxidant protection can impair the barrier function against antigen entry.

A chemically defined diet can provide a period of relative absence of antigens to achieve reduction of endogenous toxin exposure. Formulations are widely available containing chemically defined, balanced proportions of major nutrients supplemented with nutrients that support detoxification. The ultimate elimination of toxin entry via the gut may require total water fasting. Those with high body burdens of toxins must be managed carefully to successfully employ fasting. The absence of food intake can increase the mobilization of stored toxins whereas the heightened detoxification activity demands extra nutrients.

In addition to the issue of reducing toxin loads, detoxification metabolic rates should be addressed. One effective way to increase the rate of removal of toxins from the body is to stimulate hepaticobiliary flow and maintain optimal intestinal transit time. Use of dietary oils (especially olive oil) can be helpful as cholegogues to stimulate bile flow and gall bladder emptying.[190] Patients who have undergone cholecystectomy will respond more slowly to cholegogues since the capacity for bile storage is greatly reduced.

High-fiber diets help maintain speedy intestinal transit to assist toxin removal in feces and avoid re-acquisition via entero-hepatic cycling.[191-193] Fast colonic transit time is essential to reduce microbial toxicant production and to lower the reuptake of toxins released in bile. When stool testing reveals increased intestinal bacterial glucuronidase levels, all compounds that had been processed through Phase I and II systems to form glucuronides may be released for reabsorption (Chapter 7). Unabsorbed dietary fiber binds many toxins and allows them to be removed in stools. The National Cancer Institute recommends between 20 and 35 grams of fiber daily. The average American eats less than half of this recommended intake. High-fiber cereals are one of the easiest sources to find fiber, as one serving size can be as high as 8 to10 grams. Other measures suitable for most patients are listed in Table 8.22.

The skin is a large organ of detoxification via the loss of toxins in sweat.[194, 195] Use of sauna and massage can accomplish stimulation of toxin losses through increased blood flow and sweating.[196] Finally, it must be emphasized that the gut is an organ of detoxification at least as important as the liver or skin. The hepatic detoxification reactions discussed in this chapter also occur in the mucosal cells of the gut.[197-202] Clinical laboratory assessment of gastrointestinal function is discussed in Chapter 7, "GI Function."

Chronic ethanol consumption has a dramatic effect on the conversion of 5-hydroxytryptophan to serotonin in the brain.[203] This inhibitory effect may explain the clinically beneficial action of maintaining low concentration of ethanol during alcohol detoxification.[204] The

Table 8.21 — Methods for Increasing Rates of Detoxification

Functional Enhancement	Intervention
Rate of mobilization from soft tissue and bone	Intravenous DMPS Intravenous chelation (EDTA, Penicillamine) Oral DMSA Oral vitamin C
Circulation and sweat flow	Exercise, sauna, massage
Hepatic metabolic rates of toxin conversion	Cruciferous vegetables
Urinary excretion	Fluid intake, diuretic
Fecal excretion	Cholegogues (olive oil), dietary fiber

Table 8.22 — Steps to Increase Dietary Fiber	
1	Eat more fruit. Apples, bananas, apricots, grapes, peaches and strawberries are rich in fiber.
2	Consume more vegetables. Broccoli, cabbage, cauliflower, tomatoes and zucchini all are rich in fiber.
3	Eat legumes. Not only are beans rich in fiber, but many are also high quality protein sources and low in fat.
4	Use whole grains. Increasing whole-grain bread and wheat bran effectively incorporates fiber.
5	Eat cereals high in fiber. Check the food labels on your breakfast cereal for dietary fiber.
6	Consume more berries. Add berries to pancake batters and muffin-mix recipes.
7	Eat brown rice in place of white rice.
8	Eat nuts. Many nuts are high in insoluble fiber.
9	Replace processed fruit juice with whole fruit.

suppression of serotonergic pathways in the brain is a chemical phenomenon relevant to the addictiveness of alcohol. Amino acid testing can be helpful in alcoholic detoxification programs to evaluate the status of neurotransmitter-related amino acids: tryptophan, tyrosine, GABA, and glutamate (see Chapter 4, "Amino Acids").

Since detoxification pathways are used to clear endogenously produced compounds, there are circumstances where it may be desired to decrease the rate of clearance. For example, androstenedione, a potent male anabolic hormone is cleared by hydroxylation and oxidation enzymes that are inhibited by increasing intake of olive oil. This effect appears to be mediated by various phenolic compounds in olive oil, and it is an example of how diet influences hormone levels.[205] Since the effects can be specific to hepatic CYP isoforms, metabolism of ingested toxins and sex hormones may vary depending on the principal dietary oil. Activity of CYP450-3A2 is stimulated by soybean oil, whereas olive and corn oil can decrease CYP2C11 class activities.[206] These dietary variables add to the overall individual variability of response to toxicants and endogenous metabolites.

The concept of genetic influences modified by diet is relevant in assessment of toxicant exposures. Early initiation of dietary and drug restrictions has been recommended for patients with X-chromosomal mutations involving MAO. Individuals with Norrie's disease have a deletion in chromosomal region Xp11.3 that causes non-detectable monoamine oxidase activity. They can show up to 100-fold elevations in the urinary levels of MAO substrates phenylethylamine, o-tyramine and m-tyramine.[207] Dietary restriction should include bananas because of their high levels of dopamine. Dopamine and other biogenic amines are normally modified by intestinal MAO.

Conclusion

Because of modern lifestyles, environmental toxicants play ever-increasing roles in human health. Health threats from the environment are overlaid on detoxification systems that may already be in difficulty due to endogenous or iatrogenic sources of toxicants. Our growing knowledge of genetic influences brings another dimension to patient management through specific measures that might correct impaired detoxification capacities or that offset the results of defective toxicant processing. Laboratory evaluations can provide multiple levels of insight about toxic loads, detoxification status and metabolic effects of toxicant exposures. By combining evaluations of toxic exposures and detoxification capacities with evaluations of individual nutritional insufficiency, new approaches are being developed for managing clinical effects of toxicants.

Notes:

Case Illustrations

Case Illustration 8.1 — Diet-Induced Transient Toxic Symptoms

An otherwise healthy, symptom-free 60-year-old male complains of a highly predictable sleep disturbance that occurs only when he drinks a glass of wine with a late, high-protein dinner followed by a cup of coffee. Such meals are always followed by waking from sleep about 4 hours later feeling hot, sweaty and thirsty with rapid heart rate. Return to sleep is possible only 30 to 45 minutes after drinking water. When dinner is eaten earlier without wine, he can drink coffee an hour before bedtime and have a normal 6- to 7-hour sleep period.

Test	Results		95% Reference Interval
Bilirubin, total	1.8	H	0.1–1.0 mg/dL
Bilirubin, direct	0.36		0.1–0.4 mg/dL
AST (SGOT)	19.0		15–37 U/L
ALT (SGPT)	42.0		30–65 U/L
Alkaline phosphatase	126		50–136 U/L

Tests results show that he has a mild form of Gilbert's syndrome with chronically elevated total bilirubin and normal direct (unconjugated) bilirubin, showing a mild weakness in glucuronidation. His total bilirubin values had ranged from 1.6 to 2.4 over the past decade. A detoxification challenge profile shows that his caffeine clearance rate is high, glucuronidation is slightly low and other conjugation rates are within normal ranges.

Here we have a case where the imbalance of oxidation and conjugation is mild enough to be entirely benign except when the extra load of alcohol, polyphenols and other ingredients in wine are added along with stimulation of Phase I oxidation with caffeine. Then the accumulation of oxidized products requiring glucuronidation is great enough to produce polyuria and transient cardiac and brain neurotoxic effects. Since this is primarily a manifestation of genetic polymorphism affecting glucuronidation, the obvious simple solution is to avoid the offending meal combination. ❖

Notes:

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Chapter 9

Oxidant Stress

Richard S. Lord, J. Alexander Bralley and Kara N. Fitzgerald

CONTENTS

Notes:

Origins and Effects of Oxidative Stress

Oxidative stress is a condition in cells where excessive production of highly reactive molecules called free radicals damage cellular components. When production of free radicals becomes excessive, the antioxidant mechanisms of the cell become overwhelmed and free radical action is not controlled, leading to impaired cell function. Potential targets include lipid membranes; proteins, leading to enzyme inactivation or receptor malfunction; and DNA, leading to mutations and potential cancers. Since oxidant stress can affect cellular function at many levels, it has been thought to be an etiological factor in many disease states,[1] including diseases of the eye,[2] Alzheimer's disease[3–5] and other types of neuronal injury,[6] atherosclerosis, cancer, rheumatic diseases, allergic inflammation,[7, 8] cardiac and cerebral ischemia, respiratory distress syndrome, various renal[9] and liver disorders,[10] irradiation, thermal injury, and toxicity induced by certain metals, solvents, pesticides, and drugs.[11–14] Even in healthy individuals, the constant, normal degradation due to reactive oxygen species (ROS) are thought to explain common effects of aging.[5, 15, 16] The reaction depicted in Figure 9.3 leads to potential accumulation of oxidized fats in cell membranes. This mechanism has been proposed to alter membrane fluidity and affect cell-mediated immunity.[17] The importance of membrane fluidity was discussed in Chapter 5, "Fatty Acids."

Antioxidants are ubiquitously distributed in various cellular compartments to protect against the pathological consequences of ROS accumulation, as depicted in Figure 9.2. The evidence for disease protection from a single antioxidant, vitamin E, has led to the launching of large-scale studies of vitamin E supplementation in patients with early Parkinson's disease,[18] and as a suppressant of free radical damage during cardiopulmonary bypass operations.[19] Although many studies have reported clinical benefits from single antioxidants used at high doses, unless such interventions are specifically indicated, balanced levels of the various antioxidants required for tissue protection is important in long-term patient management.

Free radicals are partially reduced metabolites of oxygen or, in some cases, nitrogen. The free radical molecule contains an unpaired electron in its outer orbital, making it highly reactive and unstable (see Figure 9.1). Other molecules that encounter the radical tend to lose single electrons, becoming oxidized. When a single electron from the mitochondrial electron transport system is captured by molecular oxygen, a radical form called

Table 9.1 — Summary of Testing for Oxidant Stress

	Category	Test	Abnormal	Intervention
Markers of Oxidant Damage	Lipid oxidation	Lipid peroxides – serum or urine	H	Increase balanced antioxidant intake and decrease oxidant load. Use high-dose individual antioxidants as indicated by laboratory testing.
		Isoprostanes – serum	H	
		HNE – serum	H	
		Oxidized LDL – plasma	H	
	Protein oxidation	3-Nitrotyrosine	H	
		Methionine sulfoxide – serum	H	
	Nucleotide oxidation	8-Hydroxy-2'-deoxyguanosine – serum	H	
		DNA strand breakage – WBC	H	
Antioxidant Nutrient Testing	Metabolic end products	Uric acid – serum	L	Antioxidant nutrients
		Albumin – serum	L	Antioxidant nutrients; Find reason for albumin loss
	Glutathione	Sulfate – urine	L	N-Acetylcysteine, Methionine, Taurine, Glycine (check plasma levels)
		Pyroglutamate – urine	H	
		α-Hydroxybutyrate – urine	H	
	Fat-soluble vitamins	Vitamins A & E, β-Carotene, Coenzyme Q_{10} – serum	L	Add individual anitoxidants as indicated by laboratory testing
	Antioxidant minerals	Selenium, Zinc, Copper – RBC, hair, urine, serum	L	Trace elements, digestive support

TABLE 9.2 — RELATIVE REACTIVITIES OF REACTIVE OXYGEN SPECIES

Reactive Oxygen Species	Reactivity
Singlet oxygen (O_2)	Lowest
Superoxide radical (O_2^-•)	
Hydrogen peroxide (H_2O_2)	Moderate
Lipid peroxyl radical (LOO•)	
Hydroxyl radical (OH•)	Highest

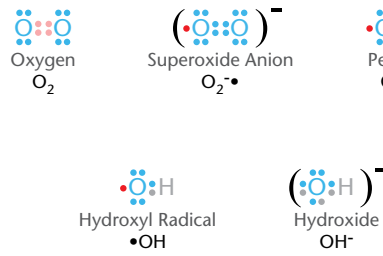


FIGURE 9.1 — Some Reactive Oxygen Species

Except for the hydroxide anion, each of the molecules shown are radicals because they have unpaired electrons (•). Oxygen contains an unusual bonding where unpaired electrons from each atom occupy bonding orbitals (•). In biological systems, free radicals have high tendencies for extracting another electron from nearby molecules. One-electron oxidations frequently create another free radical that perpetuates the sequence.

OF FURTHER INTEREST...

Oxygen continually perfuses human tissues principally in order to serve as the mitochondrial final electron acceptor in cellular respiration. The strongly electrophilic nature of molecular oxygen (O_2) is due to the presence of unpaired electrons on each oxygen atom. Thus, molecular oxygen is a di-radical, stabilized by the double-bond character of orbitals occupied by the two unpaired electrons. Generally, those species with multiple oxygen atoms to disperse the unpaired electron are less reactive than the hydroxyl radical where single oxygen carries the unpaired orbital.

superoxide is created. All such molecules containing oxygen as the acceptor of the single electron are called reactive oxygen species (ROS), several of which are ranked by their reactivity in Table 9.2.

When the unpaired electron is transferred to another molecule, it generates a second free radical. Intramolecular transformations occur frequently, producing structures altered from their original biological form. The altered forms may still contain single electrons that can react with new molecules, creating a chain reaction of propagating ROS and amplifying the deleterious effect manyfold.[20] Most of the damaging metabolic by-products that initiate these sequences are ROS. Other chemicals, such as carbon tetrachloride, can easily become damaging free radicals, even though they do not contain oxygen. Chemists define free radicals as any atom or molecule containing one or more unpaired electron (radical) that has escaped the solvent cage or enzyme active site in which it was generated (free). Read "Of Further Interest" for more background on redox and radical generation.

Although the issue of pathological damage due to excessive ROS is of great concern, it should be noted that ROS also act as normal intracellular messengers that regulate growth and development.[21] The normal mechanism of invading microbe destruction by macrophages requires an oxidative burst where intense ROS formation occurs.[22] Also, numerous normal metabolic intermediates function as either pro-oxidants or antioxidants. For example, elevated circulating bilirubin levels cause oxidative stress that can lead to neurotoxicity.[23] As discussed in Chapter 8, "Toxicants and Detoxification," the porphyrias can cause pathological oxidative stress. In 23 patients with porphyria cutanea tarda, an 8-week treatment trial with 200 mg/d of tocopherol acetate gave improvements in markers of oxidative stress while lowering urinary uroporphyrin levels.[24]

The major energy-yielding step in central energy pathways is the final transfer of the electrons carried by NADH to molecular oxygen via the coenzyme Q_{10} and iron-cytochrome system of the inner mitochondrial membrane. The mitochondrial electron transport chain transfers electrons one at a time, inevitably forming the transient O_2 radical called superoxide (O_2•). The great majority of superoxide radicals thus formed never leave the mitochondrial surface until they have received additional electrons and protons, forming stable H_2O. However, the small fraction of superoxide that diffuses

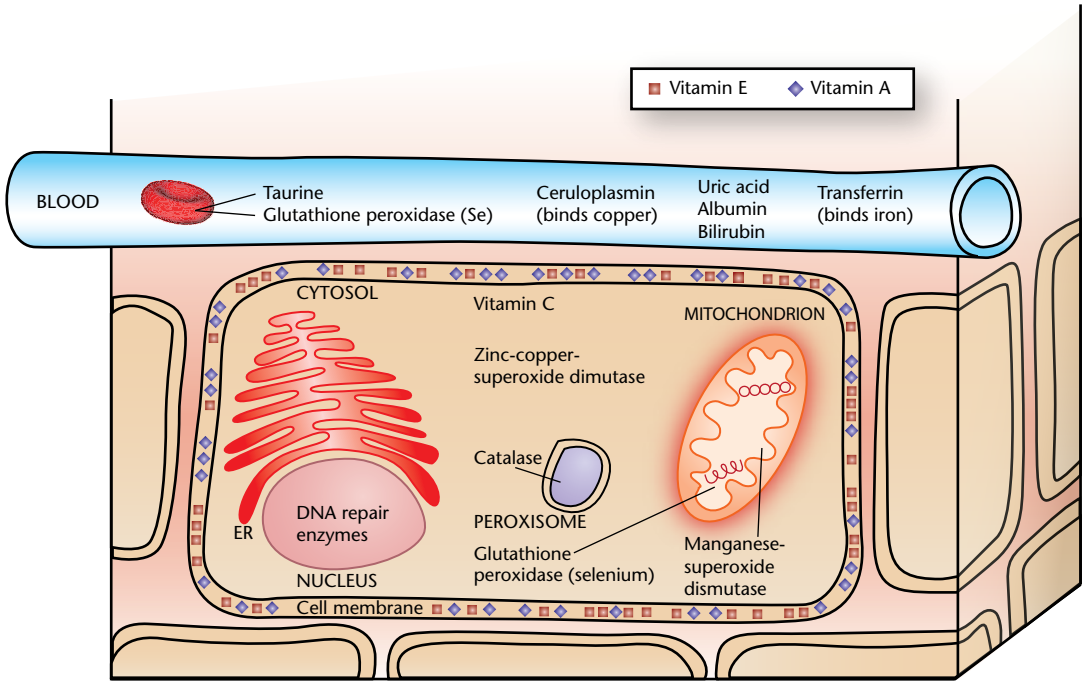


FIGURE 9.2 — Distribution of Antioxidants

The blood, cytosol and organelles contain multiple antioxidant systems to protect against oxidative damage. In structures with high lipid content such as the cell membrane (shown enlarged relative to organelle membranes), the less polar antioxidants such as vitamin E, vitamin A and carotenoids predominate. The aqueous cytosol and interior of organelles are sites where glutathione, vitamin C and the enzyme systems operate. The mitochondria are particularly susceptible to oxidative damage due to their high rate of redox reactions transferring electrons to oxygen. The release of cytochrome C from damaged mitochondria initiates apoptosis. In the blood, protection of proteins and small molecules is needed to protect soluble proteins, erythrocytes and endothelial cells against oxidative damage. Blood is especially susceptible to oxidative damage due to the high amounts of oxygen that are constantly in flux from erythrocyte hemoglobin-binding sites. In addition to the other cellular antioxidants, the plasma is protected by high concentrations of albumin and uric acid. The interior of erythrocytes contains high levels of taurine and selenium-glutathione peroxidase.

away can create considerable trouble for the cell.[25, 26] Thus, the largest source of ROS is mitochondrial respiration.[27] Even slight insufficiencies of CoQ_{10}, iron, heme or cytochromes can cause disruptions in the massive flow of electrons through these systems.

A major mechanism for removal of superoxide is the action of superoxide dismutase. The product hydrogen peroxide is less reactive, but, in the presence of free ferrous iron (Fe^{2+}), it is converted into the extremely reactive hydroxyl radical. Hydroxyl radical attack on a polyunsaturated fatty acid is depicted in Figure 9.3.

Notes:

FREE RADICAL DAMAGE AND PROTECTION

Free radical chain reactions can be terminated when antioxidants donate a single electron to the free radical, stabilizing the compound (Figure 9.4). The antioxidant reactant is either permanently altered or recovered by an electron transfer from another antioxidant. Antioxidant effects are also achieved by sequential reactions catalyzed by enzymes with their elemental cofactors acting in concert with antioxidant vitamins and normal metabolic intermediates to quench oxidative stress. High levels of a single antioxidant with insufficient levels of another may not provide adequate protection. Laboratory evaluations of antioxidant levels and oxidative stress markers can help to guide treatment protocols.

The pathology found in Down's syndrome illustrates how a perturbation at a single locus of the antioxidant protection system can produce adverse cellular effects. The genetic origin of Down's syndrome is the presence of duplicate copies of chromosome 21 (thus the alternate designation as trisomy 21). Since the superoxide dismutase (SOD) gene resides on chromosome 21, affected individuals produce levels of this enzyme that are 40 to 50% higher than controls.[28] Although the SOD-catalyzed reaction reduces levels of superoxide, its product is hydrogen peroxide, which is still quite reactive. Increased rates of oxidative damage have been found in various tissues of Down's patients, including the brain.[29, 30] Results from cell culture studies indicate the presence of stress on the glutathione system in cells with higher SOD levels.[31] The glutathione response is highly adaptable to varying levels of oxidative stress, but the oxidative challenge in Down's syndrome is too great to offset with up-regulation of the glutathione and other antioxidant systems. The oxidative stress also results in lower levels of plasma vitamin E, and the damage to chromosomes is reduced by supplementing vitamin E.[32]

Since the most intense and continuous site of redox activity is the mitochondria, understanding their changes in disease and their response to therapy is needed. Studies on isolated mitochondria have shown that metabolic stimulation by succinate causes dramatic rise in ROS formation.[33, 34] These results demonstrate how any therapy that stimulates oxidative metabolism of glucose or fatty acids can places stress on the mitochondrial electron transport chain. If mitochondrial CoQ_{10} is unable to meet the demand for increased electron flux, then ROS formation increases. Thus, a patient requiring initial therapy with carnitine and B-complex vitamins for fatty acid mobilization and oxidative metabolism can enter into a state of oxidative challenge because of insufficient mitochondrial CoQ_{10} or cytochromes due to lag in porphyrin synthesis. If the porphyrin pathway is inhibited by environmental toxins, the risk of oxidative challenge is further increased. See Chapter 6, "Organic Acids," and Chapter 8, "Toxicants and Detoxification," for further discussions of markers for assessment of mitochondrial metabolism and porphyrin pathway status, respectively.

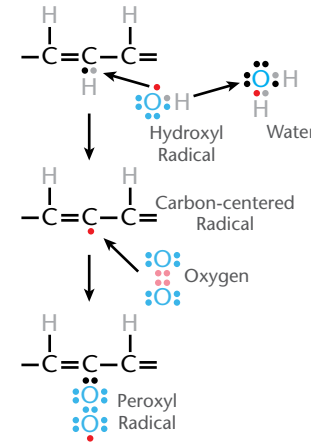


FIGURE 9.3 — Hydroxyl Radical Attack on a Polyunsaturated Fatty Acid

The multiple double bonds of PUFA molecules in cell membranes afford abundant, easily removed electrons. A hydroxyl radical is shown extracting a single electron and capturing the proton to form water. The fatty acid radical then associates with an oxygen molecule, forming the peroxyl radical.

ANTIOXIDANTS

Antioxidants are compounds that prevent oxidative damage in biological systems. The term generally implies the nutritionally essential or conditionally essential elements (i.e., selenium) and compounds (i.e., vitamin E), whose primary function is free radical scavenging. A frequent broadening of meaning is to include the enzymes

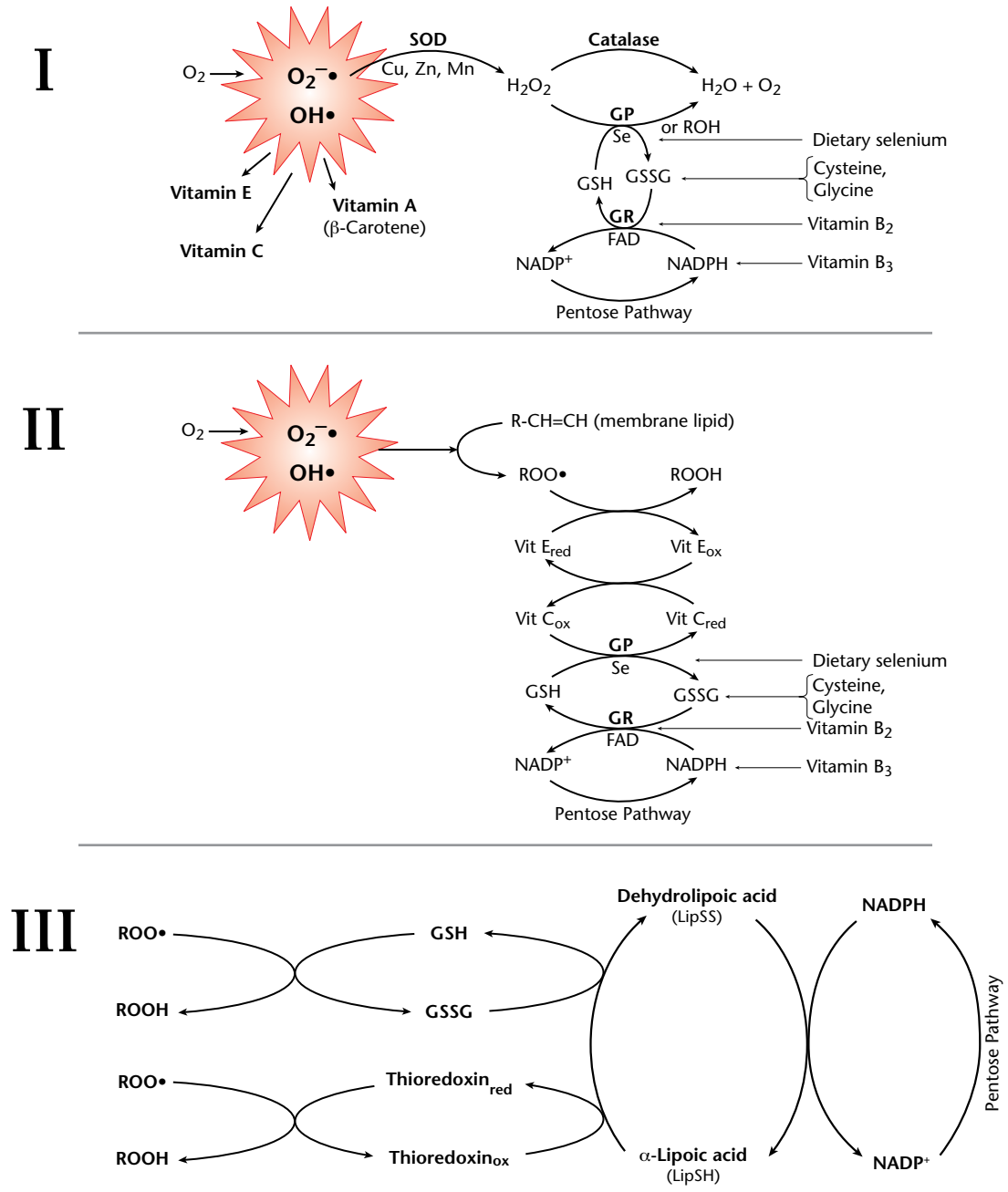


FIGURE 9.4 — Scenarios of Radical Formation and Removal

Reactive oxygen species, such as O_2• and OH•, can damage tissues unless they are removed by electron transfer to vitamins A, C and E, or by enzymatic conversions of superoxide, first to hydrogen peroxide and then to harmless water. The enzymatic conversions are dependent on adequate supply of amino acids, vitamins and essential minerals.

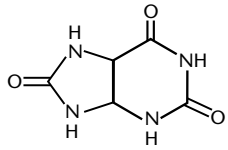
Scenario I shows the removal of hydrogen peroxide by either direct conversion to water or by oxidation of glutathione. The pentose phosphate pathway assures the supply of NADPH-reducing equivalents. In scenario II, an electron transfer from a membrane fatty acid is processed through antioxidants of decreasing electron acceptor potential, but of increasing cellular concentrations and regenerative capacity. Scenario III shows the potential involvement of lipoic acid for regeneration of oxidized forms of glutathione and thioredoxin, two critical components of cellular response to oxidative stress.

like SOD that catalyze single electron redox reactions. A perfectly legitimate further broadening that is rarely implied is to include those products of normal human metabolism that have similar actions toward ROS.

ANTIOXIDANT ACTIVITIES OF METABOLIC PRODUCTS

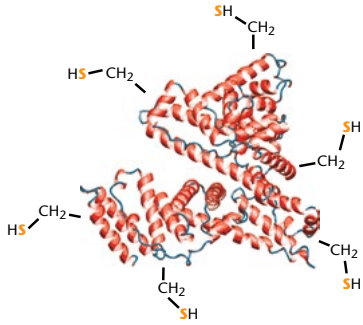
Just as natural products in foods constitute the majority of toxicants that enter the body, products of normal human metabolism provide the largest part of total protection against tissue damage from oxidative stress.

Uric Acid



Blood plasma normally contains relatively high concentrations of molecules with unique antioxidant properties. Uric acid is one such component.[35-37] Blood uric acid may help protect premature infants from ROS damage,[38] and it serves as part of the protective mechanisms in Down's syndrome.[39] Uric acid levels are lowered in schizophrenia,[40] diabetes[41] and ischemic stroke,[42] and it may help to protect against some forms of cancer.[43]

Serum Albumin

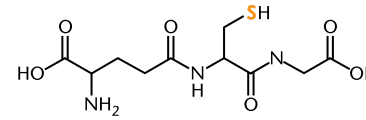


Approximately 10% of the total ROS scavenging activity of plasma is due to the presence of serum albumin. A large fraction of the antioxidant property of albumin is due to the numerous cysteine thiol groups that it carries on its outer surface.[44] The vascular complications of diabetes are partly explained by glycation-mediated changes to albumin resulting in loss of its antioxidant potential.[45] Albumin antioxidant capacity can be protected by increased levels of low-molecular-weight antioxidants.[46] Paradoxically, the binding of copper to albumin causes increased rates of serum LDL oxidation. The binding of Cu^{++} to albumin seems to cause it to have pro-oxidant enzyme activity.[47] The alteration of albumin by copper may, in turn, be diminished by the binding of serum copper to bilirubin.[48] This sequence of interactions illustrates ways in which multiple factors affect a patient's overall response to oxidative stress. Patients with conditions producing low serum albumin (Table 9.3) are at increased risk of oxidative stress effects.

TABLE 9.3 — CONDITIONS LEADING TO LOW SERUM ALBUMIN

Condition	Mechanism
Malnutrition	General restriction of protein synthesis
Liver disease	Loss of hepatocyte function
Nephrotic syndrome	Excess albumin excretion
Menetrier's syndrome	Protein losing enteropathy
Burns	Plasma loss and protein synthesis for repair
Acute diseases	Albumin is a "negative acute phase protein"

Glutathione



Some details of how glutathione (GSH) is used in human detoxification systems was presented in Chapter 8, "Toxicants and Detoxification." The rate of glutathione synthesis can vary greatly, depending on levels of oxidative stress.[49] Although accurate values for the total-body capacity for glutathione synthesis have not been reported, we can get some idea of the magnitude from studies in cell suspensions of the brassicacae *Arabidopsis*. When those cells are treated for an extended period beyond the initial 2- to 3-hour surge with the oxidizer monochlorobimane, they produce up to 8.9 nmol GSH/g fresh weight. This amount corresponds to more than 27 kg for a 70 kg human. Even allowing a 100-fold superiority for *Arabidopsis*, daily output of even 250 g is a massive peptide synthesis level. The limiting factors

under chronic oxidative stress stimulation become the supply of cysteine (or methionine) and glycine.

The disulfide chemical group in glutathione makes a particularly suitable, soft landing point for electrons that cause damage if left associated with reactive oxygen molecules. This chemical uniqueness allows understanding of why glutathione is a universal cell reduction-oxidation balancing molecule. It is ubiquitous in human tissues, and deficiencies are related to many and varied causes of disease. Low levels of the sulfur amino acids methionine, taurine and homocysteine in plasma may be due to chronic high demand for glutathione synthesis.[50, 51] Similarly, levels of glycine, serine and threonine may fall when glutathione synthesis is chronically demanded.[52] Other markers of glutathione adequacy (α-hydroxybutyrate, pyroglutamate and sulfate) are discussed in Chapter 6, "Organic Acids." Supplementation of glutathione, L-cysteine and anthocyanins restored immunologic function with reduction of symptoms in 12 children with allergic asthma and recurrent respiratory diseases.[53] These results give evidence regarding the clinical significance of glutathione restriction in patients with conditions that generate chronic oxidative stress.

Antioxidant Nutrients

Human cells possess intricate protective systems against oxidative damage.[54] An important concept in antioxidant interventions is the potential for imbalance in antioxidants because they function in concert. Oxygen free radicals are normal components of mitochondrial membranes and are a problem only when they are formed in an uncontrolled fashion. Similarly, if antioxidants like beta-carotene and vitamin E are consumed in amounts out of proportion with the total spectrum of electron acceptors, the antioxidants can become a part of the problem.

Although there is no single pathway that can be predicted for the electron transfers that are initiated by ROS, several commonly used steps have been identified. The highly reactive ROS of Table 9.2 can initiate electron transfer to any compound of lower redox potential. However, the biological systems tend to direct the electrons in orderly sequences, such as those shown in Figure 9.4.

At each level, antioxidants transfer electrons to another member of lower oxidative potential in the chain of electron acceptors until a stable compound is formed. The radical electrons ultimately leave the body as water or a reduced form of an antioxidant. The electrons must be passed down in a controlled process from one acceptor to another. Vitamin E is one of the major protective compounds in cell membranes because it can accept high-energy electrons from compounds like hydroxyl radicals. For example, transfer of a single electron from a hydroxyl radical to vitamin E gives a molecule of lower redox activity. The vitamin E radical is still an unwanted oxidant, however, that should pass the electron to a carrier that has intermediate reactivity like that of β-carotene or cytochromes. Studies associating an increased rate of lung cancer in smokers who supplemented β-carotene may relate to this phenomenon. Scenarios of typical free radical chain breaking with antioxidant restoration are shown in Figure 9.4.

Ascorbate, vitamin E, selenium, zinc and the cysteine-containing tripeptide glutathione are some of the more important compounds that protect against radical damage. As discussed in Chapter 3, "Amino Acids," even insufficiency of glycine can restrict glutathione synthesis. Important enzymes (and their cofactors) include catalase (no cofactor), glutathione peroxidase (selenium), glutathione reductase (riboflavin) and superoxide dismutase

Table 9.4 — Antioxidants Found in Human Serum

Antioxidant Metabolite	Solubility	Concentration in Serum (μM)	Concentration in Liver (μmol/kg)
Ascorbic acid	Water	50–60	260
Glutathione	Water	325–650	6,400
Lipoic acid	Water	0.1–0.7	4–5 (rat)
Uric acid	Water	200–400	1,600
Carotenes	Lipid	β-carotene: 0.5–1	5 (total carotenoids)
		Retinol (vitamin A): 1–3	
α-tocopherol	Lipid	10–40	50
Ubiquinol	Lipid	5	200

(copper, zinc, manganese) as shown in Figure 9.4. Multiple nutritional factors may afford protection from oxidative damage.[55] For example, magnesium deficiency may enhance hydrogen peroxide production and oxidative damage.[56] Adaptive systems can increase rates of enzyme synthesis, especially those required for glutathione production.[57] Laboratory evaluations are available that allow assessment of the current rate of oxidative damage and the adequacy of antioxidant protection against further damage. Markers of oxidant damage to fatty acids (membranes), proteins and DNA are available as well as various indicators of overall or specific antioxidant protection.

REFER TO CASE ILLUSTRATION 9.1

Vitamins A, C and E and β-Carotene

Laboratory evaluations for these nutrients are discussed in Chapter 2. They all play critical roles in the chain of electron acceptors that allows removal of oxygen radicals. Vitamin A and its precursor β-carotene have independent actions in this process, and the various isomers of tocopherol likewise operate with redox potentials uniquely beneficial to specific tissues.

Vitamins C and E are major players in antioxidant protection. The clinical value of β-carotene testing may be as a marker of good dietary habits rather than as a direct indicator of antioxidant status, since it may be an insignificant factor in free radical protection.[58]

Profiling fat-soluble vitamins in serum has been reviewed in Chapter 2. Such testing provides direct concentration measures for vitamins A and E, along with β-carotene and coenzyme Q_{10}. These are the principal molecules examined for deficiencies to reveal need for augmenting dietary intake. More comprehensive profiles are available that include the various minor isomers of vitamin A (carotenoids) and vitamin E (tocopherols).

Isoflavones

The polyphenolic isoflavones, such as genistein and daidzein found in soy, have extensive antioxidant properties. Because they undergo extensive metabolism in the intestine, however, as discussed in Chapter 6, "Organic Acids," it is not possible to infer systemic antioxidant contributions from their in vitro properties. Antioxidant potentials measured for the common products that are absorbed after bacterial metabolism suggest substantial biological antioxidant activity in individuals who regularly consume isoflavones-rich foods.[59] Plasma antioxidant status, homocysteinemia and endothelial response have been shown to be improved by increasing dietary isoflavone intake.[60-62] Other dietary polyphenols such as the catechins, may contribute appreciable antioxidant protection also.[63]

Copper, Manganese, Selenium, Zinc and Riboflavin

Copper, manganese, selenium, zinc and riboflavin are considered antioxidant nutrients because they play specific roles as cofactors for the enzymes that catalyze reactions that remove oxygen radicals. These nutrients are cofactors (or precursor vitamins) for the enzymes glutathione reductase (FAD),[64] glutathione peroxidase (Se),[65] and superoxide dismutase (Cu, Mn, Zn).[66] Total-body selenium is so largely dedicated to this role that some studies have evaluated overall oxidative protection by measuring serum and urinary selenium along with red cell enzymes.[67] Laboratory evaluations and specific metabolic roles for the antioxidant trace elements are discussed in Chapter 3, "Nutrient and Toxic Elements."

Can Antioxidant Supplements Be Dangerous to Health?

The "Of Further Interest" box on the following pages deals with the issue of poorly designed studies that can raise unnecessary questions about the safety of antioxidant supplementation in clinical practice. The particular report addressed there drew questionable conclusions that led to news headlines such as "Antioxidants Increase All-Cause Mortality."

Before presenting data that suggest real dangers, and at the risk of stating the obvious, a practical note may be worthy. Treating individual patients who have nutrient insufficiencies demonstrated by laboratory evaluations is not in any way comparable to population-based studies that examine average outcomes of disease incidence. A great many published studies use a design of supplementing subject and control populations without any qualification of the participants' initial nutrient status. This approach derives frequently from the misplaced focus on the curing of disease by simply increasing nutrient intake. Such results are categorically irrelevant to the common sense interventions to correct demonstrated nutrient deficiencies in patients with diseases (to aid recovery) or without them (to reduce risk of

incidence). The figure inset in the "Of Further Interest" box illustrates laboratory results that show contrasting scenarios where antioxidant supplements should be added or withheld.

Under certain circumstances, antioxidant supplementation may be harmful. For example, the ATBC and CARET studies have shown that individuals who smoke tobacco and regularly drink alcohol show slightly increased risk of lung cancer if they use high-dose supplements of beta-carotene without regard to how high their beta-carotene status may become.[71-73] Long-term follow-up in both studies showed a return to baseline incidence levels when supplementation was discontinued, further confirming the effect of beta-carotene. The mechanism of SOD association with Down's syndrome discussed above provides one possible explanation of these beta-carotene results. Specifically, a great excess of a single member in an antioxidant cascade can become a challenge to the rest of the system. This is particularly true in an excessively oxidative milieu, such as the lungs of a heavy smoker,[74] or the liver of an alcoholic.[75] Excessive intake of one nutrient with a deficiency of another can result in oxidative damage. Further, the antioxidant itself, when given alone, may become oxidized and damaging. However, properly balanced supplementation with the same antioxidants can produce reduced cancer incidence.[74]

A few vitamin E studies have reported increased risk of cardiovascular disease and certain cancers, although other studies have challenged the findings.[76-79] The ATBC study demonstrated that 50 mg of vitamin E in rural elderly male smokers increased the incidence of the common cold by 58%, whereas their less-smoking city-dwelling counterparts had a reduction in incidence of 46%.[80]

Although the negative findings in the beta-carotene studies were associated with smokers who drank alcohol, the vitamin E findings are less-clearly associated with such risk factors. Indeed, the ATBC findings with regards to vitamin E make drawing such conclusions tricky at best. Further confounding is the fact there was a reduced incidence of lung cancer in the ATBC control group when there was either higher baseline serum beta-carotene or increased dietary intake of beta-carotene during the study.[71] The CARET study also demonstrated a protective effect of increased dietary carotenoid intake in the placebo arm of the study, although no such difference was found in the active arm.[81] A study looking at an animal lung cancer model showed a protective effect against DNA damage and resistance to oxidative damage in those treated with beta-carotene, ascorbic acid and alpha-tocopherol,[82] supporting the conclusion that that supplementing with limited antioxidants at high doses may be problematic.[83]

Functional and direct assessment of antioxidant elements, nutrients and vitamins, as well as assessment of markers of oxidative stress will allow the clinician to design an appropriately individualized treatment plan. Degree of specific antioxidant deficiency as well as the severity of oxidative damage will guide the clinician in the dosing and duration of needed nutrients. In patients under extreme oxidative stress, such as smokers and alcoholics, a whole-foods diet with a broad complement of antioxidant nutrients is likely the safest alternative to high-dose single antioxidant supplementation.

Markers of Oxidant Damage

Oxidative effects in Down's syndrome illustrate both the origins of oxidative stress and the value of testing that allows monitoring the adequacy of antioxidant controls. The extra copy of chromosome 21 in Down's syndrome contains a gene for superoxide dismutase. This enzyme is critical for oxidant protection, but it is only one of the links in the chain of normal electron transfer. Tissue levels of superoxide dismutase can be 40% higher

Notes:

Of Further Interest...

Safety and Efficacy of Antioxidant Supplements

Numerous research papers have demonstrated the effectiveness of antioxidant supplements for disease prevention, and dosages considered to be safe have been established by the Institute of Medicine (IOM).[84–88] The question of safety and efficacy for antioxidant supplementation, however, continues to be scrutinized in prestigious medical journals with a zeal that seems to go beyond a straightforward search for truth. One such paper was published in *JAMA* in February 2007. It made international news with the sensationalistic statement that antioxidant supplements cause increased all-cause mortality.[89] An immediate influx of letters to the editors of *JAMA* called into question the methodology and findings of the study, demonstrating that the data appeared "cherry-picked" by the authors, and therefore incorrect.[90-94]

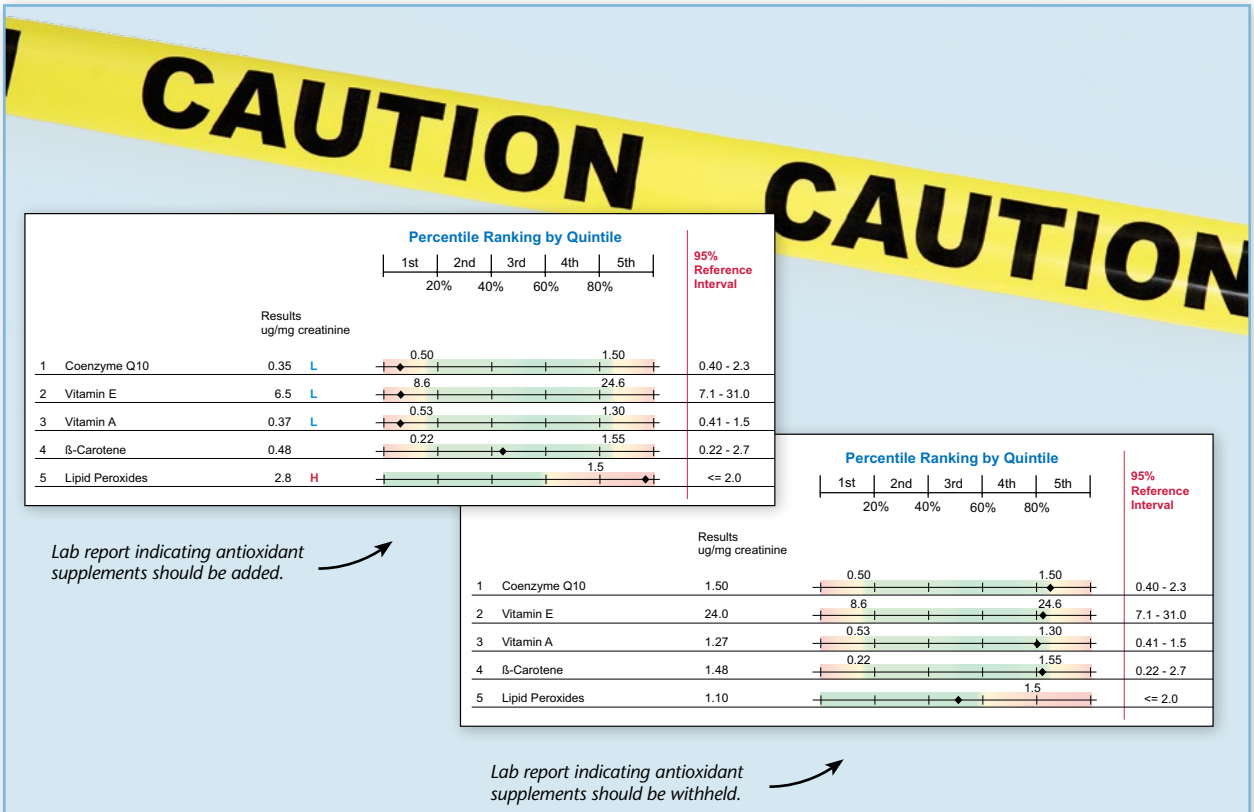
The *JAMA* study in question, "Mortality in Randomized Trials of Antioxidant Supplements for Primary and Secondary Prevention," by Bjelakovic et al.,[89] is a meta-analysis (a statistical analysis of data combined from two or more trials with the aim of increasing statistical significance by using a larger study population). The authors looked at the use of beta-carotene, vitamins A, C and E; and selenium, either singly or combined vs. placebo or vs. no intervention. The studies used in the analysis were all randomized controlled trials (RCT), which are widely accepted as the gold standard in study design, but for a number of reasons have received criticism for their inability to accurately assess antioxidant efficacy.[95-97]

> ***"Clinicians should carefully and critically read an original paper's methodology before accepting the popular media's version of the truth."***

A proper meta-analysis incorporates only like studies; however, Bjelakovic et al. allowed for any dosage, duration and combination of antioxidants, creating immense variability for comparing outcomes. Vitamin C dosage ranged from 60 to 2,000 mg; trial lengths ranged from 3 months to 12 years; antioxidant supplementation ranged from a single dose of vitamin A in one trial, to combined supplementation for 12 years. Comments to *JAMA*'s editors regarding supplement duration included the following: "dying from a supplement after only a few months of use seems biologically implausible," and "the finding that supplementation duration had no effect on total mortality diminishes the likelihood that these micronutrients increase the risk of death."[91]

The use of all-cause mortality as the end point of this analysis was suspect. Huang et al. stated that "the establishment of causality of increased all-cause mortality would require the inclusion of data not included in the study, such as temporal and dose-response relationships, evidence of effects disappearing after supplement discontinuation and recurrence after resuming supplementation."[91] Albanes notes that almost half (405 of 815) of the studies originally identified in the literature were excluded from the analysis because they observed no deaths. Of the 68 trials that did have deaths, many were small (12 trials had only 1 death), which increases the significance of a few large trials.[93] Additionally, a biologically plausible mechanism for the increase in all-cause mortality, which includes not only death from disease, but also accidents and suicide, was not hypothesized by the authors.

The process of trial selection used by Bjelakovic et al. was aggressively challenged as being biased.[90-93] To establish statistical significance, the final 68 trials used (from an original pool of 815) were further reduced by 21, which were labeled as high-biased. A high-biased trial was defined as having one or more unclear or inadequate methodological quality components. Without the elimination of these trials,



the finding of increased all-cause mortality was not significant. When considered alone, the high-biased trials demonstrated overall a significant reduction of mortality. There was a further elimination of 21 trials involving the supplementation of selenium. After the exclusion of these trials, it left only 26 trials from the original 815. The exclusion of the selenium trials also appears to support the authors' thesis of increased all-cause mortality. As Albanes states, "it would be useful to know what hypotheses formed the basis for the [exclusion] of selenium and high-biased risk trials.[93] Taylor and Dawsey pointed out the excluded "high-bias" Linxian General Population Nutrition Intervention Trial was identical methodologically to a trial included in the low-biased group, and, therefore, it should not have been excluded as high-biased.[90] Huang et al. also challenged the definition of "low-biased," stating that a lack of reporting of certain methodological details may have been mistaken as lacking high quality.[91]

The bulk of the studies included in the meta-analysis were secondary prevention trials, meaning that the subjects are already ill with specific diseases. Under this design, the supplement is being evaluated for evidence of cure for an illness and/or life extension. These types of trials cast antioxidant supplements in a poor light, since they are never promoted as cures for disease; rather, their benefit is in disease prevention. The studies were all randomized controlled trials, which have been criticized as being limited in their ability to evaluate the multifaceted effects of antioxidant supplementation for disease prevention as described in this chapter. Long-term observational studies to investigate the efficacy of antioxidant supplementation as protective or preventative is a superior design for evaluation of their efficacy.[96, 97]

The Bjelakovic report illustrates an apparent bias amongst the editors of leading medical journals against the use of nutrients in health care. Clinicians should carefully and critically read an original paper's methodology before accepting the popular media's version of the truth. Although care in choosing dosage, form and duration of any nutrient supplementation is important, appropriate use of antioxidant nutrients appears both safe and effective.

in Down's patients compared with individuals with the normal chromosome count. The increased conversion of superoxide to hydrogen peroxide leads to accumulation of hydrogen peroxide and increased rates of oxidant damage. Oxidative damage to membrane lipids and DNA can be reflected in the markers described below.[98]

Concentrations of specific antioxidants in body fluids are useful parts of the free radical pathology puzzle. However, they do not indicate to what degree the body is experiencing antioxidant damage. Laboratory procedures that challenge white cells with a pro-oxidant and then measure changes in rates of proliferation or radionuclide incorporation are of theoretical interest, but no consensus exists in the scientific community that they accurately reveal the cellular status of free radical protection.

Total Antioxidant Capacity

Because of the interactions between antioxidants in cells, the critical issue is a patient's overall capacity to resist oxidative damage. Since cancer is associated with free radical damage to DNA, many studies have focused on how antioxidant status changes cancer incidence. Although there is wide agreement that lung and breast cancer risk is lower for individuals with higher dietary intake of multiple antioxidants, raising intake of β-carotene only may increase risk of lung cancer, especially for tobacco smokers.[68-70]

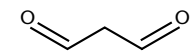
Studies that focused on single antioxidants have reported that they have either no effect or slight negative correlation with incidence of cancers of the lung and breast. It may be that the various electron acceptor/donor roles of the antioxidants must be present in proportional concentrations. If any one component, such as β-carotene, is increased dramatically, then it can become a source of electrons for further damaging radical formation. Tests that reveal inadequacy in response to oxidant stress can guide decisions for individual patient antioxidant supplementation.

For routine clinical assessments, it is impractical to attempt measuring free radicals directly since they exist for only fractions of a second. Tests that measure the by-products of free radical pathology may provide good indicators, though it is usually recommended that multiple oxidative product and micronutrient assessments be used for accuracy.[99] Individuals with insufficient antioxidant protection have increased concentrations of oxidized by-products in blood and urine.[100] Such markers include serum or urinary malondialdehyde, isoprostanes, 3-nitrotyrosine, methionine sulfoxide and 8-hydroxy-2'-deoxyguanosine. These and other compounds will be discussed in the sections below, including the mechanism of production, current research and routine clinical utility.

Lipid Oxidation

Membrane components are especially susceptible to oxidative damage because they are the sites of greatest exposure to oxygen radicals and they possess high concentrations of molecules that are easily oxidized (unsaturated fatty acids). Cell membranes are the first subcellular domains to encounter molecular oxygen when it is released from hemoglobin, and mitochondrial membranes are the site where molecular oxygen is utilized constantly for ATP production and where single electron transfer reactions occur.

Malondialdehyde (Lipid Peroxides)



Lipid peroxides are the products of the chemical damage done by oxygen free radicals to the lipid component of cell membranes.[101, 102] A serum lipid peroxide level measures the overall effect of oxygen free radical pathology, the risk of degenerative processes and the need for compensatory antioxidant supplementation and/or lifestyle modification. High serum lipid peroxide levels indicate high rates for oxygen free radical lipid peroxidation. Such effects have been demonstrated by measuring malondialdehyde (MDA) changes in obesity,[103] hypertension,[104] abdominal aortic aneurism,[105] asthma[106] and dialysis procedures.[107] A meta-analysis of multiple studies of oxidative stress in schizophrenia published up to 2006 revealed a positive relationship.[108] Other conditions where MDA has been shown to reflect increased oxidative challenge include smoking,[109] *Helicobacter pylori* infection,[110] cystic echinococcosis[111] and methamphetamine use.[112]

Evaluation of total lipid peroxides has proven to be a simple, inexpensive and accurate means of reflecting whole-body free radical activity. The assay measures malondialdehyde, a major product of lipid peroxidation, by reaction with thiobarbituric acid (see Figure 9.5). The fluorescent red adduct that is formed can be easily measured. Thus, it is also known as the thiobarbituric

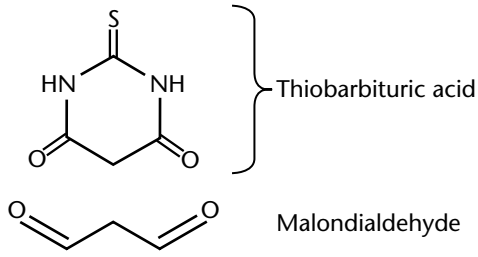


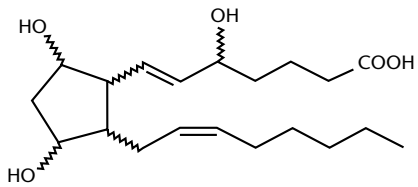
Figure 9.5 — Malondialdehyde-Thiobarbituric Acid Adduct

In the presence of thiobarbituric acid, malondialdehyde forms an adduct with a distinct absorbance maximum that lends the reaction useful for quantitative measurements that relate to oxidative damage to polyunsaturated fatty acids of membrane and lipoproteins.

acid reactive substance (TBARS) test. Whenever total antioxidant capacity is inadequate to meet the oxidative challenge, cell membrane oxidation increases, releasing lipid peroxides.

Concentrations of lipid peroxides may be measured in urine or serum, but serum has superior sensitivity to slight increases in lipid peroxidation. It has gained general acceptance in the research and clinical laboratory as a standard means of assessing the body's antioxidant capability and overall oxidative stress.[113, 114]

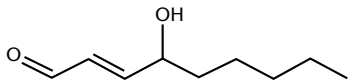
Isoprostanes



Isoprostanes are prostaglandin-like compounds produced by non-enzymatic peroxidation of arachidonic acid. Urinary excretion of isoprostane F2 (F_2-IsoP) reflects antioxidant status.[115] F_2-IsoP is thought to be a factor that contributes to the genesis of arteriosclerosis and to the activation of the clotting system.[116] Quantitation of F_2-IsoP in various body fluids is clearly established as a superior way of assessing oxidative stress.[117] Accumulation of isoprostanes may mediate the atherosclerotic effects of homocysteine because elevated fasting plasma homocysteine is associated with enhanced in vivo lipid peroxidation of LDL and high levels of isoprostane.[118] Patients with hypercholesterolemia show increased levels of isoprostanes.[119] Frequent alcohol consumption also causes elevated isoprostanes.[120]

Recent in vitro observations show the potential for formation of extremely reactive products of the isoprostane pathway. They rapidly form covalent adducts with lysine residues on proteins, which may cause enzyme inactivation or formation of antigenic macromolecules. Such reactions provide one mechanism of loss of tissue function from oxidative damage.[121] Isoprostane excretion has been used to monitor disease progression and improvement with ethanol abstinence in patients with alcoholic liver disease.[122] The marker is also an indicator of amyloid beta-peptide-induced oxidative damage in Alzheimer's disease.[123] Zinc protects against the toxicity of beta-amyloid peptide.[124] Both L-arginine and alpha-tocopherol reduce urinary isoprostane excretion and improve endothelium-dependent vasodilatation, although by different mechanisms.[125] The instability of isoprostanes makes their measurement at remote laboratories inaccurate due to specimen transport requirements.

4-Hydroxy-2-Nonenal



An aldehyde product of lipid peroxidation, 4-hydroxy-2-nonenal (HNE) produces a range of powerful biological effects. It is a highly mutagenic and genotoxic product generated during oxidative stress. HNE acts as a part of the signaling pathway in the cellular response to oxidative stress by inducing gene expression of cyclooxygenase 2, which causes increased synthesis of prostaglandins and thromboxanes.[126]

Congestive heart failure is one state of oxidative stress in which HNE is consistently elevated.[127] Aortic endothelial cells display dose-dependent inhibition of nitric oxide formation by HNE that is mediated by lowered tetrahydrobiopterin levels.[128] This effect is an illustration of how oxidative stress can alter BH_4 status, as described in Chapter 1, "Vitamins." Hyperglycemic conditions, such as diabetes mellitus, present another state of HNE elevation. The insulin-releasing response of pancreatic islet cells is inhibited by HNE. The oxidative state

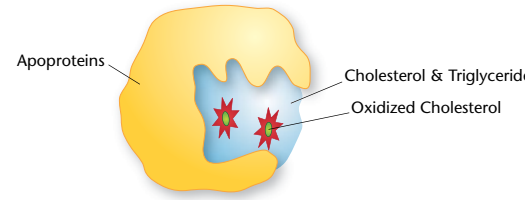
stimulated by hyperglycemia causes further inhibition of the normal glucose-lowering response with a "feed-forward" (self-propagating) effect. This linkage explains the action of lipoic acid as an antioxidant in improving insulin response.[129, 130] A similar antioxidant role of phenolic compounds from olive oil is associated with lowered HNE production in cancer patients and patients with inflammatory bowel conditions such as ulcerative colitis and Crohn's disease.[131] The fecal matrix supports abundant generation of ROS, and high HNE levels are generated in the colonic mucosa of these patients. In neurodegenerative disease, elevated HNE reflects the destructive cycle of oxidative stress and mitochondrial dysfunction.[132, 133] High HNE in Alzheimer's disease provides evidence of oxidative stress-induced pathology in the formation of neurofibrillary tangles.[134]

HNE reacts rapidly with DNA to form substituted adducts.[135] Such DNA adducts to HNE are elevated in patients with Wilson's disease and Alzheimer's disease.[136] The accumulation of copper and iron in this disease leads to increased oxidative damage and high risk of primary liver cancers. Such adducts are also greatly increased in humans placed on diets high in omega-6 polyunsaturated fatty acids without adequate antioxidant protection.[137] Thus, HNE and its DNA adducts are promising biomarkers for monitoring effects of dietary fat intake, oxidative stress and antioxidant protection. Lowered HNE also reveals an important aspect of positive health benefits from calorie-restricted diets.[138]

Plasma HNE, thus, is a marker of the progenitor effects in many diseases and, conversely, can be used to forecast wellness. The clearance of HNE requires biotransformation via omega oxidation of the aldehyde[139] and conjugation with glutathione to give excretion of a mercaptan derivative.[140] Because of the high reactivity of HNE with proteins, the measurement of protein adducts is more indicative than that obtained by attempting to measure HNE directly, even in research situations.[141]

Notes:

Oxidized Low-Density Lipoprotein



Evidence implicating oxidation products in lipoprotein particles (OxLDL) with atherogenesis is increasing.[142, 143] Advanced stages of lesions show increase of OxLDL that may associate with adaptive immunity.[144] Such a mechanism may explain the antiatherogenic effects of antioxidants, which can reduce the oxidation rate of LDL.[145-147] The ensuing immune response against oxidized low-density lipoprotein (LDL) may have a protective role in the early stages of atherogenesis,[148] but this same immune response may be a trigger for setting in motion the cascade of events that culminates in advanced atherosclerotic lesions.[149] Changes in age-related immune parameters are also mediated by oxidized cholesterol.[150]

The endothelial cells are constantly challenged to resist oxidative damage. The largest source of this challenge is oxidized plasma lipoprotein components containing oxidized fatty acids and cholesterol.[151] Cholesterol is easily oxidized to cholest-4-en-3-one. This and other partial oxidation products accumulate in lipoprotein particles where they are presented to the luminal surface of endothelial cells. Lipid hydroperoxides in plasma, for example, are carried almost exclusively (89%) in LDL. In 70 patients tested 3 months after coronary artery bypass graft surgery, plasma lipid hydroperoxide levels were significantly increased when compared with matched healthy controls,[152] providing further evidence linking oxidative damage and heart disease.

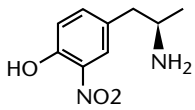
Generally, the assay of oxidized LDL is performed on lipids extracted from plasma on Liposorb gel. Results should be less than 2.5 nmol/mL. A related assay measures a subpopulation of LDL particles with relatively high density. Individuals with elevated levels of this variety of LDL are at high risk of heart disease even if their total LDL concentration is not high.[153] The two phenomena discussed here may be linked because oxidized cholesterol may accumulate in "small, dense" LDL. The LDL subfractions are measured by analytical ultracentrifugation or electrophoresis of serum lipoproteins.

Protein Oxidation

The surface of proteins tends to contain abundant amino acid side chains that can undergo oxidation. Two of the most well-recognized products are nitrotyrosine and methionine sulfoxide, arising from reaction of nitric oxide and ROS with tyrosine and methionine, respectively. These protein modifications do not necessarily lead to loss of function, but they generally signal cathepsins to degrade the modified proteins, releasing the altered amino acids.

The retina has special need for oxidative damage protection because of the free radical-generating effects of ultraviolet radiation. Soluble retinal proteins induce nitric oxide and superoxide production by macrophages. Increased production of ROS by macrophages in the presence of these soluble retinal proteins in vivo may accelerate photoreceptor degeneration.[154, 155] Vitamin E pretreatment protects against the loss of free radical defense in kittens exposed to high oxygen atmosphere.[156]

3-Nitrotyrosine (3NT)



While it is important to produce adequate nitric oxide (NO), when nitric oxide is present in the face of oxidant stress, peroxynitrite (ONOO-), a "reactive nitrogen species" (RNS), can be formed. Peroxynitrite is formed by combination of superoxide (O_2•) and NO radicals (Figure 9.6). Peroxynitrite is being increasingly proposed as a contributor to tissue injury in several human diseases.[157-164]

Tyrosine residues in proteins are nitrated by peroxynitrite, forming 3-nitrotyrosine. Nitrotyrosine formation takes place in proteins that are present in nitric oxide-stimulated cells such as endolthelium. Nitration of the tyrosine residues in proteins leads to changes of protein structure and function. The proteins are degraded at cell death to release the 3-nitrotyrosine residues that then will circulate in blood plasma.

The evidence presented for peroxynitrite participation usually includes the demonstration of increased 3-nitrotyrosine levels in the injured tissue. Increase in 3-nitrotyrosine, therefore, reflects increases in nitric oxide and superoxide production. Evaluation of 3-nitrotyrosine can provide insight about which patients need antioxidant supplementation to prevent oxidative damage.

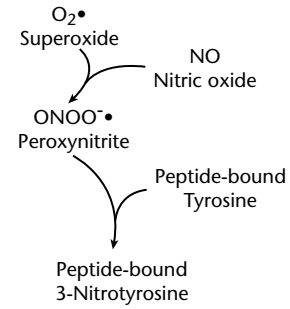


Figure 9.6 — Formation of Peroxynitrite and Nitrotyrosine

The reaction of superoxide and nitric oxide yields the very reactive peroxynitrite radical. Exposed tyrosine residues on proteins are susceptible to attack by peroxynitrite, resulting in stable polypeptide-bound 3-nitrotyrosine residues. Subsequent hydrolysis releases nitrotyrosine as a free amino acid.

Clinical Relevance. Premature infants are susceptible to bronchopulmonary dysplasia (BPD), a chronic lung disease of infancy. The disease appears to be caused in part by oxidative stress from hyperoxia. Plasma 3-nitrotyrosine concentrations are significantly higher in infants with BPD, increasing approximately fourfold during the first month of life and correlating with the fraction of inspired oxygen that the infant receives.[165] Peroxynitrite-mediated oxidant stress may contribute to the development of this disease in premature infants, and 3-nitrotyrosine is useful as an early plasma indicator of infants at risk of developing BPD. Through similar mechanisms, the oxidative stress that leads to most degenerative diseases is associated with increased 3-nitrotyrosine production. When iron deficiency is induced in weanling rats, protein tyrosine nitration was increased, along with ventricular dilation and mitochondrial cytochrome-*c* release.[166]

Nitrotyrosine formation increases with endotoxin-induced kidney injury suggesting that protein nitration may participate in renal regulation and in renal injury.[167] The concentration of 3-nitrotyrosine found in patients with sporadic amyotrophic lateral sclerosis was approximately seven times that of controls.[168]

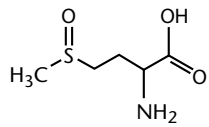
Helicobacter pylori infection is associated with gastric cancer.[169] *H. pylori* infection may contribute to DNA

damage because of up-regulation of inducible nitric oxide synthetase (iNOS) expression, and thereby radical nitrogen species, in the gastric mucosa. Nitrotyrosine in the gastric mucosa is also significantly higher in *H. pylori*-positive groups than in *H. pylori*-negative individuals.[170]

In laboratory animals, iNOS expression and NO synthesis in macrophages and cardiomyocytes are elicited in experimental inflammatory heart disease.[171] Similarly, in inflammatory bowel disease, epithelial cells and immune cells that are producing reactive oxygen species display induction of iNOS and increased 3-nitrotyrosine.[172]

We may conclude that patients with elevated nitrotyrosine are in a state of oxidative stress. Arginine supplementation is contraindicated, and, since antioxidant status is compromised, general support with antioxidant nutrients is indicated. Antioxidant nutrients, including the polyphenolic compounds abundant in tea,[173] offer protection against the formation of peroxynitrite.

Methionine Sulfoxide



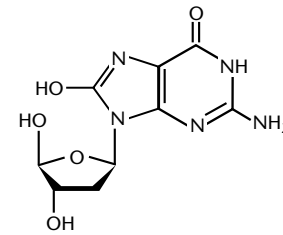
Methionine sulfoxide (MetO) is a marker of cellular oxidative stress, specifically involving enzymes and structural proteins because it is formed from methionine residues that are exposed on protein surfaces. Elevated MetO levels indicate inadequate overall antioxidant protection. Alteration of Met to MetO may be reversed by the methionine sulfoxide reductase enzymes.[174] These enzymes, called peptide methionine sulfoxide reductases (PMSR), are involved in the resistance of mammalian cells to oxidative stress.[175] MetO has been shown to cause enzyme inactivation.[176] It accumulates in the ionized calcium-regulating protein calmodulin in senescent brain under conditions of oxidative stress,[177] including Alzheimer's disease.[176, 178, 179] Dihydrolipoic acid has specific antioxidant properties and may exert a curative effect in diseases accompanied by oxidative stress by stimulating the methionine sulfoxide reductase enzyme.[180] In blood, methionine sulfoxide appears along with oxidized cholesterol as the process of oxidative damage acts on blood lipoproteins.[181]

Nutrient effects may extend to reversing, as well as preventing, oxidant damage. Oxidative damage to the protein alpha-1-antiprotease (alpha-1-AP) occurs with formation of 3-nitrotyrosine residues.[182] Oxidized alpha-1-AP has been implicated in the etiology of lung diseases. Repair of oxidized alpha 1-AP is catalyzed by PMSR, which is activated by dihydrolipoic acid to reverse oxidative damage to the damaged alpha-1-AP.[180] Thus, there is the potential to use methionine sulfoxide to monitor not only oxidative stress at the biochemical level, but also to demonstrate reversal of oxidative damage by lipoic acid. If the damage is detected early enough, reversing oxidative damage to proteins can reverse disease. However, a potentially detrimental role of excessive alpha-tocopherol is the enhancement of HDL oxidation in vitro, with more rapid appearance of methionine sulfoxide residues when isolated HDL was enriched with alpha-tocopherol.[181]

Notes:

Nucleotide Oxidation

8-Hydroxy-2'-Deoxyguanosine



The rate of oxidative DNA damage may be estimated by measuring 8-hydroxy-2'-deoxyguanosine (8-OHdG), also written as the tautomer, 8-oxoguanosine (8-oxodG). 8-OHdG is a repair product of the highly mutagenic oxidation of guanine in DNA or the cellular pool of GTP, from a 24-hour urine collection. Bus drivers from Copenhagen showed increased 8-OHdG compared with drivers from rural/suburban greater Copenhagen, which suggests that exposure to ambient air pollution can cause oxidative damage to DNA.[183] Exposure to ultraviolet light and benzo[a]pyrene can

synergistically enhance the formation of 8-OHdG in living cells.[184] Patients with the perivasculitis of the retina known as Eales disease had increased 8-OHdG that correlated with decreased SOD activity and decreased glutathione status.[185]

The intracellular antioxidants glutathione and ascorbate can protect human lymphocytes against oxidative DNA damage. The antioxidant levels are negatively correlated with the levels of oxidative lymphocyte DNA damage, and the strongest correlation observed is with glutathione.[186] Even among populations that might be considered normal, increasing intake of antioxidant vitamins with a nutrient-rich supplement reduces 8-OHdG by 22% compared with unsupplemented adults.[187] 8-OHdG also increases with high-fat diets, showing the hyperoxidative response associated with excessive fat intake.[183] Age-associated decline in mitochondrial function due to mitochondrial DNA damage is indicated by the finding of increased 8-OHdG with age in laboratory animals.[188]

Refer to Case Illustration 9.1

DNA Strand Breakage (Comet Assay)

One of the ultimate damaging consequences of oxidative challenge is damage to DNA. Such damage may be measured directly by looking for breaks in DNA strands as revealed on single-cell (WBC) gel electrophoresis in the *Comet assay*.[189] This assay measures DNA damage, particularly DNA strand breakage. It has been used to measure the oxidative damage resulting from iron overload in thalassemia.[190] The test provides a rapid measure for biomonitoring occupational exposure to DNA-damaging agents.[191]

Oxygen Radical Absorption Capacity

Although the oxygen radical absorbent capacity (ORAC) test has been offered by some laboratories for assessment of serum antioxidant capacity, it is largely of value in studies of antioxidant capacities of foods. When serum is challenged with a chemical oxidant, the time course of resistance to protein destruction is a measure of the body's total ability to resist the oxidative challenge.[192, 193] The test is useful for assessing antioxidant capacities of foods and for comparative antioxidant capacities among species of animals.[194] A difficulty of the test arises from the fact that serum components such as albumin and uric acid that are present at high concentrations make major contributions to the resistance against oxidation. Their contributions are so great that they obscure the more important factors (such as ascorbate and vitamin E) that are major factors for protection in subcellular compartments. The use of ORAC to detect susceptibility to human disease has been disappointing because of the lack of discrimination among healthy and diseased individuals. The lack of sensitivity results in serum from a range of normal individuals having ORAC values very similar to those from diabetics, cancer patients and HIV-infected individuals.[195]

Endogenous Oxidative Stress Modulators

p-Hydroxyphenyllactate

p-Hydroxyphenyllactate (HPLA) may be reported in urinary organic acid profiles. A discussion of its role as a marker of increased oxidative status is presented in Chapter 6, "Organic Acids." The compound offers a unique view of the regulation of endogenous autooxidation processes. Unlike other markers that show how much challenge can be tolerated or how much damage has occurred, HPLA reveals how much challenge is being generated within the tissues. Individuals with elevated p-hydroxyphenyllactic acid, a carcinogenic tyrosine metabolite, tend to have decreased ascorbic acid (vitamin C) in liver, adrenal glands and blood.[196]

Homogentisate

Some aspects of the oxidative stress associated with elevated homogentisate (HGA) were discussed in Chapter 6, "Organic Acids." Early knowledge about the effects of homogentisate elevation came from examination of patients with alkaptonuria. The progressive buildup of oxidation products in these patients results in the adverse pigmentation, calcification and inflammation of cartilagenous tissue called ochronosis. Administration of ascorbic acid (500 mg, BID) resulted in clinical improvement in 1 of 3 siblings with alkaptonuria.[198] The oxidation products, called plasma soluble melanins (PSM), are formed by copolymerization of dopa, catecholamines, homogentisic acid and other polyhydroxy compounds. Antioxidants delay the formation of PSM that contribute to the yellow color of plasma and urine.

Formation of PSM is found in pheochromocytoma and Parkinson's syndromes, in addition to alkaptonuria. Drugs such as the antihypertensive alpha-methyldopa that causes Parkinson's syndrome also can contribute to the formation of PSM.[199] Mechanisms other that PSM formation may add to the oxidative stress from HGA elevation.

In order to investigate the origin of metabolic lesions found in hereditary tyrosinemia type 1 (HT), 22 patients and 11 controls were challenged with 50 mg/kg HGA. The much greater elevation of serum HGA in subjects 1 hour after loading (30 vs. 19 µg/mL) suggested a mechanism of glutathione adduct formation by HGA degradation products that is exacerbated by the metabolic lesion in HT.[200] Similar effects in alkaptonuria or idiopathic HGA elevation may help explain the severe oxidative stress effects associated with HGA elevation. In the presence of copper, HGA causes DNA damage, as shown by increased production of 8-OHdG. Formation of a Cu(I)-peroxide complex due to the presence of HGA is thought to produce the DNA oxidative damage.[201] HGA causes oxidation of hemoglobin that is altered by the presence of SOD and catalase.[197]

Pathogen Invasion

Production of ROS is one result of host pathogen interactions following invasion. Neutrophil-mediated oxidative burst is a well-understood antimicrobial mechanism. The mechanism involves the rapid generation and release of reactive oxygen intermediates by the NADPH oxidase complex.[202] Increased oxidative stress has been shown to be associated with infection with a broad spectrum of infectious organisms, including *Helicobacter pylori*,[203, 204] *Prevotella intermedia*[205] and *Brucella melitensis*.[206] Another benefit to the host of increased ROS formation is the induction of apoptosis in the infected cells that significantly imparts protection to the host from the pathogen.[207]

The ability of some pathogens such as the *Cryptospordium parvum* to survive the PMN oxidative burst offers an explanation of their persistent invasiveness. Oocysts of *C. parvum* may persist within macrophage phagosomes for up to a week.[208] Improving antioxidant status may produce more effective oxidative burst in response to infection. Subjects who were given dietary supplements containing 18 mg beta-carotene, 900 mg vitamin C and 90 mg vitamin E for 7 days prior to a 2-hour treadmill run at 65% VO_2max had significantly higher oxidative burst activity than controls.[209] Also, children with recurrent upper respiratory disease and asthma who were given reduced glutathione, L-cysteine and anthocyane (a dietary flavonoid) showed elevation of interferon-gamma production, lymphocyte response to mitogens and NK cell activity, along with long-term improvement in clinical status.[53]

Pathogen responses also can generate additional oxidative stress for host cells. The global gene expression profile of *Shigella flexneri* following infection of human epithelial and macrophage cell lines suggests that ROS protection mechanisms are involved in survival and growth strategies of *Shigella* in the human host.[210] The facultative intracellular pathogen *Penicillium marneffei* that causes common opportunistic infection in AIDS patients displays highly inducible expression of genes for catalase-peroxidases.[211] Invasion mechanisms used by the yeasts *Candida albicans* and *Saccharomyces cerevisiae* involve collection of sphingolipids and sterols into lipid rafts in the cell membranes. The antioxidant virulence factor Cu/Zn superoxide dismutase (SOD1) is concentrated six- to ninefold in raft membrane fractions compared with non-raft membranes.[212] These are some of the responses involving oxidative challenge produced by both host and pathogen during infection. Patients with histories of chronic infection are at higher risk of antioxidant insufficiency.[213, 214] Numerous studies have reported favorable patient responses to antioxidants following infectious diseases.[215-219]

Treatment Options

In the case of markers of oxidant damage, the lower the test result level, the better. However, since free radicals are produced in the normal processes of metabolism, the body can tolerate a certain level without significant health risk.

Treatment of patients with elevated oxidative damage markers involves the twofold approach of reducing exposure to free radical-generating agents and increasing antioxidant levels in the body.

Reduce Pro-Oxidants

It is clear that environmental agents increase free radical activity. The oxidative stress associated with elevated body burdens of toxic elements such as cadmium was discussed in Chapter 3, "Nutrient and Toxic

Elements." The toxicity of pesticides, ionizing radiation and cigarette smoke also may be due to their free radical initiating ability. Lifestyle factors such as stress, heavy exercise, smoking, excess alcohol and infections tend to raise lipid peroxide levels.

The body burden of pro-oxidant compounds may be lowered by reducing exposure and increasing removal or detoxification. Individuals who may have greatly increased pro-oxidant responses to even slight xenobiotic exposures must take extra measures to lower environmental levels. Other sources provide more details about how to manage environmentally sensitive patients.[220]

Increase Antioxidants

If oxidative damage markers are high, supplementation of natural antioxidants is indicated. These include vitamins A, C and E; beta-carotene; selenium; coenzyme Q_{10}; taurine; B_2; B_3 and certain bioflavonoids.[55] Balanced intake of all of the antioxidants is superior to individual supplementation because of the demonstrated codependence of antioxidant reaction sequences. In this regard, it is worth considering the use of botanical sources of antioxidants such as Ginkgo biloba, Panax ginseng or other rich sources of complex mixtures of polyphenols with antioxidant vitamins.[221]

Magnesium deficiency can increase oxidant loads because of its effect of increasing stress responses.[222] Magnesium was not included above as an antioxidant nutrient because it has no specific free radical scavenging cofactor role. However, when implementing overall oxidant reduction strategies, magnesium status must be assured.

Table 9.5 — Food Sources of Antioxidants

Antioxidant	Food Source
Vitamin A	Carrots, dark green vegetables, mango, beef liver, cheeses
Vitamin E	Whole grains, seeds
Vitamin C	Fruits, potatoes
β-Carotene	Dark-colored vegetables
Anthocyanins	Blueberries, strawberries
Epigallocatechingallates EGCG	Green tea
Lycopene	Tomatoes

Other Lifestyle Factors

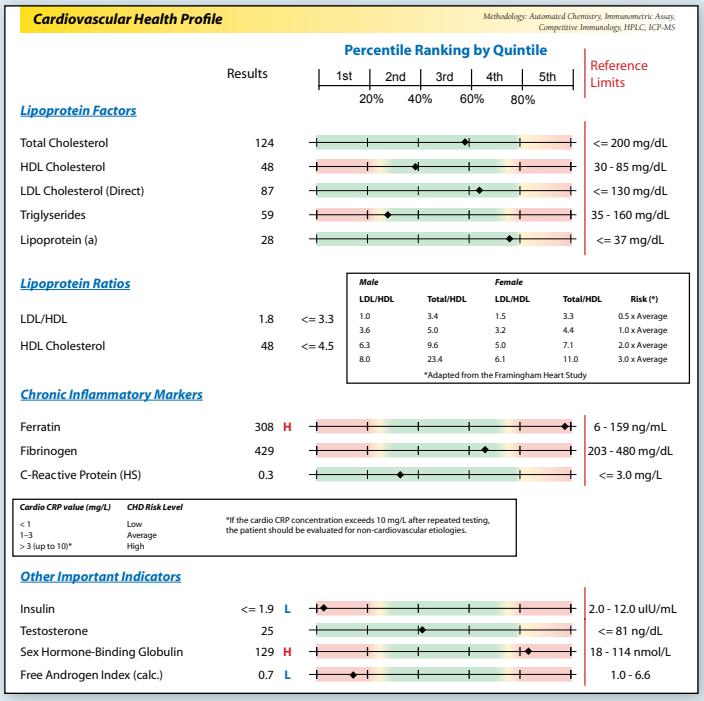
Sleep disorders interfere with normal tissue restoration and hormonal control patterns. The pineal hormone melatonin, in addition to its role as functional regulator of the hypothalamo-pituitary-gonadal system, is a highly efficient free radical scavenger and general antioxidant.[223] It is roughly twice as effective as vitamin E for protection against the peroxyl radical. Regular adequate sleep is the major controlling factor in maintaining adequate levels of melatonin.

Notes:

Case Illustrations

Case Illustration 9.1 — 8-Year Multiple Sensitivity, Oxidative Stress

This case illustrates a patient with a severe, long-standing chronic condition that defies standard diagnosis. The clinician decided to do a very thorough nutrient and toxicant evaluation. Even with multiple metabolic and stool profile data, the apparent focal problem might have been missed in the absence of key measurements. Once the nature of the oxidative stress problem was identified, aggressive interventions could be planned.



Cardiovascular Health Profile

Methodology: Automated Chemistry, Immunometric Assay, Competitive Immunology, HPLC, ICP-MS

	Results		Percentile Ranking by Quintile (1st 2nd 3rd 4th 5th; 20% 40% 60% 80%)	Reference Limits
Lipoprotein Factors				
Total Cholesterol	124			<= 200 mg/dL
HDL Cholesterol	48			30 - 85 mg/dL
LDL Cholesterol (Direct)	87			<= 130 mg/dL
Triglyserides	59			35 - 160 mg/dL
Lipoprotein (a)	28			<= 37 mg/dL

Lipoprotein Ratios

LDL/HDL	1.8	<= 3.3
HDL Cholesterol	48	<= 4.5

Male LDL/HDL	Male Total/HDL	Female LDL/HDL	Female Total/HDL	Risk (*)
1.0	3.4	1.5	3.3	0.5 x Average
3.6	5.0	3.2	4.4	1.0 x Average
6.3	9.6	5.0	7.1	2.0 x Average
8.0	23.4	6.1	11.0	3.0 x Average

*Adapted from the Framingham Heart Study

Chronic Inflammatory Markers

Ferratin	308	H	6 - 159 ng/mL
Fibrinogen	429		203 - 480 mg/dL
C-Reactive Protein (HS)	0.3		<= 3.0 mg/L

Cardio CRP value (mg/L)	CHD Risk Level
< 1	Low
1–3	Average
> 3 (up to 10)*	High

*If the cardio CRP concentration exceeds 10 mg/L after repeated testing, the patient should be evaluated for non-cardiovascular etiologies.

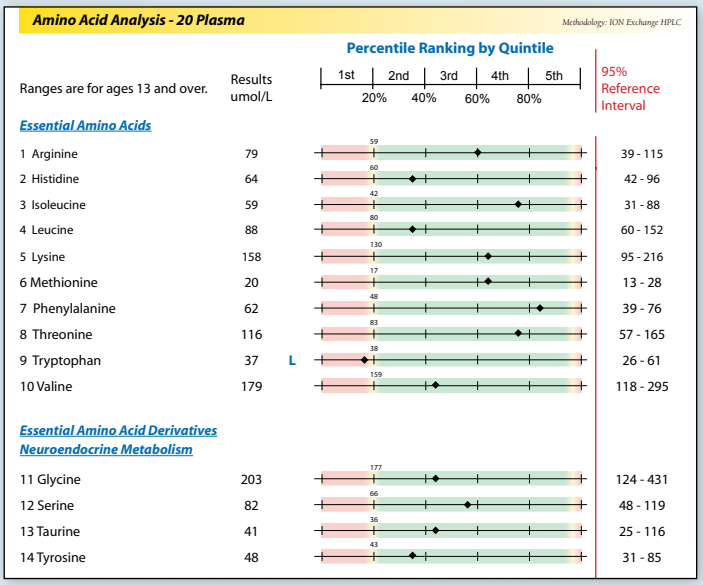
Other Important Indicators

Insulin	<= 1.9	L	2.0 - 12.0 uIU/mL
Testosterone	25		<= 81 ng/dL
Sex Hormone-Binding Globulin	129	H	18 - 114 nmol/L
Free Androgen Index (calc.)	0.7	L	1.0 - 6.6

Demographics: 51-year-old female

Presenting Symptoms:

- Food allergies and sensitivities
- Multiple chemical sensitivity
- Extreme fatigue and brain fog
- GI complaints – constipation
- Hives
- Acne Rosacea
- Cannot take supplements – too sensitive
- Duration of symptoms – 8 years



Amino Acid Analysis - 20 Plasma

Methodology: ION Exchange HPLC

Ranges are for ages 13 and over.	Results umol/L		Percentile Ranking by Quintile (1st 2nd 3rd 4th 5th; 20% 40% 60% 80%)	95% Reference Interval
Essential Amino Acids				
1 Arginine	79		59	39 - 115
2 Histidine	64		60	42 - 96
3 Isoleucine	59		42	31 - 88
4 Leucine	88		80	60 - 152
5 Lysine	158		130	95 - 216
6 Methionine	20		17	13 - 28
7 Phenylalanine	62		48	39 - 76
8 Threonine	116		83	57 - 165
9 Tryptophan	37	L	38	26 - 61
10 Valine	179		159	118 - 295
Essential Amino Acid Derivatives Neuroendocrine Metabolism				
11 Glycine	203		177	124 - 431
12 Serine	82		66	48 - 119
13 Taurine	41		36	25 - 116
14 Tyrosine	48		43	31 - 85

Notes:

Findings:

- **Gastrointestinal function stool profile**
  - Abundant *Clostridia* sp. and extremely prevalent *E. coli*
  - Fungi and protozoans present, not of typical pathogen taxonomy
  - Elevated SCFA dominated by butyrate with little acetate; low pH
  - Many WBCs with normal lactoferrin
  - Digestion & absorption appears normal

The stool assessment of anaerobic bacteria shows an unfavorable balance of predominant species accompanied by atypical fungi and protozoans. This pattern is frequently found in patients with various food sensitivities, but it does not explain the severity of symptoms in this case.

The unusual pattern found in the porphyrin profile suggests a toxicant interference similar to the effects reported for arsenic. Other origins of the interference are indicated by the nutrient profiles that follow.

- **Porphyrin profile**
  - Copro I/III quite elevated (I and III in 1st decile and > 95%ile, respectively)
  - No general porphyrin pathway stimulation apparent

- **Multiple Nutrient Assessments**
  - CARDIOVASCULAR PROFILE
    - High ferritin and peroxides with low CoQ_{10}, tocopherol and Mg, suggesting oxidative stress
    - Low free androgen index ➜ potential anabolic deficit
  - PLASMA AMINO ACIDS
    - Slightly low Trp
  - ERYTHROCYTE & WHOLE-BLOOD ELEMENTS
    - Very high Cu with low K, Mg, Zn, Mn and Se
    - Very high Al and high Pb
  - SERUM ANTIOXIDANTS & VITAMIN D
    - Low alpha- and undetectable gamma-tocopherol with very low CoQ_{10}
    - Lipid peroxides in 5th quintile with extreme elevation of 8-OHdG
    - Very low vitamin D
  - PLASMA FATTY ACIDS
    - Multiple low n-3 (ALA, DPA, DHA) and n-6 (LA, AA) PUFAs without elevated Mead
    - Elevated myristoleic and upper 4th quintile palmitoleic
    - Generally low saturated fatty acid pattern and elevated palmitelaidic with normal C18 *trans*-fatty acids
  - ORGANIC ACIDS IN URINE
    - High ratio of citrate/isocitrate, suggesting aconitase activity deficit
    - High pyroglutamate and benzoate, indicating glycine deficit
    - No upper g.i. dysbiosis, indicated by bacterial metabolic markers

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Element - Erythrocytes and Whole Blood

Methodology: Inductively Coupled Plasma/Mass Spectroscopy

Ranges are for ages 13 and over.

	Results		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval	
Nutrient Elements					
1 Potassium	891	L	1.585	1,099 - 2,492	ppm packed cells
2 Magnesium*	17	L	26	18 - 40	ppm packed cells
3 Zinc	4.1	L	5.4	4.2 - 9.3	ppm packed cells
4 Copper	741	H	329	275 - 534	ppb packed cells
repeated and verified					
5 Manganese	26	L	27	22 - 43	ppb packed cells
6 Chromium	3.7		3.0	1.3 - 7.5	ppb packed cells
7 Selenium	0.16	L	0.19	0.15 - 0.41	ppm whole blood
8 Calcium	27		27	12 - 36	ppm packed cells
Toxic Elements					
9 Aluminum	200	H	66	<= 101	
10 Arsenic	5		6	<= 17	
11 Cadmium	< 0.39		0.6	<= 2.3	
12 Lead	25		20	<= 32	
13 Mercury	3		5	<= 11	
repeated and verified					

CoEnzyme Q_{10} Plus Vitamin Panel - Serum

Methodology: High Performance Liquid Chromatography

	Results mg/L		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
1 Coenzyme Q_{10}	0.35	L	0.64 – 2.16	0.48 - 3.0
2 alpha-Tocopherol	8.1	L	9.8 – 25.1	6.8 - 31.7
3 gamma-Tocopherol	<0.02	L	0.26 – 2.06	0.06 - 2.9
4 Vitamin A	0.58		0.56 – 1.24	0.38 - 1.5
5 ß-Carotene	0.48		0.15 – 1.70	0.10 - 2.7

Lipid Peroxide - Serum

Methodology: High Performance Liquid Chromatography

	Results nmol/mL			95% Reference Interval
5 Lipid Peroxides	1.6	H	1.5	<= 2.0

8-Hydroxy-2 deoxyguanosine - Urine

Methodology: LC/Tandem Mass Spectroscopy, Colorimetric

Ranges are for ages 13 and over.

	Results ng/mg creatinine			95% Reference Interval
6 8-Hydroxy-2-deoxyguanosine	12.6	H	5.3	<= 7.6

Vitamin D - Serum

Methodology: Chemiluminescent Immunoassay (CLIA)

	Results ng/mL			95% Reference Interval
7 25-Hydroxyvitamin D	9	L	16 – 32	10 - 64

Notes:

Conclusions:

The most compelling pattern is one of extreme oxidative stress, possibly due to toxic aluminum and copper levels. Toxic element metabolic impact may explain the porphyrin pathway abnormality, although the pattern is not reported specifically associated with aluminum effects. Exposure to aluminum should be immediately investigated and minimized and, if copper excess is confirmed, phlebotomy may be employed and avoiding copper-rich foods (esp. shellfish) can be advised. Add zinc to tolerance to offset high copper metabolic effects.

Antioxidants and vitamin D appear in urgent need of repletion. The full range of lipid and water-soluble factors can be helpful to relieve the oxidative stress and restore redox balance. Use PUFA supplements with caution, in spite of the depressed tissue status, because of the potential for exacerbating oxidative stress. Supplementation of PUFA-rich oils can be started as soon as follow-up testing indicates relief of the oxidative stress.

The oxidative stress may be causing general anabolic process suppression as indicated by low free androgen index and low levels of saturated (and other) fatty acids that can reflect suppressed hepatic LDL export. Aggressive addition of nutrient elements that are low in erythrocytes may be poorly tolerated because they stimulate the anabolic processes to produce greater oxidative stress. ❖

Fatty Acids - Plasma — Methodology: Capillary Gas Chromatography/Mass Spectrometry

Ranges are for ages 13 and over.	Results uM		Percentile Ranking by Quintile (1st 20% · 2nd 40% · 3rd 60% · 4th 80% · 5th)	95% Reference Interval
Polyunsaturated Omega-3				
1 Alpha-Linolenic (18:3n3)	27	L	37	22 - 144
2 Eicosapentaenoic (20:5n3)	45		44	19 - 362
3 Docosapentaenoic (22:5n3)	40	L	46	31 - 112
4 Docosahexaenoic (22:6n3)	168	L	172	95 - 333
Polyunsaturated Omega-6				
5 Linolenic (18:2n6)	862	L	1,571 – 2,807	1,305 - 3,300
6 Gamma-Linolenic (18:3n6)	10.8		8.9 – 38.1	5.2 - 58.0
7 Eicosadienoic (20: 2n6)	24		18 – 37	14 - 45
8 Dihomogamma-Linolenic (20:3n6)	99		88 – 225	64 - 294
9 Arachidonic (20:4n6)	322	L	330 – 633	260 - 750
10 Docosadienoic (22:2n6)	1.8		1.2 – 2.9	0.9 - 3.8
11 Docosatetraenoic (22:4n6)	11		11 – 38	7 - 51
Saturated				
20 Capric (10:0)	4.2		2.1 – 6.6	1.5 - 9.8
21 Lauric (12:0)	9.5		7.5 – 24.9	6.0 - 41.9
22 Myristic (14:0)	45		44 – 127	34 - 180
23 Palmitic (16:0)	1,422	L	1,610 – 2,946	1,364 - 3,525
24 Stearic (18:0)	511	L	662 – 1,086	557 - 1,234
25 Arachidic (20:0)	27		26 – 48	22 - 57
26 Behenic(22:0)	38	L	66 – 127	53 - 157
27 Ligoneceric (24:0)	26	L	52 – 104	42 - 130
28 Hexacosanoic (26:0)	< 0.62		0.62	<= 0.81
Trans				
34 Palmitelaidic (16:1n7t)	9.7	H	3.0	<= 5.2
35 Total C:18 Trans	68		101	<= 154

TOXICANTS AND DETOXIFICATION

Lipoprotein Factors
(Arg, NAC, Met, Mg and antioxidants)

29 2-Methylhippurate	0.005		0.047	<= 0.106
30 Orotate	0.9		1.0	<= 1.6
31 Glucarate	1.7		7.0	<= 11.9
32 α-Hydroxybutyrate	0.3		1.2	<= 2.2
33 Pyroglutamate	57	H	43	< 72
34 Sulfate	250		123 – 343	89 - 432

COMPOUNDS OF BACTERIAL OR YEAST/FUNGAL ORIGIN

Bacterial - general

35 Benzoate	5.2	H	2.5	<= 8.2
36 Hippurate	254		542	<= 1,099

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Chapter 10

Hormones

Marcus N. Miller, Cheryl K. Burdette and Richard S. Lord

CONTENTS

Notes:

Introduction

Because of their complex interactions, hormones are not easily ordered into a sequential presentation. The adopted approach favors a quasi-life-cycle topical organization of growth, maintenance and reproduction. The main endocrine hormones are discussed under the headings of "Growth and Homeostasis," "The Stress Response," and "The Sex Hormones." Each section introduces concepts of master-gland control followed by discussions of hormone function and clinical assessment. Specific hormone coverage is followed by aspects of test selection, hormone delivery, bioidentical hormone therapy and hormonal biotransformation. The final section of the chapter briefly touches on some other mechanisms of cell control, concluding with the example of cytokine activity in the AKT signaling pathway for apoptosis.

The endocrine system has historically been defined as consisting of eight primary organs: pituitary, thyroid, thymus, adrenal, pancreas, pineal, ovary and testes. Hormonal synthesis and release also takes place in other organs, such as the stomach, small intestine, heart, liver and placenta. A web-like interplay of hormones works to maintain physiological homeostasis as part of the total neuroimmunohormonal regulation of the body. Classic endocrinology is concerned with long-range interactions in which chemical messengers produced in one organ exert control on specific target tissues outside of the site of synthesis. The full spectrum of cellular controls also includes paracrine and autocrine signal molecules that exert control on nearby cells or the same cell in which they were produced. Examples of these include eicosanoids (see Chapter 5, "Fatty Acids") and nitric oxide (see Chapter 4, "Amino Acids").

A brief discussion of other levels of cell controls and the details of a representative cytokine-stimulated phosphorylation pathway is included at that end of this chapter. Some hormones are able to behave in both a paracrine and an autocrine fashion. For example, testosterone, which is produced in the Leydig cells of the testes, can stimulate nearby spermatogenesis as well as distant target organ responses. Hormones that act on distant target tissue cells are released in greater quantities than are paracrine or autocrine signal molecules, and hormone half-lives vary from milliseconds to days. Those released in the greatest quantity are epinephrine, cortisol and insulin. Some of the most important hormones are regulated by the hypothalamus and pituitary and include growth, steroid, thyroid and stress hormones.

Table 10.1 — Hormonal Effects on Nutrient Status

Hormone	Hormone Abnormality	Impaired Nutrient	Hormone Effect on Nutrient(s)
Thyroid	High	Vitamins C, E	Increased utilization
	Low/high	Vitamin A, beta-carotene	Abnormal carotene conversion
	Low	Iodine	Indication of deficiency
	High	CoQ_{10}	Increased utilization
	Low/high	Tyrosine	Thyroid function
	Low	Selenium	Thyroid function
Testosterone	Low (M)	Zinc, amino acids	Increased utilization
Estrogen	Low (F)	Calcium, magnesium, vitamin D	Increased mineralization of bone
ACTH	High	Amino acids	Increased utilization
Cortisol	High	B-complex vitamins, amino acids, minerals, iron, vitamin C	Increased loss
	High	Fatty acids	Lowered omega-6 levels
DHEA	High	Vitamin E	Increased oxidant stress
Growth Hormone	Low	Fatty acids, amino acids	Decreased fat oxidation
		Vitamin D	Increased catabolism
Insulin	High	Minerals, amino acids, fatty acids	Increased loss
	Low	Glucose	Decreased metabolic utilization and increased urinary loss

Evaluation of hormones and other cell regulators is a growing area of laboratory science. The catalog of one specialty laboratory lists over 350 separate single hormones or multiple hormone profiles.[1] Well over 100 direct hormone and challenge response tests are available for gastrointestinal hormones alone. Endocrinology is an ever-evolving field, where the elucidation of cell differentiation and regulation has led to an expansion of knowledge about the rapidly growing list of compounds used for cellular communication. We will confine our discussion primarily to the tests that are most commonly utilized by the integrative medical practitioner.

Nutrient and Hormone Interactions

The output of hormones in response to physiological stresses can impact the demands for specific nutrients, as summarized in Table 10.1. Thus, laboratory assessment of hormones is pertinent to the practice of nutritional medicine because the hormonal influences on metabolism alter nutrient demands. Hormones can also have direct effects on nutrient availability. For example, thyroxine is involved in the conversion of beta-carotene to vitamin A. Abnormal levels of vitamin A have been found in hyper- and hypothyroidism.[2, 3] Hyperthyroidism has also been shown to cause increased utilization of coenzyme Q_{10} in children, due to increased electron transport-chain activity.[4] Vitamins C and E have also been shown to be deficient in the hyperthyroid state.[5]

Not only do hormone actions alter nutrient status, but also hormone production can be altered by nutrient deficiencies because of their actions on enzyme activities and gene expression.[6] Some of these relationships are summarized in Table 10.2. Nutrients are known to play a significant role in modulating the formation, clearance and tissue responses to hormones. Nutritional adequacy is thus a prerequisite to appropriate hormonal function. Returning to the example of thyroid function, selenium and zinc are both cofactors in key thyroid enzymes, and have been shown to be abnormal in various thyroid conditions. Iodine deficiency has long been recognized as a cause of goiter due to excessive thyroid stimulation in an attempt to synthesize thyroxine (see Chapter 3, "Nutrient and Toxic Elements").[7]

Hormones may be classified by their structural similarities. For example, cholesterol provides the starting material for the steroid hormones, including the reproductive hormones as well as cortisol, DHEA and aldosterone. When cholesterol levels are lowered, as with statin drugs, effects of reduced precursor concentrations on production of these hormones may explain increases in risks of depression, impulsive behavior and suicides.[8] Via multiple hormone-hormone interactions, such effects can spread to other structural classes, such as the tyrosine-derived catecholamines, dopamine and homovanilate, that may rise with decreased serum cholesterol.[9]

TABLE 10.2 — NUTRIENT INFLUENCES ON CELLULAR CONTROLS

Nutrient or Dietary Component	Cellular Control Factor(s)	Effect of Low Nutrient Supply on Hormone
Iodine	Thyroxin	Reduced biosynthesis
Amino acids	Tryptophan – Serotonin	Reduced biosynthesis
	Tyrosine – Thyroxin	
	Arginine – Nitric oxide	
Essential fatty acids	Eicosanoids	Reduced biosynthesis
	$T_4 \rightarrow T_3$	Reduced conversion rate
Zinc	Growth hormone	Reduced concentrations
	Testosterone	Primary failure of Leydig cells
	Thyroxin	Reduced synthesis and reduced tissue conversion to T_3
	Corticosteroids	Impaired ACTH response
Selenium	T_4	Reduced conversion to T_3
Dietary fiber and probiotics	Butyrate	Unregulated colonic epithelial growth
Soy products	Isoflavone derivatives	Estrogen mimetic

GROWTH AND HOMEOSTASIS

Rates of hormone synthesis and release are regulated by the hypothalamus. Neuronal-level control governs secretion of releasing factors that travel from the hypothalamus to the anterior lobe of the pituitary gland, causing release of hormones that stimulate specific endocrine glands that, in turn, secrete hormones for stimulation of target tissue responses as illustrated for the stress response in Figure 10.1. Through its hormones, the hypothalamus plays a major role in the control of metabolic activity throughout the body, including basic functions such as hunger, thirst, body heat and sleep.

Growth hormone releasing hormone (GHRH), also known as somatocrinin, is carried by the hypothalamo-hypophysial portal circulation to the anterior lobe of the pituitary gland, where it stimulates secretion of growth hormone (GH), also known as somatotropin. GHRH and GH are both released in pulses. GH promotes growth of the body via protein synthesis and fat breakdown. GH stimulates the production of insulin-like growth factor-I (IGF-I), also known as somatomedin C, which is produced primarily by the liver as an endocrine hormone.

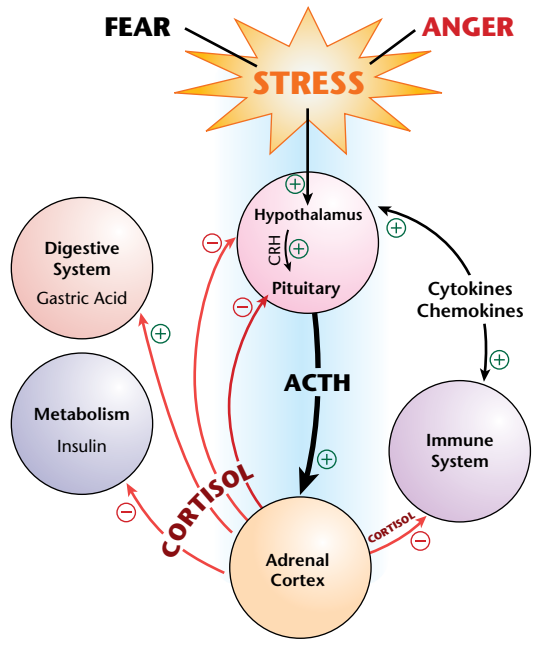

FIGURE 10.1 — **Metabolic and System Responses to Stress**

The hypothalamic, pituitary and adrenal glands respond in tandem to elicit multiple tissue responses when stress signals are received. When the brain perceives the removal of the threat, then the initiating neuronal signals subside and the system returns to basal levels of activity.

GROWTH HORMONE (GH) AND INSULIN-LIKE GROWTH FACTOR-I (IGF-I)

GH: Single-polypeptide chain of 191 amino acids
IGF-I: Single polypeptide chain of 70 amino acids

Effects on Target Tissue Cells	Laboratory Evaluations
Incorporation of amino acids into protein	Insulin resistance
Releasing FFA from adipocytes	Elevation of serum phosphate
Elevated basal and random GH level	Hypercalciuria
Inhibiting glucose uptake by tissues	Hyperprolactinemia
	Glucose-suppressed GH concentration
	GH response to insulin-induced hypoglycemia
	GH response to arginine infusion
	GH response to GHRH or somatomedin-C infusion

Functions: The principal effect of GH is to stimulate the liver and other tissues to synthesize IGF-I.[10] Thus, IGF-I mediates the action of growth hormone. The levels of IGF-I, unlike growth hormone, are relatively constant during the day, making its measurement more reliable. The greater stability of serum IGF-I is due in part to the buffering action of IGF-binding protein that is also present in serum. The presence of a binding protein raises the question of whether bound, free or total forms should be measured. Salivary IGF-I levels measure the active, free form and reflect the GH status of the patient.[11] The concentrations of serum IGF-I are low at birth (< 100 ng/mL) and rise steeply during childhood and puberty, reaching values as high as

Notes:

1,000 ng/mL by age 15. Levels generally begin to fall by the mid-twenties, and they continue to decline thereafter.[10,12] Although the absence of GH and IGF-I may contribute to the phenotype of aging, increased IGF-I is an important risk factor in age-related pathologies, including lung, breast and prostate cancer. This apparent contradiction raises the question of whether levels of these hormones change in response to other factors as a way of normalizing physiological stressors. Growth hormone has recently been recognized for its powerful endothelium-dependent effects as well. Studies looking at endothelial function in the aged versus the young showed an increase in aortic media thickness with age, which resulted in a decreased vasodilatory response. When GH was given, vasodilatory response improved and reduced media thickness. GH also appears to restore amino acid status in animal studies. In older rats, an increase in glutamine, arginine and aspartate were noted with an increase in citrulline. GH treatment restored amino acids to levels of those in young rats.[13,14]

Clinical Assessment: Excessive production of GH causes gigantism, acromegaly or other malformations. Deficient production of GH has differing effects depending on when in a person's development the deficiency begins. In young children, a deficiency of GH results in growth failure that can be dramatically relieved if GH is given before puberty. In adults, a GH deficiency is associated with metabolic syndrome, osteoporosis, muscle wasting, impaired quality of life and increased incidence of cardiovascular events.[15] GH response has been found to be negatively correlated with body mass index, increasing age and, in the absence of additional pituitary hormone deficits, hypothalamic-pituitary disease.[16, 17] GH has been successfully used in reducing cholesterol,[18,19] body fat,[20] blood pressure, and anxiety, and in increasing lean muscle,[21] bone density, HDL, and energy levels.[22] The diagnosis of adult GH deficiency (GHD) often includes the addition of provocative testing, such as the insulin tolerance test, the arginine test or the GHRH-arginine test, although clinical signs and symptoms must be included in the evaluation.[16, 23] IGF-I may not be as sensitive a marker in adult onset GHD.[17] The evidence for partial deficiency of GH, also referred to as GH insufficiency (GHI) is increasing. Some researchers have noted that the condition may be more common in adults with concurrent hypothalamic-pituitary disease.[17] Signs often associated with GHI are abnormal body composition, dyslipidemia and insulin resistance.[23]

Several factors have been identified to explain improvements of tissue function in aged animals resulting from administration of GH. Individual differences in IGF-I synthesis are significant. Dose titration to the upper-normal mid-life range reduces the incidence of side effects and GH insensitivity. Women have lower sensitivities than age-matched men and are likely to require slightly higher doses of GH. Starvation, cachexia, hyperalimentation and insulin-dependent diabetes mellitus are associated with functional GH resistance in which, despite normal or high GH levels, circulatory IGF-I levels are low. In such conditions, human recombinant IGF-I is available for direct intravenous or subcutaneous administration,[24] but doses must be adjusted carefully to avoid side effects. Caloric restriction induces moderate endocrine compensatory mechanisms, including increased GH and decreased IGF-I. It has been proposed that the decline in pathologies and increased life span observed in caloric restriction result from these changes causing decreased stimulus for cellular replication.[25]

Plasma amino acid concentrations reflect changes in amino acid metabolism associated with changes in IGF-I and IGF-binding protein levels. In rats, lower dietary protein intake and lower plasma EAA/NEAA ratios were associated with lower IGF-I.[26] Arginine functions as a secretagogue in its ability to stimulate the release of growth hormone and subsequently raise IGF-1 levels.[27, 28] In a study of 8 adult men, arginine supplementation stimulated GH release, though to a lesser degree than seen with exercise alone. The greatest GH release was from the combined effect of arginine administration before exercise.[29] GH administration to older adults has also shown to increase exercise capacity.[30]

GH is involved in lipid metabolism.[31] A negative feedback loop between plasma free fatty acids and growth hormone has been suggested.[32] Research studies have found decreased free fatty acids have been shown to result in an increase of growth hormones,[32] whereas free fatty acid elevations inhibited GH secretion.[33]

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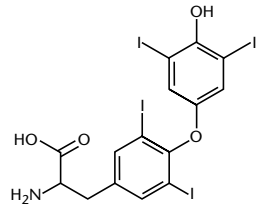
Other Relationships and Effects: GH treatment reduced postmenopausal osteoporosis by up-regulation of intestinal vitamin D receptors, in a manner similar to the action of estrogen.[34] In a case-control study that compared healthy control subjects with GH-deficient participants, compared with GH-deficient females, GH-deficient males were found to have significantly reduced bone mineral density and bone mineral content. The authors suggest that females are able to compensate for the lack of GH via estrogen substitution, an effect testosterone does not seem to possess.[35] Both GH and IGF-I have been shown to modulate renal vitamin D through regulation of inorganic phosphate balance.[36] Low IGF-I will also decrease carboxylated osteocalcin synthesis. This decrease will result in a lack of bone mass mineralization. In a 10-year study of 87 adults with GHD, GH replacement induced a sustained increase in bone mass and bone density.[37] Vitamin K is indicated in patients with a low IGF-I because it has been shown to increase carboxylated osteocalcin and, therefore, improve bone mass.[38] Zinc also modulates IGF-I. Zinc links to a peptide hormone cell-service receptor to form a complex that stimulates mRNA synthesis and gene expression for IGF-I.[39] In HIV patients, acetyl-L-carnitine at 3g/d for 5 months resulted in an increased IGF-I level and a concomitant decrease in lymphocytic apoptosis.[40] Omega-3 fatty acids up-regulate IGF-I activity in bone and should also be considered when IGF-I levels are low.[41] Since IGF-I potentiates uptake of magnesium into red blood cells,[42] low red blood cell magnesium may be related to low IGF-I levels. A direct correlation between deficient levels of vitamin A and both 24-hour GH output and IGF-binding protein was found in short, slowly growing children.[43] Dietary conjugated linoleic acid (CLA) increases serum IGF-I and IGF-binding protein concentrations and reduces bone formation in rats fed n-6 or n-3 fatty acids.[44] This observation suggests adjunctive use of IGF-I in cases where CLA is used for reduction of body fat.[45]

When considering hormonal dysfunction, toxicity—primarily heavy metals—should always be ruled out. Heavy metals are stored in fat; therefore, the fatty nature of glandular tissue makes endocrine glands particularly susceptible to toxicity. High levels of lead in children's blood can hinder their growth. Children with the highest levels of lead had the lowest levels of IGF-I, and after each chelation treatment, a rapid growth spurt occurred.[46]

THYROXINE



Thyroxine or Tetraiodothyronine

Metabolic Effects	Laboratory Evaluations
Increases oxygen consumption	Serum T3, T4, TSH
Promotes glucose oxidation	Serum thyroxine-binding globulin
Increases gluconeogenesis	Response to TRH
Promotes digestive juice secretion	Iodine uptake scan
Uncouples phosphorylation (heat release)	Thyroid antibodies
Increases osteoblastic expression of osteocalcin and alkaline phosphatase mRNA	

TRH, also called thyrotropin-releasing factor, is secreted from the hypothalamus and stimulates the anterior pituitary to secrete thyroid-stimulating hormone (TSH) and prolactin. TSH stimulates the release of thyroid hormones from the thyroid gland, located in the neck. Thyroid hormones contain two aromatic rings of tyrosine linked together with the addition of iodine. The addition of iodine at three places makes T_3 (triiodothyronine), the addition of four iodine atoms makes T_4 (thyroxine/tetraiodothyronine) as previously illustrated in Figure 3.13 of Chapter 3.

Functions: Primary functions of thyroid hormones include controlling basal metabolic rate; protein synthesis; regulation of protein, fat and carbohydrate metabolism; and increasing the body's sensitivity to catecholamines (such as adrenaline). The gene-stimulating ability of thyroxine is demonstrated by the increased production of osteocalcin and alkaline phosphatase mRNA in osteoblasts from femur.[47] In the liver, thyroxine sustains the transcriptional rate for production of cytochrome-*c* apoproteins.[48] The thyroid gland utilizes iodine in combination with tyrosine to produce the thyroid hormones T_4 and T_3 (T_1 and T_2 are also synthesized), which are then released into the bloodstream and regulate metabolism (see the section "Iodine" in Chapter 3, "Nutrient and Toxic Elements").

T_4 is a hormone with diminished biological activity relative to T_3. It is converted into T_3, the form that performs most of the thyroid function in the body. Generally, there is significantly more T_4 (80%) than T_3 (20%).

T_3 and T_4 participate in a negative feedback loop; when levels are low, the pituitary gland produces TSH, and the TSH stimulates the thyroid gland to produce more T_3 and T_4. When the levels of T_3 and T_4 rise, the pituitary decreases TSH production.

Clinical Assessment: The most common thyroid dysfunction is hypothyroidism. Table 10.3 summarizes signs and symptoms of hypo- and hyperthyroidism. There are several forms of hypothyroidism, which are generally referred to as being primary, secondary and tertiary. Primary hypothyroidism is due to a decreased production of T_3 and T_4 at the level of the thyroid gland itself. Primary hypothyroidism can result from a dietary deficiency of iodine or impaired iodine uptake by the thyroid gland. Primary hypothyroidism can also occur as a result of autoimmune destruction of thyroid tissue. Autoimmune destruction of thyroid tissue is often referred to as Hashimoto's thyroiditis. In the United States, Hashimoto's thyroiditis is the most common cause of primary hypothyroidism, whereas worldwide, iodine deficiency is the most common etiology of primary hypothyroidism.[49] Secondary hypothyroidism refers to inadequate stimulation of the thyroid to produce thyroid hormone as a result of insufficient TSH due to pituitary dysfunction. Secondary hypothyroidism occurs far less frequently than primary hypothyroidism. Tertiary hypothyroidism occurs as a result of hypothalamic dysfunction, wherein decreased release of the TRH results in inadequate TSH release.[50] Secondary and tertiary hypothyroidisms are frequently associated with tumors in the pituitary or hypothalamus. Functional hypothyroidism displays decreased T_3 with normal TSH and T_4, secondary to decreased conversion of T_4 to T_3 in peripheral tissues. Reasons for decreased conversion include increased conversion to reverse T_3 or selenium deficiency. Reverse T_3 is an isomer of T_3 that is made under conditions of stress and has little to no bioactivity.

Thyroid hormone evaluation was one of the first assessments to be routinely done in the clinical laboratory. Protein-bound iodine (PBI) analysis was replaced by T_4, followed by thyroid profiles of free and bound T_4 along with the metabolites T_3 and TSH. Calculated values of T_3 uptake and free thyroxine index have been added to improve discrimination of primary thyroid disorders and conditions that stimulate the synthesis of serum thyroid-binding proteins. In examining laboratory reports, it is important to differentiate total T_4 and free T_4. The total T_4 test has been used for many years. Its drawback is that it can be affected by interference, such as the amount of protein in the blood. Free T_4 is the active form of thyroxine and is not affected by protein levels.

Upper reference limits for serum TSH steadily declined during the first 20 years after introduction of the test. Initial values of approximately 10 declined to 4.0 to 4.5 mIU/L prior to the year 2000. In its recent guidelines, the National Academy of Clinical Biochemistry (NACB) recommended 2.5 mIU/L as a reference level for serum TSH. This was done because reference populations, on which the definition of the reference range is based, contain persons undergoing an initial phase of autoimmune thyroid disease. Primary hypothyroidism is characterized by low or low-normal levels of T_4 and T_3, and high TSH. Primary hypothyroidism affects the entire

TABLE 10.3 — SYMPTOMS AND SIGNS OF HYPOTHYROID AND HYPERTHYROID

Hypothyroid		Hyperthyroid	
Symptoms	**Signs**	**Symptoms**	**Signs**
Fatigue	Pale, dry skin	Palpitations	Fast heart rate
Weakness	Coarse, dry hair	Heat intolerance	Trembling hands
Weight gain or increased difficulty losing weight	A puffy face	Nervousness	Weight loss
Hair loss	Hoarse voice	Insomnia	Muscle weakness
Cold intolerance	Elevated blood cholesterol level	Breathlessness	Warm, moist skin
Muscle cramps		Increased bowel movements	Hair loss
Constipation		Light or absent menstrual periods	Staring gaze
Depression		Fatigue	
Irritability			
Memory loss			
Abnormal menstruation			
Decreased libido			

body, mentally and physically, so symptoms can vary significantly. Thus, it is important to also use clinical judgment, patient history and symptoms, and physical exam when making a final evaluation of thyroid status. Many clinicians agree that measuring TSH alone is not adequate to rule out thyroid dysfunction. In addition to a TSH, running a free T_3, the most biologically active form of the hormones, alongside a free T_4, will give a better understanding of activity. In early hypothyroidism, the level of thyroid hormones (T_3 and T_4) may be normal. Additionally, it may be necessary to also measure reverse T_3. In patients making antibodies to thyroperoxidase, thyroid function may appear normal because of compensation of the impaired glandular output.[51] An abnormal serum TSH concentration, with normal T_4 and T_3 may be indicative of a subclinical thyroid disorder (see Table 10.4). Subclinical hypothyroidism was found in up to 16% in some populations, and is more prevalent in elderly women.[52] Hypothyroidism can have a significant effect on metabolic control and hyperinsulinemia, and is considered an independent risk factor for atherosclerosis.[53] In case-control studies of patients with subclinical hypothyroidism, those who experienced normalized thyroid function had improved cardiovascular and insulin markers.[54] The relationship may be explained by a decrease in hepatic lipase with an increase of TSH.[55] Older populations may have greater rates of undiagnosed hypothyroidism.[56]

Hyperthyroidism (thyrotoxicosis), detected as high levels of free T_3 (FT_3) or free T_4 (FT_4), is most often autoimmune in origin, although favorable response to selenium supplementation has been reported.[57] In hyperthyroid patients, TSH will be low or suppressed, whereas T_3 and T_4 are generally increased.[58] The TSH is lowered because the pituitary decreases output of TSH in response to elevated circulating hormone levels.

A pattern of elevated TSH with normal (or low) T_3 and T_4 indicates potential essential nutrient limitation of T_4 synthesis. Specific results that exemplify this condition are shown in Table 10.4. These thyroid profile results are typical of those found for a patient with impaired TSH response, which may be due to insufficiency of iodine or tyrosine to sustain adequate rates of thyroglobulin synthesis. Since iodine and tyrosine are the direct precursors for thyroid hormone synthesis, supplementation may help to maintain normal thyroid status in patients with this pattern. When a plasma amino acid profile is available, low phenylalanine and tyrosine are indicators that corroborate tyrosine limitation for T_4 synthesis.

Table 10.4 — Iodine or Tyrosine-Deficient Thyroid Profile

Analyte	Result	Range
Total T_3	0.86	0.45–1.37 µg/mL
T_3 Uptake	34	23–40%
T_4	6.3	4.5–12.0 µg/dL
T_7 (FT_I)	2.1	1.4–3.8
TSH	5.3 (H)	0.3–4.7 µIU/mL

Thyroid hormone displays complex interactions with other hormones. Schmidt's syndrome involves concurrent autoimmune thyroiditis and Addison's disease. Although this occurs in a relatively small percent of the population with a thyroid condition, variants of this may be more common. When treating a thyroid condition, the adrenals must also be considered. Addison's patients may have a higher occurrence of hypothyroidism.[59, 60] In someone with autoimmune thyroiditis, giving cortisol has been shown to lower titers of antithyroid antibodies.[61] Improvements in adrenal function may therefore facilitate improvement in thyroid function and improve glucose tolerance. Increased 16-hydroxyestrogen activity compared with 2-hydroxyestrogen has also been associated with proliferative thyroid disease.[62]

Physiological responses to circulating T_4 depend on its peripheral conversion to T_3 by enzymes that require selenium.[63] Patients with normal circulating hormone levels who display clinical hypothyroid symptoms may be selenium depleted. Thyroid function is particularly dependent on essential element status. Single and multiple deficiencies of selenium, zinc and iodine have distinct effects on thyroid metabolism and structure.[64–66] Zinc supplementation was found to favorably effect thyroid hormone levels, particularly total T_3.[65] Human populations have developed Keshan disease, Kashin-Bek disease and hypothyroid cretinism when they've become deficient in iodine and selenium simultaneously.[67] Adequate dietary supply of iodine is essential for proper thyroid function. High blood levels of fatty acids

Notes:

can inhibit the conversion of T_4 to T_3. Extrathyroidal conversion of T_4 to T_3 is inversely correlated with plasma free fatty acid concentration in patients with various non-thyroidal illnesses, including diabetes mellitus, liver cirrhosis, chronic obstructive pulmonary disease and chronic heart failure.[68] The effect is enhanced by adding oleic acid, especially in patients with lower serum albumin. This effect indicates that the composition of fatty acids, not just their total concentrations, determines the magnitude of inhibition.[69] On the other hand, fatty acid composition is influenced by the action of thyroid hormone. Hypothyroid patients have inhibited delta-6- and delta-5-desaturase pathways that cause alterations of membrane fatty acid composition.[70] Conversion of T_4 to T_3 was decreased by the administration of alpha-lipoic acid in animal studies. This decreased conversion apparently resulted from enhancement of tissue responses to T_3, since the treatment was accompanied by decreased triglyceride levels and stable glucose concentrations.[71] Other studies have indicated low levels of free and esterified carnitine in serum and urine of patients with thyroid dysfunction. Weakness is common in both hyper- and hypothyroidism, and skeletal muscle L-carnitine may play a role in this regard.[72] Adding to the lipoic acid effect, carnitine modulates thyroid hormone action in peripheral tissues.[73] Possibly acting to offset its mitochondrial stimulation, carnitine antagonizes thyroid hormone action by inhibiting both T_3 and T_4 entry into the cell nuclei. This is relevant because thyroid hormone action is mainly mediated by specific nuclear receptors.[74] Collectively, the results summarized here demonstrate the potential for improving thyroid hormone function by normalizing nutritional factors that impact cellular responses.

TABLE 10.5 — GOITROGENIC CHEMICAL CLASSES AND FOODS[81]

Class	Instance	
Xenobiotics	Sulfurated organics (thiocyanate,* isothiocyanate, goitrin,* disulphides)	
	Resorcinol*	
	Dinitrophenol*	
	Phthalate esters and metabolites	
	Polychlorinated biphenyls (PCBs) and Polybrominated biphenyls (PBBs)*	
	Polycyclic aromatic hydrocarbons (PAHs)	
Foods	Cruciferous Vegetables	Broccoli
		Brussels sprouts
		Cabbage
		Cauliflower
		Kale
		Kohlrabi
		Mustard
		Rutabaga
		Turnips
	Other Foods	Babassu (a palm-tree coconut fruit)
		Cassava
		Mandioca (a Brazilian food staple)
		Millet
		Peaches
		Peanuts
		Radishes
		Soybean and soy products, including tofu
		Spinach
		Strawberries
Nutrients	Inorganic iodine in excess*	
	Lithium in high doses*	
	Flavonoids (polyphenols), polyhydroxyphenols and phenol derivatives	
	Pyridines	

**Strongest goitrogenic action in human studies*

Decreased skeletal muscle breakdown, measurable as falling urinary 3-methylhistidine, low insulin and increased TSH levels have been demonstrated in thyrotoxic patients and in animals under protein-calorie malnutrition.[75, 76] These effects on protein turnover suggest that amino acid evaluation is indicated for patients with abnormal thyroid status. An evaluation of vitamin A status should be considered in patients with hypothyroidism, because decreased thyroid levels can impair the conversion of beta-carotene to vitamin A.[5]

In hyperthyroid patients, the risk of oxidative damage is increased due to the higher metabolic rate. Research studies found lipid peroxidation was increased in participants compared with controls, and activities of antioxidant enzymes were altered. These effects were significantly reduced when ascorbic acid was supplemented at 1,000 mg/d.[77] Other markers of oxidative damage provide insight about how well a hyperthyroid patient is controlling oxidative damage (see Chapter 9, "Oxidant Stress"). Thyroid hormones stimulate synthesis of enzymes, such as mitochondrial cytochrome-*c*, that regulate cellular ATP turnover rate. If the various nutrients, such as coenzyme Q_{10}, B-complex vitamins,

carnitine and elements, are not available to support metabolic oxidative pathways, the mitochondria cannot keep up with increased demand. Thus, clinicians treating patients taking thyroid supplements should assess the patient for nutrient insufficiencies that could make it more difficult for the thyroid hormone to elicit normal responses. Complete evaluation of both hypothyroidism and hyperthyroidism also includes appropriate thyroid antibody assessment.

The prevalence of goiters is higher in areas with greater iodine deficiency, although they also result from impaired thyroid synthesis, or increased intake of goitrogens. Goiters also develop as a result of impaired thyroid hormone synthesis or increased intake of goitrogens. The body attempts to normalize thyroid hormone levels by increasing production of both TRH and TSH. Persistent stimulation of the thyroid gland by TSH may result in increased cellularity and hyperplasia of thyroid tissue, which can lead to goiter development. The most important differential diagnoses are cancer and autoimmune thyroid disease. Patients being treated for hypothyroid should be evaluated for low iodine status, especially as iodinated salt use has decreased (see the section "Iodine" in Chapter 3, "Nutrient and Toxic Elements"). Goitrogenic foods contain substances that can interfere with the uptake of iodine and the utilization of iodine in the synthesis of thyroid hormone. For patients consuming balanced diets with varied compositions, intake of dietary goitrogens are not of concern unless there is an iodine deficiency or evidence of thyroid disease. Thus, cruciferous vegetables should not be avoided out of a fear of goitrogenic effects. Goitrogens listed in Table 10.5 act directly or indirectly on the thyroid gland. Of the goitrogens that act directly on the gland, mechanisms include inhibition of iodide transport into the thyroid (thiocyanate and isothiocyanate); interference with oxidation and organic binding of iodide in the gland; and interruption of proteolysis, release of hormones, or dehalogenation.[58, 78-80] Goitrogenic effect of foods is usually destroyed when the foods are cooked. High thiocyanate intake relative to iodine intake correlates with goiter prevalence.[81] In rats, thiocyanate intake reduced circulating T_4, and when thiocyanate intake was reduced, circulating T_4 normalized.[80] Typically, iodine must be deficient in the diet and goitrogen intake must be significantly and chronically elevated to bring about goiter.[82] Iodine deficiencies can also be aggravated by deficiencies of vitamin A, selenium or iron.

A strong association has been found between thyroid dysfunction and non-thyroid autoimmune diseases.[83–86] Patients with altered thyroid function should be evaluated for autoimmune conditions, and inflammatory markers. Thyroid function may also be a significant factor in the metabolic syndrome. Waist circumference was associated with free T_3 and TSH, independent of insulin status and metabolic markers in euthyroid women.[87] Elevated levels of TSH have also been positively and significantly associated with hemoglobin A_{1c}.[54] Animal studies have found blood glucose levels to remain elevated for more than 24 hours following a glucose load in rats with altered thyroid status.[88]

INSULIN

Insulin: Disulfide-linked A and B polypeptide chains containing 51 amino acids

Effects on Target Tissue Cells	Laboratory Evaluations
Increased cellular uptake of glucose	Fasting plasma insulin
Increased protein synthesis	Insulin response to glucose challenge
Increased glycogen synthesis	Proinsulin response to glucose challenge
Increased fatty acid synthesis	
Increased amino acid uptake	
Increased potassium uptake	
Decreased protein degradation	
Decreased lipolysis	
Decreased gluconeogenesis	

Functions: The pancreas is both an endocrine and exocrine gland. The pancreas functions as an endocrine gland by secreting hormones such as insulin, glucagon and somatostatin. The pancreas functions as an exocrine gland by producing and releasing enzymes that are utilized in digestion.

Insulin is an anabolic hormone that functions to increase growth, DNA synthesis and cell replication. Its primary function is to regulate the cellular uptake of plasma glucose. Insulin-stimulated increase of glucose uptake is accompanied by increased lipogenesis,

Notes:

diminished lipolysis and increased amino acid uptake. Insulin is first produced as the much larger polypeptide preproinsulin. Preproinsulin is converted to proinsulin for pancreatic islet-cell storage. Proinsulin release involves cleavage of a portion called C-peptide to produce circulating insulin. Proinsulin is homologous with IGF-I and -II, which can bind the insulin receptor with 10% affinity of insulin.[89] Insulin lowers circulating glucose levels by increasing the number of membrane glucose transporters (see Figure 3.17 under "Chromium" in Chapter 3, "Nutrient and Toxic Elements"). There are four glucose transporters, referred to GLUT1, GLUT2, GLUT3 and GLUT4. All require insulin to transport glucose, although GLUT4 can also be activated by exercise.[90, 91]

Insulin may affect neural tissue and modulate neural metabolism, synapse activity and feeding behaviors.[92-94] The arcuate nucleus of the hypothalamus is considered the insulin-sensing center. There are two major types of neurons in the arcuate nucleus that are believed to increase or decrease transcription, and thus hyperpolarization based on insulin action. They are the orexigenic neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons and the anorexigenic pro-opiomelanocortin/cocaine amphetamine-related transcript (POMC/CART) neurons.[95] It is believed that K+-ATP channels in the hypothalamus may lead to insulin secretion similar to that found in the beta cells of the pancreas.[96]

When insulin-sensitive tissues fail to respond and lower circulating glucose, the pancreas attempts to secrete greater levels of insulin. The resulting hyperinsulinemia in a patient with normal blood glucose is referred to as insulin resistance.[97] As the resistance increases, more insulin is secreted for the same glucose-lowering response. The excess insulin in circulation leads to significant health problems. Fasting insulin levels vary, depending on many factors, including level of insulin resistance, weight, exercise and dietary glucose load. Normal-weight women were found to have a mean fasting insulin level of approximately 10 mU/mL, obese women had 15 mU/mL, and levels ranged from 20 to 35 mU/mL in those with insulin resistance. Within 1 hour of an oral glucose load, insulin levels increased up to 50 mU/mL in normal-weight women, up to 60 mU/mL in obese women, and 120 to 180 mU/mL in those with insulin resistance.[98] In those with the most extreme insulin resistance, fasting circulating insulin levels went as high as 200 mU/mL, and have been documented as high as 1,400 to 2,000 mU/mL following a glucose load.[99] A higher circulating insulin concentration affects energy metabolism by increasing synthesis of triglycerides and cholesterol, leading to elevations of triglycerides and LDL cholesterol. Insulin resistance is related to increased risk of cardiovascular disease,[100] obesity, non-alcoholic fatty liver disease,[101] hyperuricemia,[102] prehypertension,[103] breast cancer,[104] aging and small gestational age at birth.[105]

Clinical Assessment: Fasting serum insulin concentrations above 10 IU/mL indicate possible insulinemia associated with insulin insensitivity or resistance. Elevated plasma triglycerides and free fatty acids are considered a hallmark of insulin resistance (see Case Illustration 5.6 in Chapter 5, "Fatty Acids"). Insulin resistance can also result in high levels of palmitic and stearic fatty acids by inhibiting delta-9 desaturase. Delta-9 desaturase converts saturated fatty acids to monounsaturated fatty acids. Follow-up testing with a 2-hour glucose tolerance test with insulin readings aids in the differential diagnosis of dysglycemia and dysinsulinemia. Diets designed to maintain low glycemic load can help to lower insulin output and reduce the impact of hyperinsulinemia. Consuming a low glycemic-load diet may be especially important to individuals with elevated insulin levels trying to lose weight.[106] The hemoglobin A_{1c} (HbA_{1c}) test provides a measure of average blood sugar levels over the past 2 or 3 months, although identifying the average or mean may not be an adequate assessment for those patients with extremely high or low blood glucose levels. Rises in fasting plasma glucose and HbA_{1c} may also be seen with increased age as a result of a decrease of glycemic control, independent of diabetes status.[107]

High glycemic-load diets induce insulin secretion, leading to lowered concentrations of amino acids in plasma and increased deposition of body fat. Restriction of high glycemic foods with adequate protein supplied in several small meals may help to stabilize blood sugar. Amino acids such as arginine have also been shown to improve insulin output.[27] Omega-3 fatty acids may improve insulin sensitivity.[108-111] Chromium and vanadium have been found effective in aiding insulin responsiveness of type-2 diabetics, and a higher level of intracellular magnesium has been found to correlate with greater insulin responsiveness.[42, 112, 113] Rather than routine introduction of these nutrients, the results from

testing of fatty acids, amino acids and trace elements can provide effective guidance regarding which diabetic patients have focal need for specific nutrients.

The Stress Response

The allostatic load, defined as the cumulative physiologic effect of the body's long-term response to stress, has been correlated with degenerative diseases and aging.[114-117] These physiologic responses result in inflammation and immune stimulation from factors such as anxiety, competition and poor personal relationships. Laboratory evaluations can give direct clinical insight about the level of stress and other adverse lifestyle factors that bear on the outcomes from nutritional interventions. The physiological responses to stressors of various origins are initiated by modulating the output cellular controls such as hormones, neurotransmitters, nitric oxide, and eicosanoids and immunomodulators. Other chapters have covered testing for amino acid neurotransmitter precursors, organic acids and fatty acid flow into eicosanoid pathways. Measuring polypeptides, catecholamines, thyroid hormones and steroid hormones adds valuable clinical information that can indicate specific nutrient interventions. The well-known "fight-or-flight response" involves stimulation of both the cortical and medullary aspects of the adrenal gland, with an increased synthesis and release of both cortisol and epinephrine. Short periods of adrenal stimulation favor the survival of an organism under threat. Persistent stimulation of the adrenal gland has been shown to play a role in the development of visceral obesity,[117] as well as insulin resistance.[118] Glucocorticoid elevation due to chronic stress is associated with increased bone resorption in humans[119] and increased urinary losses of water-soluble vitamins in animal studies.[120]

Corticotropin-Releasing Hormone (CRH) and Adrenocorticotrophic Hormone (ACTH)

CRH: Polypeptide containing 41 amino acids

Metabolic Effects on Pituitary Cells	Laboratory Evaluations
Increased secretion of ACTH and β-endorphin	Plasma CRH

ACTH: Polypeptide containing 39 amino acids

Metabolic Effects on Adrenocortical Cells	Laboratory Evaluations
Increased secretion of corticosteroids	None

CRH, also called corticotropin-releasing factor (CRF), is a hormone produced by the hypothalamus in response to stress that stimulates the anterior lobe of the pituitary gland to secrete adrenocorticotropic hormone (ACTH), also known as corticotropin. ACTH stimulates the adrenal cortex. Impulses from the sympathetic nervous system are the primary stimulus for release of the catecholamines, epinephrine and norepinephrine, and to a lesser extent, dopamine from the adrenal medulla. Stimulation of the adrenal cortex leads to the synthesis of corticosteroids and androgens as illustrated in Figure 10.2. Corticosteroids include glucocorticoids (cortisol) and mineralocorticoids (aldosterone).

Notes:

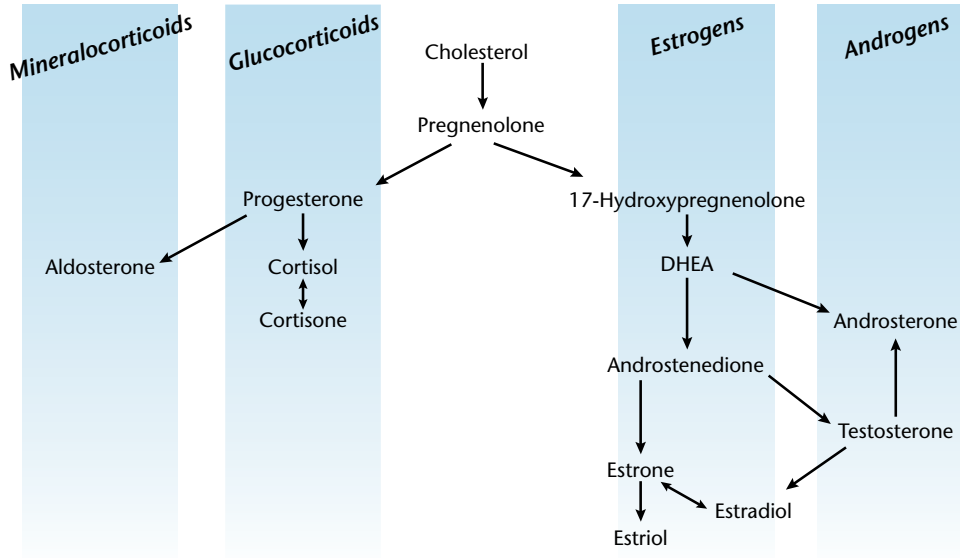
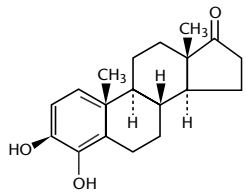


FIGURE 10.2 — **Biosynthesis of Steroid Hormones**

Four classes of steriod hormones are produced from cholesterol. Key intermediates are pregnenolone, progesterone and DHEA.

DEHYDROEPIANDROSTERONE (DHEA)

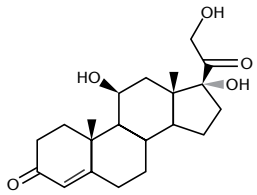


Function	Laboratory Evaluations
Precursor of steroid hormones	Serum, saliva or urine DHEA

DHEA is the precursor to testosterone and estrogen. It is involved with development, growth, immune response and cardiovascular function. DHEA production, and its sulfated form (DHEAS), decrease with age and may contribute to physiological changes that are sex-hormone dependent. A randomized, double-blinded, controlled trial of 140 older adults on either 50 mg/d DHEA or placebo found DHEA replacement therapy for 1 year improved hip bone mineral density (BMD) in older adults and spine BMD in older women.[121] Other studies have found DHEA replacement to increase in BMD, as well as partially reversing age-related changes in fat mass, and fat-free mass.[122] DHEA is the most abundant steroid in the body. It is produced via cholesterol through two cytochrome P450 enzymes to pregnenolone, followed by a conversion to DHEA through the CYP17A1 enzyme. The production takes place in the adrenal gland.

Because DHEA is a major adrenal precursor for other hormones, variability of DHEA levels will affect other hormones as well. When 50 mg of DHEA was given to postmenopausal women, it increased steroids that are derived from DHEA, such as estrone, estradiol, androstenedione, testosterone and DHEA sulfate. In another study, women with Addison's disease given 50 mg of DHEA not only normalized DHEA levels, but also improved IGF-I levels and normalized circulating levels of testosterone. DHEA may therefore be an effective hormonal replacement treatment.[123] DHEA has immunomodulatory properties as well, further demonstrating the interconnected web of cell regulators in the body. DHEA interacts with gamma-aminobutyric acid type-A receptors in the brain, as do benzodiazepines. It may have some sedating activity, which explains why excessive levels of DHEA may be an early indication of depression.[124] When supplementing with DHEA, clinicians should be aware that even with patients on standard doses of DHEA, such as 125 to 250 mg/d, and with DHEA blood levels in normal range, patients may experience elevated levels of DHEA metabolites. Testing of DHEA levels may be used to titrate down their current dose of DHEA.

CORTISOL



Effects on Target Tissue Cells	Laboratory Evaluations
Decreased glycogenesis (hepatic)	Serum, saliva or urine cortisol
Increased proteolysis	Dexamethasone suppression test
Increased lipolysis	Stress response markers
Reduced interleukin-1 response (T cells)	–Serum sIgA
Altered leukocyte distribution	–Urinary catecholamine turnover
Reduced osteogenesis (bone)	–Serum cholesterol
Sensitization to norepinephrine and epinephrine (endothelium)	–Serum triglycerides
Reduced histamine secretion	–Serum insulin

Cortisol is the primary glucocorticoid involved in the regulation of glucose metabolism and the body's response to stress. During times of stress, cortisol levels increase and accelerate the breakdown of proteins to provide the fuel to maintain body functions. This catabolic activity needs to be balanced with periods of rebuilding to maintain good health. Under the influence of cortisol, the body can adapt to new stresses and survive until the physiological defense mechanisms are exhausted, or until the stress subsides. Selye, a pioneer in the study of the effects of stress on the body, termed this phenomenon the "general adaptation syndrome."[125] The ability to avoid multiple stress effects from mineral and water imbalances is due to adrenal steroid hormones. Animals can survive without adrenal glands if they are given small doses of adrenal hormones, but they are unable to adapt to any major chemical or physical stress, and cannot tolerate temperature extremes, starvation, infection, sensitizing agents or noxious chemicals.[126]

Although variation in cortisol output is related to stress, the circadian rhythm of cortisol is linked to the sleep-wake cycle and controlled by the suprachiasmatic nucleus in the hypothalamus. Thus, interactions with other compounds can have significant effects. Secretions are characterized by a steep increase in the morning, peaking at approximately 8 a.m., followed by a gradual tapering off until about midnight, when circulating levels are at their lowest (Figure 10.3). The episodic secretion of cortisol is caused by the intermittent transformation of cortisol from its precursors in the adrenal cortex under the influence of ACTH.[127] This means that the brain is the primary site for control of the diurnal rhythm. The ratio of the highest levels (8 a.m.) to the lowest (midnight) is about 10 for saliva and only 3.3 for plasma. Patients with adrenal insufficiency show the greatest depression of cortisol output in the morning, whereas the hypercortisolemic response of Cushing's syndrome is most prominent in the late evening.[128] Morning levels may increase with increased psychosocial and environmental stressors in those with sufficient adrenal function.[129] Symptoms of cortisol deficiency and excess are shown in Table 10.6.

The pituitary-adrenal response can be tested by measuring serum or salivary cortisol after an oral dose of the cortisol mimic dexamethasone taken in the evening. In the normal response, serum cortisol levels drop to low values by the next morning because the dexamethasone causes the pituitary gland to release less ACTH. Cushing's syndrome patients sustain high cortisol levels, and those with other conditions such as major depression and post-traumatic stress response may produce sustained high cortisol levels after the dexamethasone suppression test.[130-132]

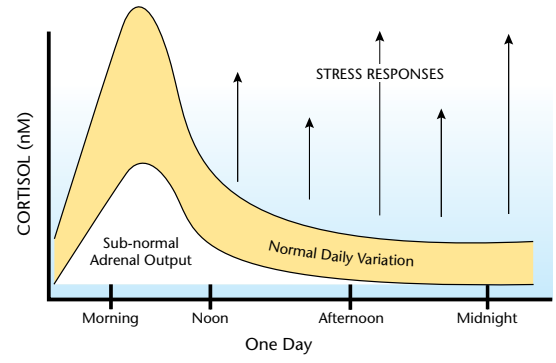


FIGURE 10.3 — **Circadian Cortisol Rhythm**

The area between the two curves is the normal range. When cortisol concentrations remain above normal during the afternoon and evening (arrows), deleterious affects of hypercortisolemia increase.

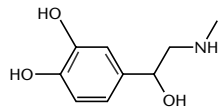
Notes:

TABLE 10.6 — SYMPTOMS OF CORTISOL DEFICIENCY AND EXCESS

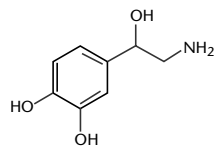
Deficiency	Excess
Chronic weakness	Sugar craving
Weight loss	Central obesity
Fatigue	Insulin resistance
Decreased stress tolerance	Weakness
Anorexia	Shakiness between meals
Alternating diarrhea and constipation	Irritability
Salt craving	Hypertension
Postural dizziness	Easy bruising
Muscle or joint pain	Amenorrhea
Hyperpigmentation	Impotence
Hypotension	Oily skin
Anemia	Sleep disturbances
	Headache

EPINEPHRINE AND NOREPINEPHRINE

Epinephrine (Adrenaline)



Norepinephrine (Noradrenaline)



Effects on Target Tissue Cells	Laboratory Evaluations
Vasoconstriction	Serum epinephrine and norepinephrine
Increased glucose release	Urinary catabolic product (VMA)

Increased output of catecholamines to modulate the autonomic nervous system is the most rapid chemical response to psychological stressors. In Chapter 6, "Organic Acids," we described the association of low central nervous system (CNS) levels of catecholamine neurotransmitters with low urinary levels of vanilmandelate (VMA), the catabolic product of epinephrine and norepinephrine.[133] An example of the relationship of these hormones to mental stress is the 42% increase in urinary VMA in cows in response to the stress of exposure to flies.[134] Sympathetic nerve endings in the adrenal gland release epinephrine, and those in all other tissues release norepinephrine. Treatments aimed at improving protein digestion and supplementation of the amino acid precursor tyrosine can normalize neurotransmitter levels in the CNS.[135] The precursor relationship of tyrosine to epinephrine also gives rise to the antihypertensive role of tyrosine.[136] Patients with hypertension show lower VMA and 5-hydroxyindoleacetate (5-HIAA) levels when treated with a selective serotonergic antagonist.[137] Thus the levels of VMA in urine are shown to be responsive to agents designed to block hormone action as well as to externally imposed stress.

PATTERNS OF ADAPTIVE RESPONSES

The hypothalamic-pituitary-adrenal axis (HPA axis) refers to the complex interactions between the hypothalamus, the pituitary gland and the adrenal gland. The interactions between these three organs are a major part of the neuroendocrine system that controls reactions to stress and activates the HPA axis (Figure 10.1). The adrenal steroids are a family of hormones that permit us to make long-term adjustments to stress and include cortisol and DHEA, both produced in the adrenal cortex. The adrenal gland normally produces much more DHEA than cortisol. Cortisol and DHEA affect carbohydrate, protein and lipid metabolism; serve as anti-inflammatory agents; modulate thyroid function; and increase resistance to stress. Thus, fluctuating amounts of DHEA and cortisol may signal important alterations in adrenal function that can profoundly affect an individual's energy levels, emotional state, disease resistance and general sense of well-being. In health, when stressors are removed, the hormone levels fall back into the normal range. The supply of precursors may become limited, and under conditions of chronic stress, the adrenal glands enter a compensated phase in which the production of the stress hormones is altered. The quantitatively more demanding DHEA pathway is frequently the first to experience the repercussions of this situation. Because of such differences in response to ACTH, the production of DHEA usually declines with chronic stress, whereas, initially, cortisol remains elevated. Patients who maintain elevated levels of cortisol, even under psychologically non-stressful situations, have a habituated endocrine

stress response, which may lead to adrenal fatigue (Figure 10.4). Patients with elevated cortisol may have greater urinary losses of B-vitamins, lowered mineral status, and reduced absorption of calcium, and a greater need for vitamins C and E.[138,139] Basic physiology can explain the relationship between stress and nutrition. If one is always in a heightened state of stress, they exist in the sympathetic state, which may impede proper digestion, further compounding nutrient insufficiencies. Several dietary changes are also recommended in adrenal fatigue, such as eating by 10 a.m. each morning; eating small frequent meals; and avoiding caffeine, high glycemic index foods and alcohol.

The entire process is shown graphically in Figure 10.4, where the initial stress response is labeled "A1." As higher cortisol is required to shut down adrenal responses and bring ACTH into the normal range, the negative feedback of cortisol on the hypothalamus is lost. The resulting condition, known as "physiological hypercortisolemia," affects enzyme activities and can lead to central adiposity.[140] Primate studies show effects of lowered serum phosphate and increased estrogenic responses with increased cortisol levels.[141] Monozygotic, female twin pairs showed a high degree of stability in a.m. and p.m. cortisol values collected daily over a 14-day period. Salivary cortisol was significantly higher for those siblings with a major history of depression than for their non-depressed twins.[142] Cushing's disease is characterized by constantly elevated levels in the upper right of quadrant "A1." Nighttime salivary cortisol testing is an accurate way to screen for hypercortisolism in children.[143] Stress-induced hormonal secretion is greater in older individuals than in their young counterparts, and increases in blood pressure with stress become greater with age.[144]

Later phases of compensated response may go through the progression from "A2" to "A5." The progression has been called "stress fixation." The arrow shows a progression from the upper right to upper left because DHEA is the first to decrease. Because DHEA has much higher normal concentrations, the supply of precursors, especially pregnenolone, can become difficult to maintain. Adrenal cortical output of DHEA falls from high to normal to low. Such a profile of low DHEA with high cortisol is typical of a high-achiever lifestyle. As long as cortisol output is sustained at elevated levels, such intense demands for physiological function may be maintained, but the low DHEA has long-term negative

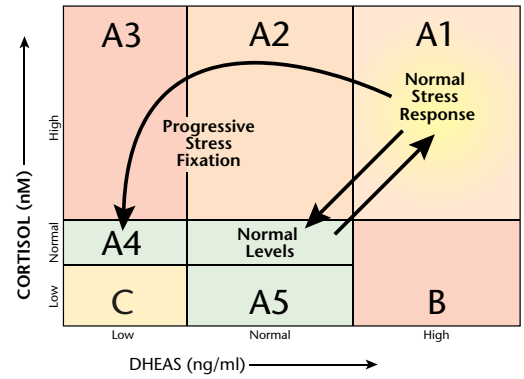


FIGURE 10.4 — **Stress Responses of Cortisol and DHEA**

The values that are plotted are the average of noon and 5 p.m. levels for salivary cortisol and DHEA. The quadrants represent normal and abnormal patterns, with A1 to A4 representing progressive failure of the normal ACTH response.

effects. Stress-induced damage to the hippocampus, an area involved in memory processes and especially affected in Alzheimer's disease, is prevented by DHEA.[145] Low DHEA further contributes to conditions associated with aging, such as osteopenia, decreased lean muscle and increased fat mass. When patients in their 70s took 50 mg of DHEA for 6 months, fat-free mass increased, fat mass decreased and bone mineral density increased.[122] Chronic fatigue syndrome (CFS), fibromyalgia syndrome (FMS), chronic pelvic pain, post-traumatic stress disorder, and other stress-related disorders may also be characterized by alterations in HPA-axis activity.[146]

The same progression for cortisol may ensue if pregnenolone becomes exhausted. If the stress is prolonged,

Notes:

the production of both hormones falls into the sector labeled "C." Individuals affected with Addison's disease, where the adrenals are unable to produce stress hormones, have values that fall into the C sector. The more infrequent finding of elevated DHEA with normal or low cortisol (sector B) is found in hypothalamic dysfunction, and these individuals should avoid high-stress occupations. Cognitive stress management intervention can reduce the rate of progression toward the stress fixation state. A 23% reduction in cortisol and a 100% increase in DHEA were found in individuals who demonstrated response to treatment by improved scales of guilt, hostility, burnout, anxiety and stress.[147] DHEA increases by 10% after exercise, mandating lifestyle changes as a part of a healthy adrenal response.[148]

Muscle protein breakdown caused by increased stress hormones can be reduced by administration of an amino acid solution. Increased adrenal activity also leads to greater loss of B-complex vitamins that should be replaced. Finally, vitamin C should be added to prevent tissue depletion, because it can be rapidly diminished by the adrenal stress response. Vitamin C has an enhancing effect on adrenal glucocorticoid production.[149, 150] A vitamin C deficiency can cause an elevation in cortisol that could lead to suppression of the inflammatory response, impairment of wound healing and decrease in bone matrix formation.

Omega-6 fatty acids are specifically depleted in individuals with high cortisol output.[151] Dietary supplements of linoleic or gamma-linolenic may be used to replenish tissue status of these fatty acids, but there is an important question of balance of omega-6 fatty acids with the omega-3 family in order to maintain the proper tissue responses to challenges. Patients with elevated cortisol may need to be evaluated for polyunsaturated fatty acid status (see Chapter 5, "Fatty Acids"). Licorice consumption potentiates the effects of cortisol, and hypokalemic hypertension can be induced by licorice addiction.[152] The effect is not a result of adrenocortical stimulation, but rather, a reduction in the conversion of cortisol to cortisone.[153] As the degree of stress fixation becomes greater, these interventions become more important, and aggressiveness of nutrient support should increase. Other evaluations of overall nutrient status allow optimization of adrenal response and improvement of total-body response to, and need for, the adrenal cortical hormones. Of course, cognitive stress reduction training is of value at any point in the process.

Assessment of Adrenal Hormones

About 1 to 10% of the steroids in the blood are in unbound or free form. The rest are bound to carrier proteins such as cortisol-binding globulin, sex hormone-binding globulin and albumin. Subtle structural abnormalities due to genetic variation can lead to primary endocrine abnormalities because of impaired binding-protein affinities. Adrenal hyperfunction has been associated with impairment of cortisol binding.[154] Since only unbound steroids can freely diffuse into various target tissues in the body, they are the only hormones that are considered biologically active. Saliva testing measures the free-circulating, biologically active hormones.[155] The relationship of serum and salivary cortisol has been thoroughly examined.[156] Using single measurements of 11 p.m. salivary cortisol, elevated levels identified primary depressive patients with a sensitivity of 62.5% and a specificity of 75%.[128] Salivary cortisol concentrations at 11 p.m. have been shown to predict bone loss in elderly men,[157] demonstrating the long-term effects of cortisol on bone mineral maintenance. Circadian variations are more marked in saliva than in plasma. The cortisol response to stress is developed at birth. Salivary cortisol determination in neonates has been proposed as a helpful measure to control for neonatal stress and birth asphyxia.[158]

ACTH Challenge Test

An ACTH challenge test will give information about how well the adrenal gland responds when recruited by the pituitary to respond to stress. This challenge test assesses the ability of the adrenal cortex to respond to ACTH by an appropriate response in increased cortisol production. Blood or serum levels of cortisol are obtained 1 hour before and 1 hour after injection of ACTH. Cortisol should increase to at least 20 µg/dL in serum, 1 hour post-ACTH challenge. Serum and salivary levels have been found to be highly correlative.[159] Salivary samples may be of more clinical utility because of the less invasive method.[160] It has been hypothesized that the stress of a blood draw may be enough to alter cortisol output, especially when serial samples are involved.

Markers of Stress

Secretory IgA

Secretory IgA (sIgA) forms an immune barrier to protect against gastrointestinal tract infections[161] (see Chapter 7, "Gastrointestinal Function," for further

discussion). Salivary levels of sIgA may be used to monitor this immune-barrier function. Although sIgA production requires an adequate amino acid supply, dietary protein inadequacy has little effect on sIgA levels.[162] Chronic stress, however, decreases sIgA production.[163] The measurement of sIgA as a biomarker for the functional impact of chronic stress greatly enhances the interpretation of stress hormones because single-point elevations of cortisol may be only a normal, temporary stress response. Low sIgA is a sign of the impact of stress directly on the immune system, but elevated sIgA reveals another site of degeneration affected by stress.

The production of IgA is increased when dietary antigens are not blocked by the physical barrier in the small intestine. In human IgA nephropathy, there is a significant decrease in levels of IgA immune complexes after patients are put on a gluten-free diet. In patients with elevated IgA, gluten may act as a toxic lectin, increasing the permeability of the intestinal mucosa to various dietary antigens.[164, 165] Some individuals with only modest IgA deficits can have substantial mucosal permeability defects on milk challenge.[166] IgA antibodies also help to clear *Candida albicans* from mucosal surfaces. IgA levels are lower in women who show positive cultures for *Candida albicans*.[167] This connection provides an explanation for the increased incidence of invasive candidiasis in highly stressed individuals.

Antigliadin Antibodies (AGA)

Chronic stress is a factor in the onset of the gluten sensitivity characteristic of celiac disease. The corollary is also true; gluten sensitivity contributes a significant stress to the body and can add clinical value to adrenal hormone assessment. Detection of IgA-class antigliadin antibody provides an immunohistochemical marker of celiac disease latency and gluten sensitivity.[168] Early dietary treatment is important in conditions such as celiac disease to avoid malnutrition and the development of malignant disorders. Any condition that causes deterioration of the epithelial layer of the small intestine can lead to increased gliadin antibodies. IgG antigliadin antibody concentrations were elevated in subjects with small intestinal bacterial overgrowth and positive luminal antigliadin antibodies.[169]

The Sex Hormones

Gonadotropin-Releasing Hormone (GnRH)

GnRH: Decapeptide (10 amino acids)

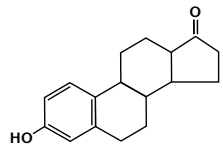
Metabolic Effects on Pituitary Cells	Laboratory Evaluations
Increased synthesis of follicle-stimulating hormone and luteinizing hormone	Serum GnRH

Activities of the sex glands are regulated by gonadotropin-releasing hormone (GnRH), a peptide hormone produced by the hypothalamus that stimulates the anterior pituitary gland to begin secreting follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH stimulates follicular maturation and estradiol secretion in the ovaries. After the follicle ruptures the ovaries produce progesterone. LH acts on the Leydig cells of the testes to stimulate the production of testosterone. The ratio of LH to FSH is determined by the frequency of GnRH pulses secreted from the hypothalamus, as well as by feedback from androgens and estrogens.[170] Low-frequency GnRH pulses lead to FSH release, whereas high-frequency GnRH pulses stimulate LH release. GnRH is secreted at different frequencies in males and females. Females have a large surge of GnRH just before ovulation, the rest of the month the frequency of the pulses varies during the menstrual cycle. Males experience a constant pulse. GnRH activity varies by age. Typically, GnRH is very low during childhood and increased synthesis and release is activated at the onset puberty. The pulsatile release of GnRH may be affected by hypothalamic-pituitary disease, elevated prolactin levels and hyperinsulinemia. In light of the obvious interrelationship with the endocrine system, achieving homeostatis with the administration of exogenous sources can be difficult.

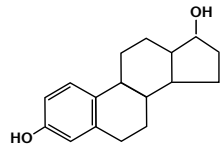
Notes:

ESTROGENS

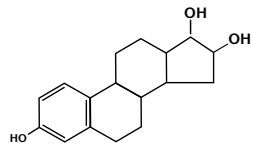
Estrone



Estradiol



Estriol



Functions: Estrogen's most basic biological function is to stimulate tissue growth, whereby it is involved in the regular restoration of the endometrium and, during pregnancy, in the expansion of breast tissue. The relatively massive, cyclic uterine tissue replacement process is a major source of macro- and micronutrient demand in menstruating women. Its cyclic nature requires control of the large shifts in rates of cell division, along with stimulation of the associated biosynthetic pathways. In the ovaries, estrogen is produced from cholesterol as shown in Figure 10.5. Redundant pathways from the pregnenolone and progesterone precursors assure routes of synthesis, even when point genetic mutations occur. Testosterone is aromatized to estradiol (E_2), which is reduced to form estrone (E_1) in a reversible reaction. Estradiol can be reconverted to estrone or further hydroxylated to form estriol (E_3) (Figure 10.6). FSH stimulates estradiol secretion in the ovaries. All three forms have varying levels of activity and specificity for different tissue. Estradiol is the most active of the estrogens responsible for actions attributed to the estrogens. Estriol has an intermediate activity and estrone is the least active of the estrogens. Estrone increases with menopause and is mostly derived from androstenedione. Estradiol plays a role in immune activation, and potentially overactivation.[171, 172] Estrogens are the major steroid anabolic hormones in females before menopause.

Estrone metabolites include 2-hydroxyestrone, 4-hydroxyestrone, and 16α-hydroxyestrone. Research has found 2-hydroxyestrone formation is catalyzed predominantly by CYP1A2, CYP1A1 and CYP1B1 enzymes; 4-hydroxyestrone formation is catalyzed predominantly by CYP1B1, CYP1A2 and CYP1A1 enzymes; and 16α-hydroxyestrone formation is catalyzed predominantly by CYP2C19, CYP1A1 and CYP3A5 (Figure 10.7).[173] In addition to estrogens and their metabolites having different levels of activities, a large part of how estrogen affects tissue depends on the receptor affinity. There are three distinct types of estrogen receptors, estrogen-α, -β and -γ. Different tissues have varying numbers of each of these receptor sites. The selective effects of estrogens and their analogs may be due to the differential distribution of each estrogen receptor (ER) subtype in various tissues.[174] The tissue distribution and/or the relative levels of ER-α and ER-β expression in rats are quite different. There is a moderate-to-high expression of ER-α in the breast, testis, pituitary, ovary, kidney, epididymis, and adrenal gland, and a relatively high expression of ER-β in the prostate, ovary, lung, bladder, brain, uterus and testis. The differential expression of ER-α and ER-β within individual tissues may contribute to the selective effects of estrogen within individual tissues. For example, in the rat and human prostate, ER-α and ER-β are differentially expressed in the secretory epithelia and stromal tissues. It is also clear that estrogens exert organizational effects on the rat and mouse prostate, since neonatal exposure to 17β-estradiol or diethylstilbestrol causes permanent changes not only in the size of the prostate, but also in the expression level of certain genes.

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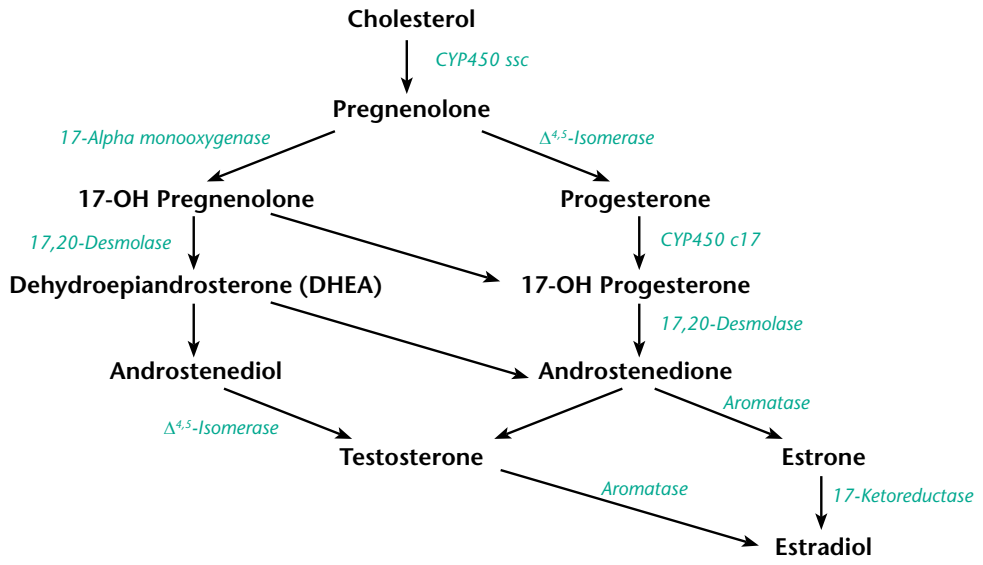

FIGURE 10.5 — **Biosynthesis of Sex Hormones**

Upon stimulation by FSH and LH, a series of oxidation, reduction and isomerization reactions converts cholesterol to the sex hormones in the ovaries and testes. Several of the reactions use the same enzymes needed for steroid hormone synthesis in the adrenal glands. The aromatase reactions produce the aromatic ring of the estrogens seen in Figure 10.6. The secreted hormones bind to sex hormone-binding globulin for delivery to target tissues, where they interact with nuclear receptors to elicit specific protein synthesis.

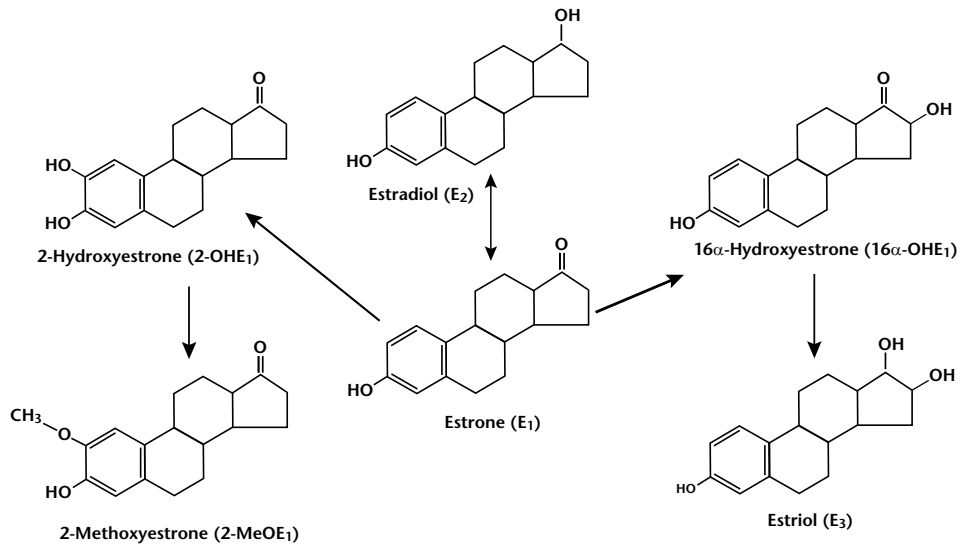

FIGURE 10.6 — **Pathways that Compete for Estrone**

Estrone has three principal fates. It may undergo reduction to estradiol or hydroxylation at the 2 or 16α positions. Reduction of the keto group of 16α-hydroxyestrone produces estriol. The 2- and 16α-hydroxyestrones are major excretory products, appearing in ratios above 2:1 in healthy states. Small fractions of these two derivatives are also methylated by catechol-O-methyl transferase and SAMe to form methoxy derivatives. A further hydroxylation at the 4 position (not shown) occurs to only small extents, but 4-hydroxyestrone has estrogen activity and carcinogenic properties.

Additionally, in the rat ovary, the levels of ER-β expression vary within compartments of ovarian tissue. ER-α is expressed at a low level throughout the rat ovary with no particular cellular localization. Finally, in primary osteoblasts isolated from the bone of neonatal rats, both ER-α and ER-β were detected, although the level of ER-β was much higher. The fact that both estrogen receptors are expressed in bone helps to explain the beneficial effects of estrogens on bone mineral density.[175] It is important to keep in mind that in addition to the type of estrogen and the type of receptor as well as binding affinity, all of these factors are affected by how functional or healthy the receptor is. For example, higher levels of inflammation can impact estrogen receptor reactivity.[176] Oxidative stress should be assessed to assure adequate hormone function. Markers for oxidative stress, such as p-hydroxyphenyllactate, 8-hydroxy2 deoguanosine, hsC-reactive protein and lipid peroxides, are discussed in Chapter 6, "Organic Acids," and Appendix A, "Comprehensive Cardiovascular Health Risk Assessment."

Estrogen is cleared by Phase I and Phase II hepatic detoxification reactions. Phase I consists of three major pathways via the cytochrome P450 enzyme system; Phase II, sulfation, methylation and glucuronidation help to further detoxify Phase I products (Figure 10.7). Amino acids status is important to consider alongside hormone therapy. The follicular phase must facilitate rapid growth of tissue. Amino acid substrate must be plentiful for this function to occur. Estrogen has anabolic effects elsewhere in the body, which are impossible to perform unless nutrition is adequate. In fact, as estradiol increases, the activity of branched-chain α-dehydrogenase complex is decreased to increase the amount of essential amino acids available. Variations in breakdown of branched-chain amino acids are seen in females, and not males, that correlate with levels of estrogen.[177]

Clinical Assessment: High levels of estrogen are characterized by symptoms such as increased anxiety, difficulty sleeping and an increase in irritability. High levels are also associated with increased risk of cancer and highly proliferative breast tissue, resulting in fibroids or cysts.[178] Many of these same symptoms are experienced with menopause, when estrogen levels fall. The fluctuations of estrogen levels may be more important than their concentrations for producing symptoms and requiring the body to establish a new "set point." Thus, the erratic levels of estrogen during menopause have been speculated to be responsible for symptoms.[179, 180] It is possible that estrogen responses are bimodal, with levels that are too high resulting in dysfunctions similar to levels that are too low. When estrogen levels are too low, breast cells will not "mature" properly. Women are born with type 1 lobules in the breast, which progress to type 2 with puberty, finally maturing into types 3 and 4 after the thirty-second week of pregnancy. This maturation, which happens with high levels of hormones, makes the tissue more resistant to mutations.

Elevated levels of estradiol can simulate an immune response. Estradiol also possesses neuroprotective and antiapoptotic properties as an *N*-methyl-D-aspartate (NMDA) antagonist. Exposure of glutamatergic neurons in cell culture to estradiol or estriol has revealed decreased rates of apoptosis and necrosis. Estriol was found to have more of a protective effect, even though estradiol is bound more tightly to the estrogen receptor.[181] Research has also found appropriate levels of estradiol to reduce reactive oxygen species,[182] increase coronary blood flow[183] and decrease bladder disorders by reducing neurogenic inflammation. However, an increase in breast cancer risk has been associated with elevated estradiol, as well as an increased growth of prostate cancer cells.[184]

The use of hormones in perimenopausal, premenopausal or menopausal women (often referred to as hormone replacement therapy, or HRT) involves treatment with either estrogen alone or estrogen administered in

TABLE 10.7 — FEMALE CYCLE SERUM REFERENCE VALUES

	Follicular	Luteal	Postmenopause
	pg/mL		pg/mL
Progesterone	< 1	5–25 ng/mL	< 1
Estrone (E1)	10–150	16–170 pg/mL	< 20
Estradiol (E2)	50–300	200–400 pg/mL	< 50
Estriol (E3)	5–50	10–60 pg/mL	< 30
Total Estrogen	70–400	70–700 pg/mL	< 60

Estrogen Pool:
Free Estrogens
& SHBG
Sulfated Estrogens
Estrogen Metabolites
Exogenous Estrogen
Reabsorbed Estrogens

Estrogen Actions:
Estrogen can have a stimulant or agonist effect depending on the receptor and/or the tissue. After estrogen engages the receptor it may be inactivated and excreted.

Estrogen Clearance:
Phase I Detox compounds must be rapidly detoxified via phase II pathways.

Phase II Detox products are water soluble, non-reacting, can be excreted in the urine or bile, and do not have the hormonal effects of their parent molecules.

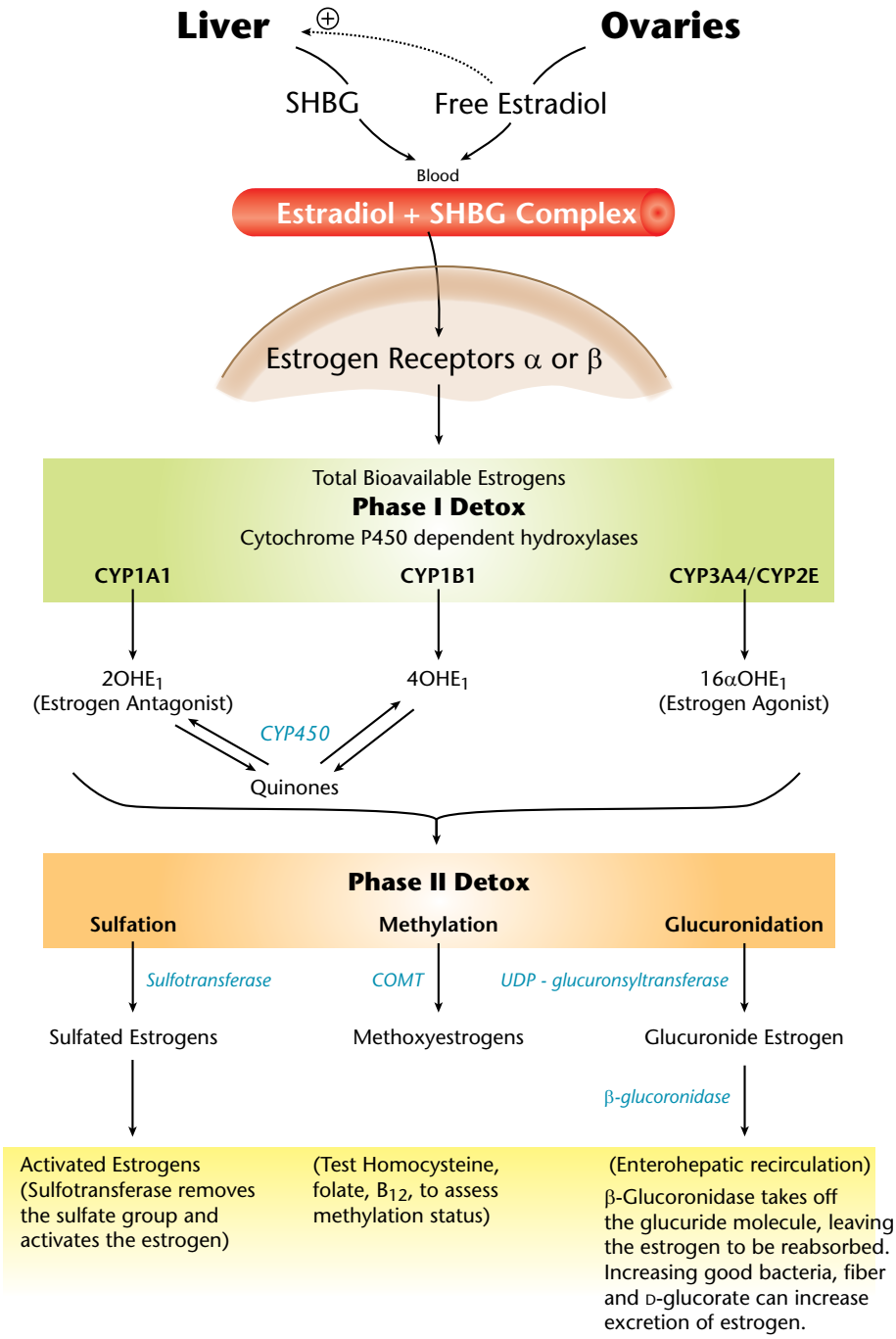

FIGURE 10.7 — Catabolism of Estradiol

The major fraction of estradiol is reoxidized to estrone. Separate cytochrome P450 enzymes carry out 2- and 16α-hydroxylation. A portion of the 2- and 4-hydroxy derivatives is converted to methoxy (MeOE) forms, depending on individual methyl donor and cofactor status.

combination with progesterone. Hormone replacement therapy has been used for decades to prevent menopausal symptoms such as irritability, "hot flashes" and increased bone loss, although clinical signs and symptoms alone are often insensitive parameters by which to judge a patient's estrogen level. Thus, testing of estrogen levels is recommended due to the potential adverse effects of either elevated or depressed levels. Synthetic hormones are not amenable to laboratory testing of "normal," and can only be assessed for therapeutic levels. Considerable concern should also be given to downstream estrogen metabolites that result as a consequence of supplementing with synthetic estrogen. Testing and adjustments can be made to non-synthetic supplemented, or natural, hormones. Testing of hormone levels must account for timing of menstrual cycle in premenopausal women (Figure 10.8). Estrogen and progesterone measurements allow the determination of peak values within broad ranges of cycle phases, and when 15 to 20 points are tested over a month interval, hormone output may be assessed by observing the degree of rise at ovulation. In order to evaluate the full physiological impact of estrogen hormones, a more complete profile that includes estrone and estriol may be done so that total estrogen can be calculated. Total estrogen reference values are shown in Table 10.7. Direct assay of total estrogens is also available.

The best evidence for the risks and benefits of menopausal hormone replacement therapy come from the Women's Health Initiative (WHI), an NIH randomized clinical trial of more than 16,000 healthy women. The trial showed that the overall risk of estrogen plus

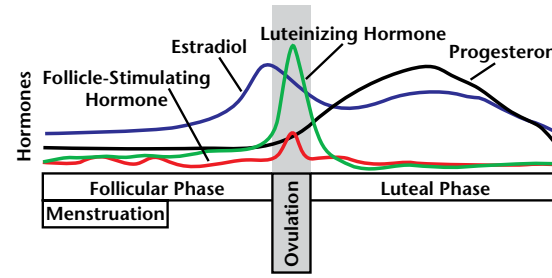

FIGURE 10.8 — **Typical Female Cycle Mapping**

Hormone assessments in menstruating women must take into account the large monthly fluctuation of circulating estradiol and progesterone. Full descriptions of the pituitary-ovarian feedback systems can be found in many sources.

progestin outweighs the benefits. Nevertheless, a risk versus benefit assessment should be made on a "per patient basis." For example, in a patient with a history of osteoporosis-related bone fractures and no personal or family history of atherosclerotic disease or breast cancer, one could make a case for the WHI study showing that the benefits of HRT outweigh the risks—in this patient. Among the risks observed after 5.6 years of follow-up were increased risks of breast cancer, heart disease, stroke and blood clots. Thus, the potential negative effects of estrogen have dampened the enthusiasm about hormone replacement. In fact, studies focused on problems with estrogen replacement have led to concerns about the risk of aberrant estrogen metabolism in premenopausal women.[185] The role played by estrogen metabolism in hormone-sensitive diseases has come from studies of estrogen-dependent neoplasms of reproductive organs. With respect to breast cell malignancies, estradiol is believed to act primarily as a promotional factor, causing increased growth rates in breast cells already transformed to a cancerous state. However, estradiol is not the only active estrogen in the human body. Other metabolites formed from estradiol have the capacity to act as estrogens, and in some cases, as antiestrogens.

The increased risk from estrogen and estrogen metabolites has led to a search for compounds that produce estrogen-like effects safely and decrease the production of 16α-hydroxyestrone ($16OHE_1$). Soy isoflavones (e.g., daidzein and genistein) are natural compounds that have come closest to meeting these criteria. They are increased by eating a diet rich in soy products.[186] Soy

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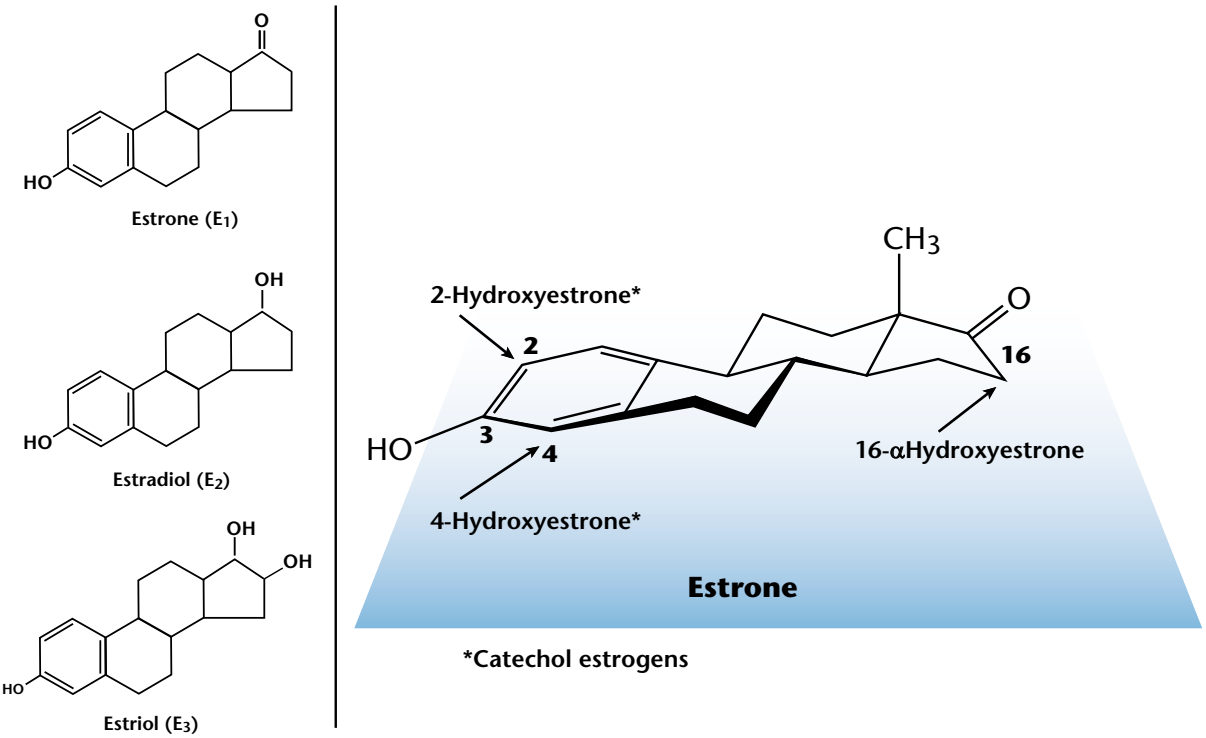


FIGURE 10.9 — **Hydroxy Derivatives of Estrone**

Both the standard structural drawing (left) and the 3D projection of estrone (right) are shown. The position of hydroxyl insertion to form the catechol estrogen metabolite is shown, and the reason for the α and β prefixes can be seen as "down" or "up " orientations at carbon 16.

products have many estrogen-like properties, though they operate through different receptor sites on target tissues.[187-189] Although much research has found soy isoflavones to have little or no effect on induction of tumors, recent studies have found that the simultaneous consumption of isoflavone supplements with Tamoxifen may increase tumor risk.[190] Dietary intake of soy products[191] and flax[192] have been shown to favorably modulate the rates of 2- versus 16-hydroxyestrone production. Though some studies have not found soy or soy extracts to lessen vasomotor symptoms of menopause, or slow postmenopausal bone loss.[193, 194]

Estrogen Clearance and the 2:16 Ratio

The major metabolites of estradiol and estrone are those hydroxylated at either the C-2 or the C-16 positions (Figure 10.9). Most, but not all, studies have found that women with a high urinary 2-hydroxyestrogen (2OHE) to 16α-hydroxyestrone (16OHE) ratio are at a reduced risk of breast cancer. The most consistent associations were observed with invasive cancer in premenopausal women.[195] A decreased risk of metastasis was also found with an improved ratio.[196] 2OHE is essentially devoid of peripheral biological activity, as shown in studies on uterine weight, gonadotrophin secretion and cell proliferation.[197] 2OHE has also been found to exert a modest antiestrogenic effect,[198, 199] and to have antiproliferative properties[200] and antiangiogenic effects, as well as to inhibit mitotic progression through disruption of spindle formation.[201] It has therefore been called "the good estrogen."[202] Not only does it modulate estrogen activity by blocking more stimulating forms of estrogen, it also interacts directly with the DNA, increasing expression of a gene that is involved in apoptosis. 2OHE has also been found to decrease lipid peroxidation of neuronal tissue, offering protection to the CNS.[203] 16OHE and estriol (E_3) are estrogen agonists[204] that have been found to initiate neoplastic change in mammary tissue.[205] Tumors in estrogen-sensitive tissues may be promoted by 16OHE. Women with breast and

endometrial cancers have been found to have marked elevation of 16OHE, which is a significant risk factor for such estrogen-dependent tumors.[206, 207] Research substantiates looking at the ratio of 2OHE and 16OHE, not only for a risk factor for breast cancer, but also for other conditions of inappropriate estrogen activity. 16OHE is more stimulating to tissue than 2OHE, contributing to mitotic and proliferative states of disease. Interestingly, it is thought that this same stimulatory activity helps to maintain bone density.[208] 16OHE has been found to be elevated in unregulated or overactive immune system conditions.[209] Estrogen metabolism should be considered with treatment of most autoimmune conditions. In a study of patients with rheumatoid arthritis and systemic lupus erythematosus, 16OHE was found to be 10 times higher in patients compared with controls. It was speculated that the elevated 16OHE may contribute to the maintenance of the proliferative state in these diseases.[210] Because there is an optimal level of both 2- and 16-hydroxyestrone, a ratio comparing the two levels can be very helpful.

The ratio of 2OHE to 16OHE (estrogen metabolite index, or EMI) should be greater than 2.0, and values in the upper-normal range are advisable (Table 10.8). Any woman using HRT who has a low EMI should be monitored closely for improvements in the urinary metabolites. In a large multicenter study of 10,786 women who were followed for 5.5 years, the results showed that the higher the 2:16 ratio, the lower the risk of breast cancer.[211] As the ratio of 2OHE to 16OHE decreases, the severity of recurrent respiratory papillomatosis also increases.[212] Testing for the urinary levels of 2OHE and 16OHE provides valuable insight regarding risk and can be nutritionally altered.[197, 199, 204]

Unlike certain risk factors for cancer, such as genetics, a 2:16 ratio is highly treatable. Lifestyle factors can influence the individual markers as well as the ratio. Increased consumption of polyphenol-containing foods and flaxseeds, and adequate intake of dietary calcium and protein, as well as decreased smoking, decreased caffeine intake, and exercise have all been shown to favorably impact both 2- and 16OHE.[213-217] Flaxseed supplementation at 10 g/d significantly increases the urinary 2-:16-hydroxyestrone ratio.[192, 218] Soy isoflavones and foods or extracts containing indole-3 carbinol (I3C) have also been found to improve the 2:16 ratio.[219, 220] Even though a large body of research exists demonstrating safety with I3C, more recent studies may begin to favor diindolylmethane (DIM), a derivative of I3C, because it is not dependent on acidification in the gut, and it does not increase 4OH of estrone (Figure 10.10).

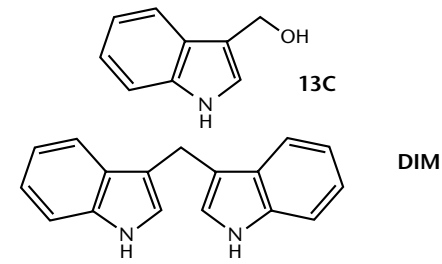


FIGURE 10.10 — 13C and DIM Structures

The glucosinolate form of I3C that occurs in foods is hydrolyzed in the stomach to I3C, which then undergoes dimerization to form DIM.

DIM and I3C are both used to help modulate estrogen metabolism. Estrogens are metabolized by cytochrome P450 enzymes that are inducible by compounds found in vegetables of the *Brassica* family, such as cabbage, brussels sprouts and broccoli.[221] Two phytochemicals contained in these foods, I3C and DIM, have been identified as active inducers of certain P450 isozymes.[222] The reaction catalyzed by these P450 isozymes produces 2-hydroxylation of estradiol. Induction of P450 by I3C results in decreased concentrations of several metabolites known to activate the estrogen receptor.[223]

TABLE 10.8 — EXPECTED VALUES FOR FIRST MORNING URINE ESTROGEN METABOLITES (NG/MG CREATININE)

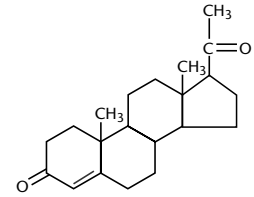
Analyte	Premenopause	Postmenopause	Postmenopause with HRT
16OHE[1]	3–30	2–8	5–25
2OHE[1]	3–40	2–10	10–75
Urinary Estrogen Metabolites (UEM) Index	4–110	5–18	6–158
Estrogen Metabolite Index (2OHE/16OHE)	> 2.0 (Healthy pre- and postmenopausal women)		

One of the acidification products I3C is speculated to be responsible for an increased production of 4OHE, which exerts a stimulatory effect on estrogen-sensitive tissue. If the P450 enzyme is not sufficiently active, a competing pathway causes 16-hydroxylation, leading to an increase in 16OHE. Other constituents in the cruciferous family are also speculated to aid in estrogen metabolism. Glutathione S-transferase has also been found to be up-regulated by the sulfur constituents in cruciferous vegetables. *Brassica* vegetables also improve glucuronidation, aiding with elimination of estrogen metabolites. Compounds that aid estrogen metabolism were found to decrease DNA damage, quantifiable by 8-hydroxy-2-deoxyguanasine, an oxidative marker discussed in Chapter 6, "Organic Acids." Women who have a low EMI may be treated with I3C and DIM supplements and sulfur-containing supplements, as well as with ground flaxseed and soy. Some in-vitro evidence suggests that I3C may increase 4-hydroxylation of estrone, a potentially pro-carcinogenic effect. However, when dosing is properly adjusted and monitored, the effect is largely a reduction in CyP1B1 that sharply inhibits 4-hydroxylation.[224, 225]

Altering the ratio with IC3 has shown promising therapeutic effect, not only for estrogen receptor positive cancers, but also for estrogen receptor negative cancers.[196] Research regarding the 2:16 ratio was also of clinical value for other cancers. In a group of 8 cervical cancer patients, 4 had complete remission, as their 2:16 ratio improved in a dose-dependent fashion. The greatest response was seen in the group taking 200 mg/d of I3C.[226] Because metabolites are being considered, the preferred method for measuring 2:16 ratio is in the urine. The 2:16 ratio does not fluctuate with timing of the menstrual cycle or with menopausal status. Results remain consistent regardless of changes in levels.[227] Expected values can be seen in Table 10.8.

Notes:

PROGESTERONE



Effects on Target Tissue Cells	Laboratory Evaluations
Endometrial transformation (stimulated epidermal growth factor)	Serum progesterone
Vaginal and cervical epithelial stimulation	
Decreased uterine contractility	
Neurosteroid: Synaptic agonist & myelinization	
Neuroprotectant: Apoptosis regulation	
Immunoregulatory	

Functions: Progesterone is known to affect reproduction, sleep quality, respiration, mood, appetite, learning, memory and sexual activity. It is primarily thought of as a reproductive hormone. It increases during the luteal phase of the menstrual cycle, and levels increase significantly with pregnancy. As a reproductive hormone it is progesterone's role to maintain the lining of the uterus. Progesterone increases up until ovulation, and if a fertilized egg implants on the uterine wall, it is the corpus lustrum's job to maintain the increasing levels of progesterone to keep the endometrial tissue intact. If there is no fertilized egg, progesterone levels will fall, causing the lining to shed and menstruation to begin. A symptom of low progesterone is dysfunctional uterine bleeding. When progesterone levels are adequate, there will be less uterine contractions, cramping and pain. Progesterone increases sensitivity of the uterus to estrogen by modifying estrogen receptors in the uterus. It also has a number of regulatory effects on estrogen, including down-regulating estrogen receptors, inhibiting estrogen transcription, increasing sulfurtransferases, and inducing apoptosis and cell differentiation. Progesterone receptors function similarly to estrogen receptors, via nuclear receptors that initiate DNA transcription.

Progesterone has a key role as an intermediate in the biosynthesis of androgens, estrogens and the corticoids. Progesterone is synthesized from pregnenolone, a derivative of cholesterol, and can be converted to cortisol, aldosterone, testosterone or estrogen, depending on the

tissue. FSH stimulates follicular maturation, leading to the production of progesterone. A small amount is also produced in the adrenal glands, testes and brain. After menopause, the adrenals are a primary production site.

Clinical Assessment: Abnormally high levels of progesterone will affect activity of other hormones in the body, altering an individual's biochemistry. Increased levels can increase sulfatase activity, leading to an increase in free estrogen. Excessive progesterone may overly antagonize estrogen, resulting in urinary incontinence. Excessive levels can also cause decreased coordination, slowed reflexes, and impaired memory and reasoning skills. Some research has shown excessive progesterone to cause migraines. Chronic fatigue patients should monitor progesterone levels closely, as higher levels were found in this population group, especially when depression was present.[228] Excessive levels of progesterone also increase the risk of diabetes.[229] It appears that progesterone administration is most effective short term, or in a pulsed fashion. Continuous dosing can result in paradoxical effects.[230]

Research studies have not always distinguished between progesterone and progestins, something that could have a significant impact on interpreting the results.[231] Progestins are synthetically produced progestogens and have been around since the 1950s. A micronized capsule version of natural progesterone (derived from yams) is also available.[232] Natural progesterone was found to cause negative mood effects similar to those induced by synthetic progestogens.[233] Bioidentical progesterone, when compared with synthetic progestins, has not been shown to have a negative effect on blood lipids or vasculature, and it has been shown to offer other benefits.[234] Some progesterone metabolites are extremely potent, and their effects should be considered when adding the hormone. Because too little or too much can be an issue, progesterone should be monitored and tailored. Progesterone and estrogen receptors are both found in the same areas of the brain and include the hypothalamus and limbic system. Thus, balancing progesterone and estrogen is essential. Males synthesize less progesterone than women, although it is still vital. In a study of 38 morbidly obese men, progesterone was negatively associated with markers of obesity such as BMI, waist circumference and subcutaneous adipocyte diameter.[235]

There are many natural treatments that will increase production or use of progesterone, such as smilax, damiana and vitex. Vitamin C has been shown to increase serum progesterone levels. This may be because of vitamin C's ability to improve the adrenal gland function of making precursors to reproductive hormones.[236]

Progesterone has anti-inflammatory properties. By inhibiting the enzyme in the prostaglandin cascade responsible for production of COX, matrix metalloproteinases and different cytokines, progesterone modulates immune function.[237] COX-2 plays an important role in mammary carcinogenesis and angiogenesis in human breast cancer. Studies have shown overexpression of COX-2 in breast cancer.[238] Research has compared COX-2 mRNA expression with hormone receptor status in breast cancer. COX-2 mRNA expression was associated with progesterone receptor positivity in human breast cancer.[239] COX-2 has been hypothesized to upregulate aromatase activity. Aromatase is a recognized promoter of hormone receptor-positive breast cancer. Progesterone's ability to alleviate inflammatory and lipid peroxidation responses has also been shown to improve the recovery and disability of patients with acute severe head injury.[240]

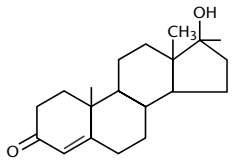
Progesterone is a neurosteroid that modulates neuronal excitability. Much research has been done with progesterone modulating neurological function. Progesterone has been found to reduce anxiety. It acts in the amygdala to modulate anxiety, fear and pain response.[241] Clinical trials have found progesterone to be a stronger anxiolytic than diazepam.[242] It has been proposed that it modulates GABA receptors.[243] Research has also shown progesterone to decrease seizures in women with epilepsy. It may help to regulate myelin synthesis in glial

Notes:

cells. Progesterone-activated pathways provide neural protection mediated through brain-derived neurotrophic factor.[244] Other neurologic mechanisms of progesterone include an increased production of dopamine. In a patient with low dopamine turnover, indicated by a low homovanillate, testing of progesterone levels may be indicated. Progesterone also decreases release of substance P and decreases neurogenic edema.[245]

Sleep-disordered breathing is more common in men and postmenopausal women. Progesterone has been associated with a decrease in sleep apnea episodes and can act as a potent respiratory stimulant to induce sleep.[246-248] Sleep-disordered breathing in premenopausal women is also affected by time of menstrual cycle, with fewer episodes associated with the luteal phase.[249]

TESTOSTERONE



Effects on Target Tissue Cells	Laboratory Evaluations
Promotes protein synthesis, increasing muscle and bone mass	Serum testosterone
Promotes male secondary sex characteristics (penile, scrotum and axillary and pubic hair growth)	
Increases sebaceous gland activity	
Cardioprotective via androgen receptor and (after peripheral conversion) estrogen receptor effects on many tissues	

Functions: Testosterone is an anabolic steroid that is synthesized from cholesterol. Cholesterol is metabolized into pregnenolone, which is converted to either androstenediol or androstenedione, both of which are then converted to testosterone. Testosterone is primarily produced by the action of LH on the Leydig cells of the testis, although small amounts of testosterone are secreted by the adrenal glands. Testosterone can then be converted to estradiol or dihydrotestosterone (DHT). 5-Alpha-reductase is the enzyme that drives the conversion to DHT, and aromatase is the enzyme that drives the conversion to estradiol (see Figure 10.5). Testosterone has many positive health associations. It has been shown to improve cognitive function[250] and lower inflammation,[251] and is associated with an increase in hematocrit and hemoglobin,[252] and a decreased risk of Alzheimer's.[253]

Clinical Assessment: Most, 97 to 98%, of plasma testosterone is bound to sex hormone-binding globulin (SHBG) or albumin. Albumin is easily dissociated and is bound to 33 to 54% of testosterone.[254] SHBG binds a greater proportion of testosterone, 44 to 65%, and binds much more strongly. It interacts with cell surface receptors and may initiate a cascade reaction, though it does not bind to the androgen receptor.[255] Only 2 to 3% of available plasma testosterone is free. Free testosterone and albumin-bound testosterone are available to bind to the androgen receptor, and is sometimes referred to as the bioavailable testosterone or BAT.[254, 256] Testosterone assessments should therefore identify the SHBG level as well as the total testosterone level.

Testosterone testing may be done in saliva, which reflects the free testosterone in serum. Total serum testosterone is also useful to determine whether there is normal response of the Leydig cells to FSH and LH. Simultaneous determination of total testosterone and SHBG allows the calculation of free testosterone concentration, sometimes called the free androgen index (FAI). Bone mineral density is positively correlated with FAI in elderly males.[257] Measurement of FAI in infertile women reveals an association of ovarian dysfunction with hyperandrogenism.[258]

When testing testosterone in women, one should be mindful of the fact that women have a midcycle peak in serum testosterone. Unbound testosterone increases in the follicular phase.[259] Additionally, time of day should be considered. Mean testosterone concentration has been noted to drop as much as 50% from morning to evening. Testing should be done at the same time of day to provide accurate baseline and follow-up information.[260] Serum testosterone levels were found to be stable throughout the morning and early afternoon for men in a large population study.[261]

Testosterone levels in men decrease with age, obesity and diabetes.[261, 262] Levels reach a peak at around 20 years of age and decline steadily thereafter. Andropause is a change in hormone levels experienced by men, similar to menopause in women.[263, 264] Symptoms include diminished libido, decreased feeling of general well-being, osteoporosis, decreased quality of life, anemia, depression, cognitive decline and sexual dysfunction, as well as

others.[265] The negative effect of testosterone deficiency on nitrogen balance and muscle development may be the major impact of testosterone on nutrient status. Myopathy has been reported in patients with low testosterone.[262, 266] Muscle loss may be reversed by treatment with testosterone and by assuring amino acid adequacy (see Chapter 4, "Amino Acids"). Testosterone hormone replacement therapy is becoming more common.[267] A small double-masked, placebo-controlled, randomized study found long-term low-dose testosterone supplementation (100 mg IM every 2 weeks) on healthy older men to increase testosterone, nocturnal GH secretions, morning concentrations of IGF-I and IGFBP-3, and lower SHBG.[268]

Although typically people feel concerns about giving higher levels of testosterone because of cancer risk, a recent study demonstrated that testosterone levels within normal ranges are not correlated with stimulation, development or growth of prostate cancer cells. The relationship of testosterone to prostate cancer showed a bell-shaped distribution. Both extremely low levels and extremely high levels were associated with the greatest risk of acquiring prostate cancer.[269] It appears that metabolites of testosterone such as DHT and estrogens may be correlated to prostate cancer risk more than testosterone itself. Because a prospective study of testosterone replacement therapy and prostate cancer risk has not been conducted, replacement therapy should be administered with caution.[270]

In response to low testosterone levels, the HPA axis becomes hyperresponsive, and cortisol levels will be abnormally high post-ACTH stimulatory tests.[271] Adrenal output of testosterone contributes to the overall pool, thus low levels of adrenals will affect adequate levels of testosterone. Hypothyroidism can impact testosterone levels as well. An underfunctioning thyroid results in a low metabolic rate and can lead to a lower testosterone level. Nutrients such as boron and zinc, and an adequate supply of arginine and branched-chain amino acids (see Chapter 4, "Amino Acids") are associated with adequate testosterone levels.[272, 273]

Recent studies have demonstrated that hypogonadism in men may be more prevalent than previously thought, and is strongly associated with metabolic syndrome and may be a risk factor for type-2 diabetes and cardiovascular disease. Thus, testosterone may be an independent predictor of insulin resistance and diabetes.[274] Studies have shown low testosterone can increase the risk of acquiring diabetes threefold.[251] Even subtle derangements in testosterone could contribute to metabolic syndrome and its pathogenesis.[251] Testosterone increases levels of adiponectin, which is inversely correlated with obesity and insulin resistance.[275] Clinical studies have shown that testosterone replacement therapy in hypogonadal men improved metabolic syndrome indicators and cardiovascular risk factors.[276] A systematic review and meta-analysis of 30 trials found testosterone use in men with low testosterone levels did not lead to significant changes in blood pressure, serum lipids or glucose.[277]

Other Levels of Cell Controls

The endocrine system encompasses those tissues specialized to form products that act on distant tissues through the presence of specific receptors, such as hormones. Many other levels of cell regulations occur through products produced within the cell or by nearby cells. These regulators include cytokines, biochemical modulators, allosteric feedback regulation, phosphorylation/dephosphorylation, translational controls, nitric oxide, eicosanoids and reactive oxygen species. Cytokines will be reviewed as an example.

Cytokine Signaling Pathways

Cytokines are protein or glycoprotein cell products that act like hormones in their ability to elicit cellular responses. Cytokines are produced by a wide variety of cell types and can alter cell function locally or globally.[278–280] Understanding signaling pathways leads to a broadened awareness of the full range of cellular control mechanisms. Their importance in explaining cell controls can be shown in many areas. Research is now looking at the synergistic effects of hormones and cytokines. One recent study proposed that the interaction of the hypothalamic-pituitary-adrenal (HPA) axis and proinflammatory cytokines determines the level of sleep/arousal within the 24-hour cycle in obese adults. The study found that when cortisol and cytokines were both elevated, there was a positive association with low sleep efficiency and fatigue.[281] Types of cytokine receptors and examples of each are shown in Table 10.9. Insulin is found alongside erythrocyte growth factor and platelet-derived growth factor because they all elicit intracellular action by binding to a protein tyrosine kinase receptor.

TABLE 10.9 — TYPES OF CYTOKINE RECEPTORS

Receptor	Examples of Activators
Protein tyrosine kinase	Insulin, erythrocyte growth factor, platelet-derived growth factor
Protein serine kinase	MIS, TGF-β, activin
Receptors without kinase activity	The cytokine receptor superfamily
G-protein coupled receptors	Neurotransmitters, prostaglandins, interleukins, interferons
Ion-gated receptors	Neurotransmitters, amino acids

TABLE 10.10 — PROTEINS IN THE AKT SIGNALING PATHWAY

Abbreviation	Name or Description
PDGF	Platelet-derived growth factor
PI3K	Phosphatidylinositol 3-kinase
PIP2	L-α-Phosphatidylinositol-4,5-bisphosphate
PDK	3-Phosphoinositide dependent protein kinase-1
AKT	Murine thymoma viral oncogene (protein kinase B)
BAD	Bcl-associated death promoter
CASP9	Caspase 9, apoptosis-related cysteine protease
FKHRL1	Forkhead family of transcription factors

The AKT Signaling Pathway for Apoptosis

Growth hormone inhibition of apoptosis is another example of cytokine effects. Binding of growth factors to their protein tyrosine kinase receptors initiates a sequential pathway of phosphorylation reactions as illustrated in Figure 10.11. Full names for the abbreviations used in the figure are given in Table 10.10. The transmission of signals by cytokines is largely by means of phosphorylation and dephosphorylation reactions involving phosphatidylinositol in cell membranes and serine and tyrosine residues of cytosolic proteins. The key sequence in this process is called the AKT signaling pathway because phosphorylation of AKT initiates multiple additional conversions that directly govern the action of cell survival or apoptosis. The serine/threonine protein kinase Akt (or Protein Kinase B) is the cellular homologue of the viral oncogene v-*Akt* and is activated by various growth and survival factors. The AKT pathway has been a focus of investigations of numerous forms of cancer.[282-284] The antifungal antibiotic Wortmannin is an inhibitor of phosphatidyl-inositol 3-kinase that also inhibits the growth of mammary tumors.[285] In addition to inhibition of apoptosis, activation of the AKT pathway signals multiple other metabolic outcomes as summarized in Table 10.11.

The study of cytokines and signaling pathways is revealing details of molecular events within cells that determine activities of biosynthesis, response to external stimuli and cell survival. Tests for cytokine levels are already being offered by some laboratories for investigative clinical use. Cytokines are highly transient, however, so their measurement is largely of academic interest currently. At the current rate of expansion in knowledge

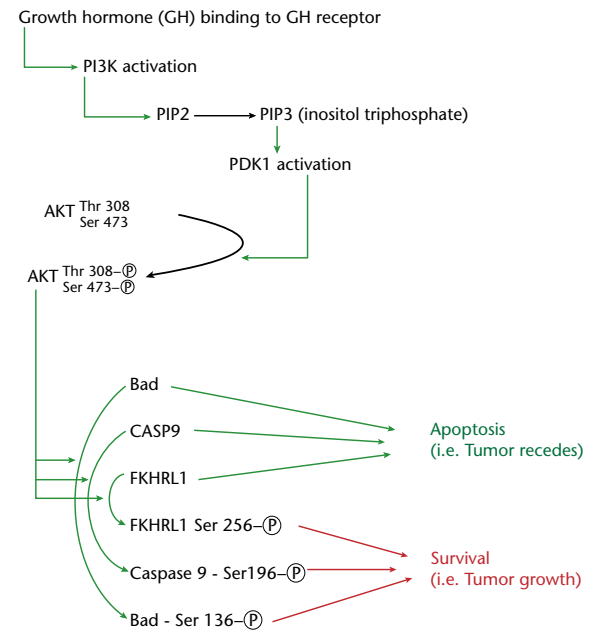


FIGURE 10.11 — The AKT Signaling Pathway for Apoptosis

The overall function of this signaling pathway is to mediate cell survival and growth. Binding of growth hormone to cell surface receptors induces the production of second messengers that activate PI3K. PI3K generates PIP3 in the cell membrane that activates PDK, a kinase that phosphorylates Thr308 of membrane-bound protein kinase B (Akt). Akt inhibits apoptosis by phosphorylating Bad. Phosphorylated Bad causes dissociation of a nuclear complex, allowing cell survival. Akt phosphorylation of FKHRL1 and caspase prevents apoptosis. Akt also ultimately leads to NF-κB activation and cell survival. In the absence of growth hormone, the Akt -mediated steps subside, allowing turnover of cells and regression of tumors.

Table 10.11 — Consequences of AKT Activation

Stimulation	Inhibition
Polymorphonuclear macrophage respiratory burst for pathogen destruction	Glycogen synthesis
GABAergic neuron synaptic signaling	Androgen receptor-mediated apoptosis
Endothelial nitric oxide synthase for cardiovascular tone	Ribosomal protein synthesis

of health and disease, measurement of cytokines in body fluids may soon find routine clinical relevance as a tool for guiding therapies, including nutrient interventions. Numerous references have been made in previous chapters to nutrient-dependent cell regulation events such as nitric oxide formation, neurotransmitter synthesis and eicosanoid formation from polyunsaturated fatty acids. Such instances of cell regulation act in concert with hormone output of endocrine glands to modulate tissue responses via cytokine pathways. Considering nutrient-cytokine and nutrient-hormone interactions should be integrated into the overall process of guiding patients from disease to health.

Specimen Choices

Although there is much confusion over how and when to test hormones, the necessity of this practice continues to impress itself on the medical community. With the advent of recent large-scale hormone trials showing an increased risk of breast cancer with hormone replacement, it has never been more apparent that hormone levels need to be tested systematically before therapy is started and while therapy is being taken to make sure safe levels are maintained. There are three primary samples that are used to test hormones: serum, saliva and urine. All three are viable methods of testing. Understanding the differences between specimens will help to direct which is most useful to a particular clinical question.

Hormone levels as measured in the serum are considered the "gold standard" of hormone testing. However, measurement of serum hormone levels is not without its limitations. Serum changes more slowly, so the effects of treatment are not as quickly observable. Without the intervention of exogenous hormones, serum, salivary and urine levels are generally highly correlated. Some studies have shown correlation post-treatment,[286, 287] others have not. There are several explanations for the inability of one specimen type to reflect what is happening globally, and on a tissue level; these include the routes of delivery and transport of hormones, as well as the fact that hormones do not equally diffuse to all areas of the body. Hormone delivery may significantly affect outcomes. Oral routes of delivery are more susceptible to a first-pass response. Many practitioners prefer a transdermal application of hormones because it avoids hepatic metabolism and poor oral bioavailability, and minimizes the hypnotic effects of progesterone.[288] When hormones are delivered vaginally, they are less effective in women who have low estrogenization of tissue.[289] Although some studies using transdermal preparations questioned absorption, others found appropriate increases in serum levels after topical delivery.[290] An example of unequal hormone diffusion was found in a study in which progesterone was delivered via vaginal suppositories, and the levels of progesterone were found to be negligible in the serum, although a tissue biopsy of the uterus showed an increase in level after treatment. There are likely two routes of delivery, lymphatic and RBC, not mirrored by serum, which may explain this difference. Salivary levels are typically 2 to 5% of serum steroids.

Sometimes the argument is made that hormone concentrations measured in saliva more accurately reflect bioavailable levels because only the free hormone passes from plasma into saliva. However, some caution about accepting the concept is warranted. For one thing, only about 50% of sex hormone in saliva is unbound, a fact that the method of measurement should take into account.[291] Also, although progesterone changes in saliva may mirror changes in plasma, the ratio between the two is altered during the luteal phase, which was not correlated to changes of unbound hormone in the serum.[292] Free unbound progesterone was reported to be 53.7% during the proliferative phase, and 41.4% in the secretory phase.[291] Saliva is unreliable if used with troches unless 24 to 26 hours have elapsed between last dose and collection of saliva. Other hormones appear

to be reliably tested for in the saliva. The presence of mixed-function oxygenases in saliva presents another concern because their activity may alter measured hormone concentrations; likewise, bacteria in saliva may affect the levels. Conjugated steroids, that is, steroids that have gone through sulfation or glycosylation, flow into the saliva at a significantly slower rate, resulting in a lower concentration of conjugated steroids.[293]

Rapid changes are seen in saliva, whereas serum is more stable. Urinary levels offer another method of testing of hormones. Both active forms and metabolites may be measured in urine. For example, estrogen conjugates, estrone glucuronide correlates, and pregnanediol-3-glucuronide are major metabolites. Urine levels correlate with serum samples with a lag time of about 12 to 24 hours.[294] The best correlation for estrogens is seen when concentrations are normalized to creatinine.[294] An advantage of urinary levels is that this is the only specimen by which one can observe a hypersecretion of estrogen.

Hormone Replacement Therapy

When hormones such as progesterone are given orally, serum levels may overestimate true levels. This is because serum levels may also measure hormone metabolites, especially glucuronides.[295] Salivary hormone testing has been considered to be superior to serum testing in that salivary levels represent a truer measure of the tissue delivery potential, uncomplicated by the partitioning between blood transport components.[296] When progesterone is administered to the skin as a topical cream, subsequent serum progesterone levels may reflect a lower concentration than is present within the tissue. This is because the lymphatic delivery and RBC delivery routes of progesterone are not fully detected in a serum sample. It has been demonstrated that delivery of hormones from lymph can occur. Hormones will leave the ovary via the subovarian lymphatic vascular network as well as through venous blood. If hormones are delivered through this mechanism, the levels measured in the blood would be incomplete.[286] Whole blood may avoid the largest variable, as RBC hormone would be considered.

It has been proposed that RBC transports hormones and delivers them rapidly to capillary beds and tissues. It is argued that saliva is more reflective of tissue levels because it is a type of tissue bed. In vitro evidence has shown that hormones are transported rapidly across the red cell membrane and that the hormones are extracted during the capillary transit time, making free plasma concentration very low. Hormones bind with varying affinities to the red blood cell. The sequence of binding affinity from low to high is estriol, testosterone, estrone, estradiol and, finally, progesterone. Seventy to 85% of progesterone exposed to RBCs is taken up by them.[297] The lipophilic steroid sex hormones may bind to the RBC membrane, and they can traverse the RBC cell membrane in less than 2 milliseconds.[298] These molecular dynamics suggest that the timing of specimen collection for exogenously delivered hormones must be taken into consideration. In a study that compared saliva, serum and urine samples after the application of transdermal progesterone cream, progesterone was not found in the RBC, but it was measured only once, 24 hours after the last application. Because of the high rate of redistribution, even RBC levels measured within an hour, may still be too much of a delay to see the elevation.[299] The hypothesis is that most transdermal progesterone is taken up by RBCs in contrast to in vitro dialysis studies that have shown progesterone to distribute almost equally between a phosphate buffer dialysate and red cell membranes.[300] Oral progesterone does not have the same pattern in saliva. In fact, oral progesterone may be underrepresented by salivary levels, whereas transdermal is extenuated by salivary levels.

Hormones were intended to be a short-term therapy to provide symptomatic relief during a women's transition through menopause. They have grown in use and are now often prescribed long term. Justification for this long-term use is explained by a potential protective effect against cardiovascular disease, osteoporosis and Alzheimer's. Research corroborating the benefit of hormones in these cases is conflicting. Rigorous monitoring of hormones is required to use them in a manner that is consistent with the pursuit of optimizing health.

Notes:

Bioidentical Hormones

Standard menopausal hormone therapy (also referred to as hormone replacement therapy, HRT) uses substances that do not match those in the human body. HRT comes primarily from conjugated equine estrogens. The Women's Health Initiative and the Women's Health Initiative Memory Study, large epidemiological studies that focused on the effects of HRT, found significant negative effects for those women using HRT and have lead to the significant decline in equine hormone use. Bioidentical hormones, which have been used in Europe for years, are designed to match the structure and function of hormones produced in the body.[234] Bioidentical estrogens are structurally identical to estrone, estradiol and estriol.[301] When any hormones are put into a system, thought should be given to the body's ability to eliminate and metabolize them. If metabolism is not adequate in both the gut and liver, toxicity and improper balance will ensue, undermining the goal of health. The lowest level of a hormone that can be used to lower symptoms should be used, and care must be given to make sure the hormone is utilized properly once inside the system with frequent testing of hormone levels, as well as hormone metabolites.

Hormone Biotransformation (Detoxification)

Hormone metabolites are conjugated by the liver either through glucuronidation, sulfation or methylation. Liver detoxification is discussed in more detail in the Chapter 8, "Toxins and Detoxification." Hormone levels may be improperly regulated if detoxification pathways are functioning inadequately. Insufficient amino acid status can alter conjugation. For example, methionine is required for sulfate production to clear circulating thyroid hormone and to sustain turnover of S-adenosyl-methionine for hydroxyestrogen methylation.[302] Other factors also affect the enzyme UDP-glucuronyl transferase that drives glucuronidation. Factors that up-regulate UDP-glucuronyl transferase are thyroid hormone, increased blood sugar, fish oils, limonene-containing foods, dill weed, caraway and vitamin C. The enzyme is inhibited by aspirin and probenecid. Sulfation is activated by molybdenum. An underactive sulfation is associated with carcinogenic estrogen metabolites. Catecholestrogens are further degraded to quinines that can act on the DNA. This DNA damage could be increased under conditions of an impaired sulfation pathway.[303] Liver function must be considered as part of hormone therapy.

The final piece of elimination occurs in the gut. After conjugation, hormones are water soluble and are prepared to be excreted. If bowel function is impaired, hormones can sit in the gut longer, and be reabsorbed. This results in an increased level of circulating metabolites that may impact health. Also in a state of dysbiosis, the enzyme β-glucoronidase may be affected. This relationship is demonstrated by the production of daidzein from soy and other herbs that are created by human intestinal bacteria. This metabolite from beneficial gut flora and soy potently inhibits β-glucuronidase.[304] β-Glucuronidase will cleave hormones from their conjugates, resulting in increased reabsorption. Evidence of the relationship of gut flora to hormones is seen with research that associates antibiotic use with breast cancer.[305, 306] The disruption on the intestinal microflora by antibiotic use changes the way estrogen is eliminated. It also changes the way phytochemicals such as phytoestrogens and polyphenols are metabolized. Thus, a patient's gut flora status may need to be considered when evaluating overall hormone health. Dysbiosis profiles as discussed in the Chapter 7, "Gastrointestinal Function," and Chapter 6, "Organic Acids."

The interrelationships within the body's endocrine system are complex and multifaceted. The most appropriate manner by which to test for specific hormone levels continues to be an arena of ongoing research. Furthermore, knowing when to test for specific hormone levels and how to best test for these levels represents both the art and the science of practicing medicine.

Notes:

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Chapter 11

Genomics

Richard S. Lord and J. Alexander Bralley

CONTENTS

Notes:

With the sequencing of the human genome, there has been an explosion of interest in identifying the genetic components of disease processes. The subsequent ability to identify individuals with these genetic tendencies affords medical science a potential new tool to predict, prevent and intervene in many different illnesses, especially those that are chronic in nature. This chapter will give a brief overview of this emerging field and how routine genomic laboratory assessments may influence integrative, functional medicine now, and in the future.

MOLECULAR BIOLOGY 101

All living organisms utilize a system of information storage and transmission contained in the genetic material. In mammals these are organized into genes contained in the larger structure of the chromosomes. It is hard to comprehend the implications of the human genome, which revealed its amazing complexity with the initial sequencing in 2002. A thought-provoking analogy is presented in Matt Ridley's *Genome: The Autobiography of a Species in 23 Chapters*.[1]

Imagine the genome is a book.

There are twenty-three chapters,
called ***Chromosomes***.

Each chapter contains several thousand stories,
called ***Genes***.

Each story is made up of paragraphs,
called ***Exons***,
which are interrupted by advertisements,
called ***Introns***.

Each paragraph is made up of words,
called ***Codons***.

Each word is written in letters called ***Bases***.

There are 1 billion words in the book…which
makes it longer than 800 Bibles.

All genes are composed of the same compound, deoxyribonucleic acid, commonly known as DNA. All DNA is composed of a purine base (adenine and guanine) and a pyrimidine base (cytosine and thymine). The bases combine with the sugar, deoxyribose and phosphate to form the four nucleotides (A, G, C and T), which make up the double helix strand of DNA. Ribonucleic acid (RNA) is very similar in its components, except uracil is substituted for thymine and the sugar component is ribose.

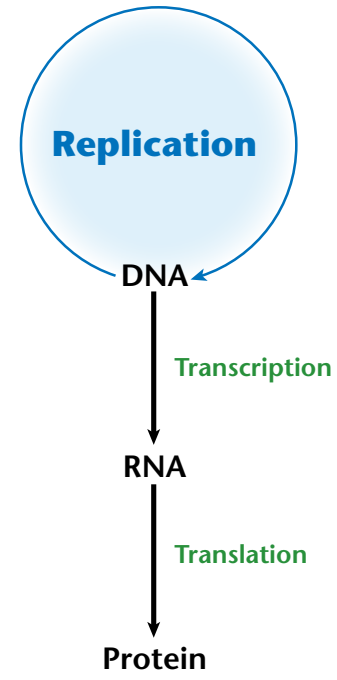


FIGURE 11.1 — The "Central Dogma" of Molecular Biology

DNA is duplicated during cell division. DNA information is transcribed to RNA. RNA then translates this to protein synthesis.

There are an estimated 20,000 to 30,000 genes contained in the human genome. Less than half of them have a known function. There are more than 3 billion nucleotides in this genome. The average gene is about 3,000 nucleotides long, although the size can vary substantially. Interestingly enough, only about 3% of the structure of the human genome is used to regulate body function through what is called the "Central Dogma" of molecular biology (Figure 11.1).

The central dogma states that the chromosomal DNA is replicated for cellular reproduction. In the newly formed cell, the DNA strand containing a gene is unraveled and transcribed into an RNA molecule, which migrates out of the nucleus into the cytoplasm. There it

attaches to a ribosomal structure that translates the three nucleotide codes into the primary sequence of amino acids of a protein. Protein functions may be classified as internal or external. Internal proteins act on substances generated by the body internally. These are the enzymes and structural proteins. Proteins that act on substances arising from outside the body are external, the primary group being the immunoglobulins that confer resistance to microbial penetration into the system. Any errors in transcribed amino acid sequences can lead to malformed proteins and inefficient or, at worst, ineffective catalytic or structural function of the protein molecule. Gene-level alterations that produce variation in protein structures are the basis of genetic disorders or inborn errors of metabolism.

Over 99% of the human genome is identical in all humans. It is the other 1% that represents the broad variations in human traits, abilities and risk of disease. The genetic structure of an individual is referred to as the genotype of the individual. The expression of this genotype is referred to as the phenotype of the individual. Variations in the genotype give rise to variations in the phenotype.

GENETIC VARIABILITY

The most common form of genetic variation is the single nucleotide polymorphism or SNP (pronounced "snip"), in which a single base is substituted for another in a DNA sequence. There are approximately 1.42 million known SNPs in the human genome and about 60,000 of these fall within exon regions.[2] A SNP may or may not have clinical significance. Some SNPs alter function in various ways. For example, if contained in the protein coding sequence of a gene, a SNP may change an amino acid in the protein sequence, altering conformation in a coenzyme-active site or in the substrate binding site. The altered structure may enhance cofactor binding or weaken it. A SNP may also alter a protein's stability, making it last longer, thereby increasing activity, or just the opposite, allowing more rapid breakdown with consequent decreased function. If a SNP resides in part of the regulatory aspect of a gene, it may affect gene expression, thereby altering overall function.

An allele is a genetic locus that confers inherited traits. Each person inherits two alleles for each gene, one allele from each parent. These alleles may be the same or may be different from one another. A SNP can reside on either or both alleles. An allele with no SNP at a particular location on the DNA sequence of the gene is referred to as "wild type," or the most common form of the gene.

If one allele has a SNP and the other doesn't, the individual is heterozygous for that gene, meaning the SNP was derived from one parent. A heterozygous gene product may or may not produce properties in the individual that may deviate from the wild type. For example, if the gene codes for a protein molecule performing an enzymatic function, this may be altered by the SNP in one of the two alleles. One allele will code for the "normal" wild-type form; the other with the SNP will code for a slightly altered protein whose function may be significantly less efficient. How these two alleles are expressed will determine the level of function the individual has. In general, heterozygotic individuals tend to maintain good function, although this is a topic of some debate. This concept can give rise to the wide range of function seen in the population regarding genetic nutritional need.

REFER TO CASE ILLUSTRATIONS 11.1–11.2

When both alleles contain the same SNP, this is referred to as a homozygous condition. In Case Illustration 11.1, the SNP variation is fully expressed since both alleles are affected (Figure 11.2).

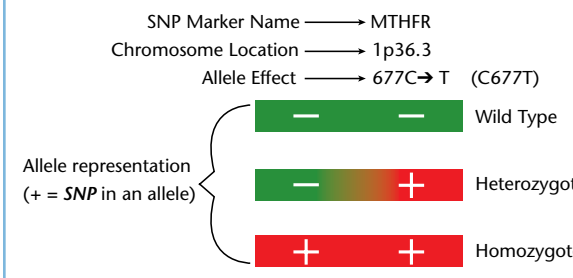


FIGURE 11.2 — SNP Representation

The allele effect denotes the nuceotide position, 677 in the gene sequence in this case. Cytidine (C) has been replaced by thymidine (T), cytidine being the wild-type alelle.

The Era of Personalized Medicine

Given the ability of modern science to easily detect and evaluate the effects of these SNPs on human health, an era of what is being referred to as "personalized medicine" is evolving. Two areas where this is happening rapidly are pharmacogenomics and nutrigenomics.

Pharmacogenomics

The conventional approach to pharmacotherapy is for physicians to prescribe drugs for an individual based on population studies and clinical trials. If a particular drug was not effective or had undue side effects, the dosage may be adjusted or another drug used. Finding an effective drug and dosage may, therefore, take several months. Physicians must rely heavily on their clinical experience and population-based studies. Therapeutic drug monitoring in blood has been used for years to guide clinicians in cases where maintaining safe and effective blood levels for the patient were critical and achievable. On the other hand, if clinicians could predict which drug would be effective and at what dose, then pharmacotherapy could be individualized. This is the goal of pharmacogenomics.

Pharmacogenomics testing attempts to predict how an individual will respond to a drug based on their genetic makeup. The two main areas to which pharmacogenomics can be applied are drug metabolism and responsiveness. The primary way in which drugs are metabolized is through the cytochrome P450 (CYP) isoenzyme system found mostly in the liver. Most drugs are metabolized by at least one CYP; many are metabolized by multiple CYPs. Specific dosing guidelines based on pharmacogenetic data are being developed and are beginning to appear in the literature to predict adverse drug reactions (ADR).[3] Genotype-based dosage guidelines have been published for a number of drugs.[4–7]

Current genetic variants of CYPs can be identified through several commercially available assays as well as through clinical laboratory-developed tests. The CYP variant can affect the metabolic phenotype through changes in protein expression, structure, functional stability and/or substrate specificity. The CYP activity is expressed as four major categories: extensive or normal metabolizer (EM), the ultrafast metabolizer (UM), the intermediate metabolizer (IM) and the poor or slow metabolizer (PM). These designations have been extensively studied for several CYPs, and clinically significant variance can be seen among ethnic groups.[8, 9] Figure 11.3 shows the proportion of drugs metabolized by different CYPs.

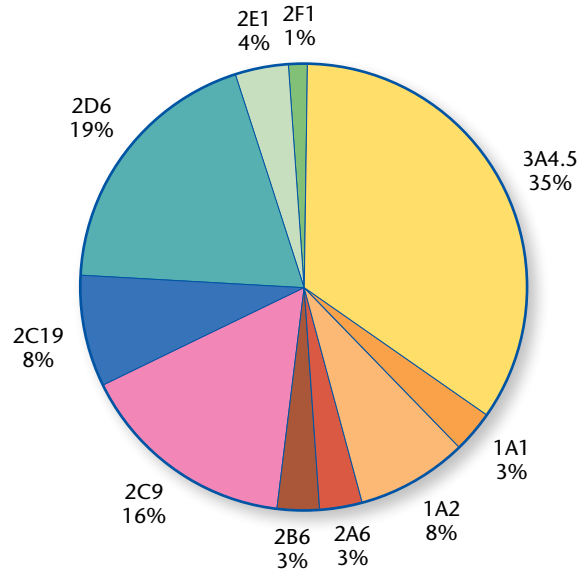


Figure 11.3 — Proportion of Drugs Metabolized by Common CYPs

The primary way in which drugs are metabolized is through the cytochrome P450 (CYP) isoenzyme system found mostly in the liver. Most drugs are metabolized by at least one CYP; many are metabolized by multiple CYPs.

The other application of pharmacogenomic testing is in the area of drug responsiveness. Population-based clinical trials for drug efficacy suffer from the same variability of drug response as previously discussed. A recent example is the drug Verceptin, developed by Genentech. In initial clinical trials on a broad population base, the effectiveness of the drug did not reach FDA standards for efficacy and was not approved for human use. However, when patients were evaluated for genetic makeup, a different picture emerged. In women who were epidermal growth factor receptor 2 (HER2)-positive, Verceptin produced significantly better effects on reducing metastatic breast cancer than in women who were HER2-negative. Genentech teamed with Dako Corporation to develop a test for HER2 positivity. Both the drug and the test received FDA approval for this application in 1998. By targeting the population sensitive to the drug, patients with this form of cancer receive a positive

therapeutic outcome, whereas general population-based analysis would have eliminated this choice to those who would benefit. This illustrates the potential promise of pharmacogenomics in drug development.

This aspect of genomic testing for pharmacotherapeutics, coupled with improved and extended therapeutic drug monitoring (TDM) techniques and biomarker assessment, can develop into a powerful strategy for personalized medicine. These approaches coupled with other integrative medicine techniques will have lasting influence on the style of medicine practice in the future.

NUTRIGENOMICS

Another aspect of personalized medicine, nutrigenomics, has been defined as the study of the interface between genes and the nutritional environment. Nutrigenomics is being proposed as a new model in healthcare, where genotyping can help determine which foods and food supplements are best consumed by an individual to optimize their health and prevent or mitigate metabolic diseases. Researchers in this field seek to define how genetic variation affects a person's response to various dietary nutrients.[10-12]

Kaput lays out five principal tenets of nutrigenomics:

> (1) improper diets in some individuals and under some conditions are risk factors for chronic diseases; (2) common dietary chemicals alter gene expression and/or genome structure; (3) the influence of diet on health depends upon an individual's genetic makeup; (4) some genes or their normal common variants are regulated by diet, which may play a role in chronic diseases; and (5) dietary interventions based upon knowledge of nutritional requirements, nutritional status, and genotype can be used to develop individualized nutrition plans that optimize health and prevent or mitigate chronic diseases.[13]

The interaction of environment and gene expression is illustrated in Figure 11.4. Genotype testing is not meant to determine who is inclined to get cancer or heart disease; rather, it is designed to predict who will benefit most from specific diet and nutrient changes. Most of the SNP testing currently done for nutrigenomics

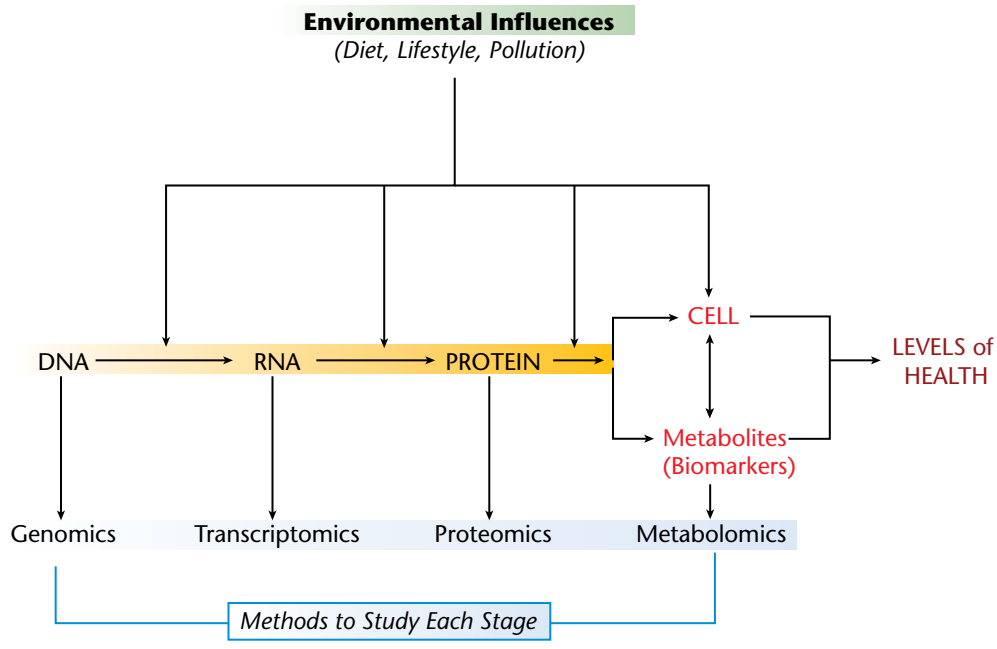


FIGURE 11.4 — Gene Expression Process

This figure illustrates how the environment can influence this sequence at different levels.

has focused on patients who are at risk of health problems associated with a particular nutrient. A relatively well-known example is folate metabolism related to the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR). This enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cofactor in remethylation of homocysteine to form methionine. Homozygous SNPs in the gene coding for this enzyme have been associated with increased homcysteine and cardiovascular disease risk and increased risk of neural tube defect births in pregnant women.[14, 15] Researchers have found the C677T homozygous SNP in the MTHFR gene is quite prevalent, occurring in 18% of Caucasians and 10% of Hispanics.[16] This homozygous form produces a protein enzyme that is more heat labile than the wild type, thereby causing reduced enzymatic activity. Nutrigenomic testing can identify those individuals who could benefit by increased folate supplementation.

Further Considerations

Although nutrigenomics holds great promise, some experts have expressed caution regarding the present scientific support for conclusions drawn from the research. The genome is exceedingly complex, and with over one million SNPs already identified, there is still not a complete understanding of the interactions that can occur to affect health. Recently an advisory action was issued by the FTC and the Government Accountability Office stating that direct-to-the-consumer (at-home) nutrigenomic testing lacked scientific evidence to fully justify conclusions and recommendations based on the test results.[17]

A principal limitation with nutrigenomic testing is the question of the phenotypic expression of genetic variations. Even if a person has, for example, an MTHFR homozygous C677T SNP, one does not know for certain to what extent overall function is affected. Is there a functional limitation that justifies suggesting that the person take folate and how much? Is it enough to compensate for the loss of enzymatic function? Are there mitigating factors that obviate the need for additional folate? To answer these questions, functional metabolic testing would still need to be done, that is, in this case, measurement of plasma homocysteine levels. This is true for many of the genomic tests that suggest functional defects, be they detoxification issues or increased risk of osteoporosis. In each case, measure of the functional metabolite is necessary to determine effective intervention procedures for the patient. In a sense, it almost begs the question of whether genomic testing is really cost-effective for patients or should they just proceed with function analysis of nutrient demands. The genetic issue may be expressed, but additional testing is still required. On the other hand, if a functional impairment is found, then the genetic testing can shed light on the need for further assessment in family lines.

Refer to Case Illustration 11.3

Conclusions

This brief synopsis of genomic testing offers a sampling of the enormous breadth of information the human genome project has generated. Some experts estimate that the majority of new medical tests in the future will revolve around genetic testing. From identifying infectious agents to assessing which drug and what dosage or which nutrient might be needed in excess, genomics will play a key role. For further reading see TIGR,[18] Science Magazine[19] and Washington University School of Medicine SNP Research Facility.[20]

Notes:

Case Illustrations

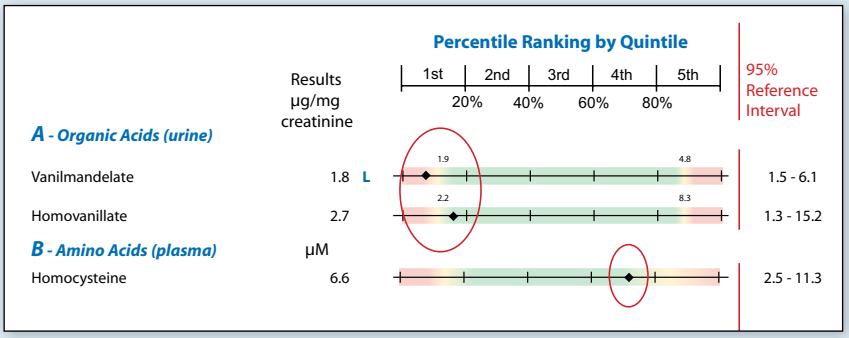
Case Illustrations in this chapter were contributed by Kara N. Fitzgerald, ND

Case Illustration 11.1 — Combined COMT and MTHFR Homozygous Effects

History: A 31-year-old female presented with long-term panic disorder with a recent onset of agoraphobia. Her family history is significant, with a mother with anxiety disorder and agoraphobia, one sister with a long-term panic disorder and alcoholism and another sister with anxiety disorder.

Genetic Testing: Genetic laboratory testing revealed that the patient is homozygous for a single nucleotide polymorphism (SNP) in the catecholamine-O-methyl transferase (COMT) enzyme, resulting in significantly reduced enzymatic activity (low activity). Her mother is heterozygous for the same COMT SNP. Further, the patient is homozygous for a SNP in the 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme, which also results in reduced enzymatic capacity. The patient's sisters have not undergone genetic testing.

COMT and MTHFR are both involved in methylation activity. COMT is an enzyme responsible for the clearance of catechol compounds, including norepinephrine, epinephrine, dopamine and some estrogen derivatives. COMT catalyzes an O-methyltransferase reaction that requires the methyl donor S-adenosylmethionine (SAMe) as a cofactor. MTHFR is responsible for the final step in the methylation of folic acid, generating 5-methyltetrahydrofolate (5-methyl-FH4). 5-methyl-FH4 is involved in the methylation cycle, where it donates its methyl group to B_{12} in the recycling of homocysteine to methionine. Trimethylglycine (betaine) is also able to donate a methyl group to homocysteine to make methionine (see Figure 2.11). One of the main metabolic fates of methionine is its conversion to SAMe. After SAMe donates its methyl group, it is converted to homocysteine and is again recyled back to methionine via folate/B_{12} or betaine. SAMe production can be induced by supplying exogenous folic acid, B_{12} or betaine. See Chapter 2, "Vitamins," for further discussion of folic acid and vitamin B_{12} in single-carbon metabolism.



Functional Testing: Section A of the patient's laboratory data shows evidence of impaired metabolic function due to the polymorphisms. Impaired COMT activity accounts for the low clearance of vanilmandelic acid (VMA), the metabolite of norepinephrine and epinephrine, and low-normal clearance of homovanillate (HVA), the metabolite of dopamine. Since COMT is involved in the catabolism of these catecholamines, it is not surprising that low turnover is evidenced in this patient with a low-activity homozygous SNP in the enzyme. It may be that the reduced catabolism of catecholamines, particularly norepinephrine and epinephrine, is contributing to this individual's clinical panic disorder. Low-activity COMT SNP has been associated with increased incidence of anxiety and panic disorder, particularly in women.[21,22]

On the other hand, evidence for lack of functional impact of the MTHFR polymorphism is found by the normal homocysteine shown in section B of her laboratory data. She has no history of hyperhomocysteinemia, so her MTHFR appears to be functioning at a level adequate to meet metabolic demands, despite the presence of the SNP. The conversion of homocysteine to methionine via methyl transfer from SAMe is functioning adequately. Thus, her ability to sustain adequate SAMe for methylation reactions appears normal. This is an important distinction in managing this patient.

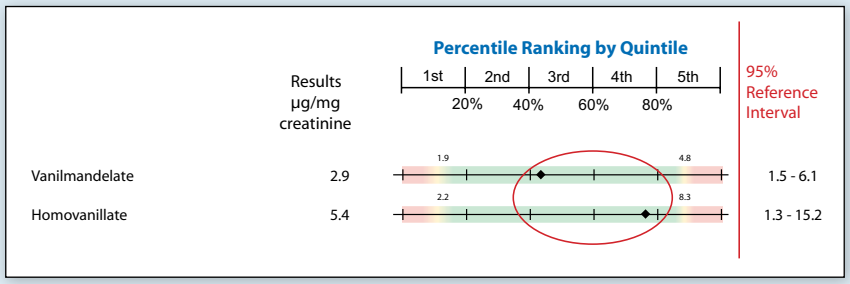
Discussion: Supplementation with high-dose methylated folic acid and betaine (trimethylglycine) was initiated based on the SNP findings prior to metabolic function evaluation. Soon after starting this regimen, the patient reported an increase in presenting complaints and the onset of agoraphobia. The symptom exacerbation could be caused by increased production of SAMe from the combined

effects of added methylated folic acid and betaine. SAMe concentrations in the brain govern the rate of epinephrine biosynthesis from norepinephrine via a non-COMT methyltransferase enzyme. Increased SAMe availability could result in increased epinephrine production, whereas the COMT SNP decreased the ability to clear the catecholamine, resulting in symptom deterioration. Upon replacement of methylated with unmethylated folic acid and discontinuation of betaine, symptoms greatly improved, including a resolution of the agoraphobia.

This case illustrates the need to determine the metabolic impact of SNPs before designing interventions. Many factors other than the presence of a SNP can alter the functional outcome in each affected patient. ❖

Case Illustration 11.2 — COMT Homozygous Effects

A 50-year-old female presented with long-term anxiety and insomnia. Genetic studies revealed a heterozygous SNP that is specific for low-activity COMT. However, her urinary levels of COMT-dependent catecholamine metabolites homovanillate and vanilmandelate are normal, indicating adequacy of COMT activity. Thus, the laboratory results demonstrating normal metabolic function suggest that the COMP SNP is unlikely to be contributing to this individual's chief complaints via elevated catecholamines. ❖



Notes:

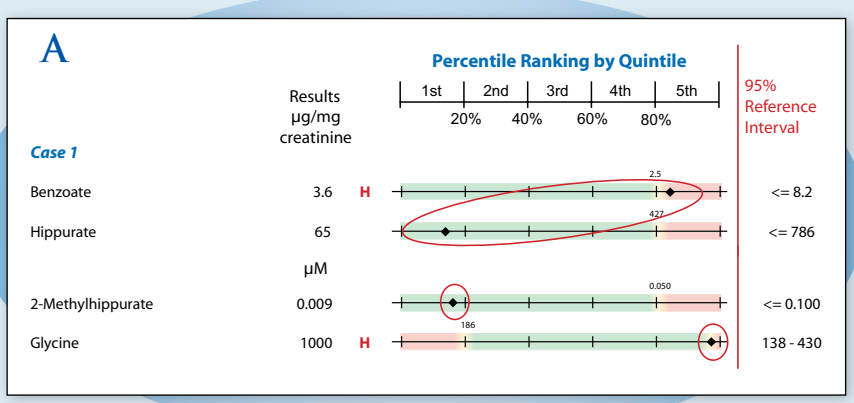
Case Illustration 11.3 — Evidence for Glycine Conjugase Polymorphism from Metabolic Data

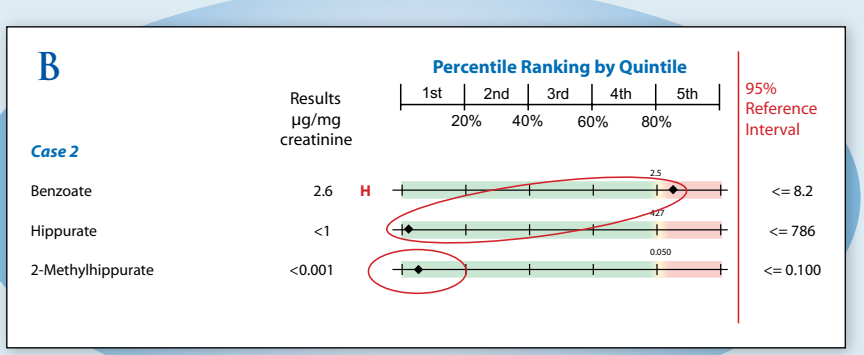
Laboratory data from urinary organic acid profiles of 2 patients are presented as sections **A** and **B** in the accompanying figure. The first patient (**A**) is a 58-year-old female who presented with migraine headaches and severe pruritic dermatitis. A primary migraine trigger for this individual is gasoline odor. The patient achieves minimal relief of pruritus with topical steroids. Her urinary benzoate is in the 5th quintile, with concurrent 1st quintile hippurate, the glycine conjugate of benzoate. Another glycine conjugate, 2-methylhippurate is also found in the 1st quintile, and plasma glycine is greatly elevated.

The second patient (**B**) is a 65-year-old female who presented with severe dermatitis. Similar, though more dramatic failure to convert benzoate to hippurate is shown by finding benzoate in the 5th quintile with concurrent undetectable hippurate. The data indicate very poor glycine conjugation activity. At the same time, an unusual absence of detectable 2-methylhippurate is found.

Discussion: Glycine conjugation (GC) is a Phase II liver biotransformation pathway, conjugating glycine with carboxylic acids. Conjugation with glycine increases solubility of the aromatic acids so that they may be eliminated. Coenzyme A derived from pantothenic acid is a necessary cofactor for the reaction.

In industrialized society, xylene exposure is difficult to avoid. A component of petroleum, xylene, is found in gasoline, automobile exhaust, solvents and cigarette smoke. In the liver, the Phase I oxidation of xylene produces 2-methylbenzoate and is conjugated via glycine peptide conjugase (GPC) to form 2-methylhippurate. Benzoate





exposure is also widespread, as it is a commonly used food preservative, a component of some fruits and a product of GI bacterial metabolism. Human detoxification pathways conjugate benzoate and 2-methylbenzoate with glycine via GPC to form hippurate and 2-methylhippurate.

Healthy individuals have a large capacity for glycine conjugation, and, under heavy loading, the reaction can cause depletion of glycine as discussed in Chapters 6, "Organic Acids," and 8, "Toxicants and Detoxification." Many patients have moderate benzoate loading. If glycine conjugation activity is normal, then they show elevated urinary hippurate with normal benzoate. Heavy loading of the conjugation pathway produces elevation of benzoate and hippurate. In both of the cases presented here, however, benzoate is high, whereas hippurate is 1st quintile (**A**) or undetectable (**B**). These findings indicate compromised conjugation capacity. This is especially evident in **A**, where high plasma glycine is found. Thus the conjugation substrate is plentiful, and the problem lies in the GPC enzyme. Since xylene exposure is virtually ubiquitous for urban residents such as these 2 patients, the low or undetectable 2-methylhippurate levels add to the suspicion of GPC deficiency. In case **A**, gasoline, a source of xylene, is associated with migraine activity.

Optimizing liver Phase II biotransformation activity, particularly alternative amino acid conjugation pathways, is a therapeutic goal for both individuals. Since glycine is proscribed due to risk of generating hyperoxaluria from greatly elevated glycine, supplement considerations include pantothenic acid, taurine and glutathione. Taurine spares glycine utilization, making it more available for conjugation reactions.

Case 1 follow-up: The patient's dermatitis resolved following a protocol including supplementation with high-dose B_5 and taurine. Her migraine headaches have been greatly reduced. ❖

Notes:

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Notes:

Chapter 12

Pattern Analysis

Richard S. Lord

CONTENTS

Comprehensive Nutritional Evaluation

Since essential nutrients are indispensable for the function of all cells and tissues, the symptoms and signs resulting from deficiencies of various nutrients exhibit a high degree of overlap. For example, conditions such as malaise, depression, or diarrhea can be affected by deficiencies of thiamin, methionine, or zinc. Thus, it is generally difficult to know which nutrient groups to evaluate based on initial clinical observations.

The ideal evaluation would provide information on the status of all 40 essential and conditionally essential nutrients, plus factors that modulate nutrient needs. Interventions could then be designed to support all areas of insufficiency necessary for optimizing energy metabolism, detoxification, redox status and other tissue functions. To approximate this ideal, a testing profile should provide sufficient data to allow reasonable assurance that areas of clinical focus can be identified. Numerous laboratory reports containing the measured parameters must be reviewed and evaluated. The evaluation of dozens of interacting parameters can be daunting and time consuming. Most clinicians surmount these obstacles by stepwise integration of single test results or profiles and use of the expertise of colleagues and laboratory experts to guide their acquisition of the expertise needed for making decisions in each case. Multi-test profiles that are widely performed are summarized in Table 12.1.

Stepwise integration should begin with the single profile that yields the most comprehensive and useful insight into critical nutrient needs. The enzyme stimulation assays discussed in Chapter 2 provide information about a single vitamin, while profiles of amino acid or organic acids yield data for multiple nutrients. Based on patient history and clinical assessment, one of the multiple analyte profiles is typically selected first. Other tests may be added to expand the range of nutrients initially evaluated or as follow-up testing to confirm or elaborate abnormalities found in an initial routine evaluation. A common approach is to order the profile of organic acids in urine as a sensitive, broad-range test that can

Table 12.1 — Commonly Performed Multi-Test Profiles

Multi-Test Profile	Specimen	Typical Number of Analytes
Fat–soluble vitamins	Serum	5–6
Nutrient and toxic elements	RBC & whole blood	8–10
	Urine	12–15
	Hair	12–15
Amino acids	Plasma	10–40
	Urine	10–40
	Blood spot	8–12
Fatty acids	Plasma	20–40
	RBC	20–40
	Blood spot	6–8
Organic acids	Urine	30–40
GI Function—Celiac markers	Urine	3–4
GI Function—Food allergy (IgE)	Plasma	10–100
GI Function—Food sensitivity (IgG)	Plasma	10–100
GI Function—Microbial populations	Stool	70–100
	Urine	10–15
Detoxification	Urine / Saliva	5–8
Hormones	Serum	3–6
	Urine	10–15
Oxidant Stress	Urine	2–3

help guide specific clinical interventions. The evaluation may then be expanded with specific antioxidant marker analysis or fatty acid profiling. Elemental or amino acid profiling can enhance confidence of having identified focal issues that become reference points for monitoring the success of interventions.

Another rational approach is to deal with issues that may have overriding impact on nutrient supplementation before focusing on other specific nutrient deficiencies. For patients with primary presentations of depression, anxiety or chronic fatigue, a plasma amino acid profile serves to guide amino acid interventions that directly influence neurotransmitter turnover. System function approaches frequently focus on GI function, hormonal and neurotransmitter functions or immune competence. Digestive and microbial stool testing, food-specific serum IgG levels, and other measures of digestive function can guide diet-specific alterations and enhance digestive tolerance. Specific nutrient deficiencies that may be present can be made worse by food restrictions. For example, food sensitivity testing frequently leads to elimination of several classes of high protein foods. The low intake of high protein foods can exacerbate amino acid deficiencies. Without adequate amino acids, restoration of gut immune barrier integrity can be difficult. This type of circular dependency is best dealt with by simultaneous evaluations of food sensitivities, amino acid status and degree of intestinal microbial overgrowth. Thus, the integration of data from multiple profiles provides a much more complete picture of areas of most critical need.

As the complexity of laboratory data increases, interpreting results to arrive at a clinical solution becomes more difficult. However, experts in nutritional science, clinical therapies and the implications of abnormal laboratory results have developed algorithms to assist in the interpretation. The most common example of such expert reliance is the computation of relative risk from cholesterol data. More recent and detailed algorithms may be used to make recommendations of individually customized essential amino acids or full vitamin-mineral formulas. Such computer-generated recommendations are always subject to the attending physician's final revision based on the patient's medical history and physical exam. Nutrient recommendations based on integration of multiple profiles are derived from knowledge of how the patterns of various direct measurements and functional markers determine the focal nutrient needs for each patient.

Notes:

Multiple Markers

The clinical relevance of laboratory data includes pathognomonic abnormalities (factors that define the presence of a disease) and risk factors that suggest the likelihood for future disease. For example, the finding of elevated fasting glucose defines the presence of diabetes, while the finding of elevated total cholesterol indicates increased risk of heart disease. Both of these tests are performed routinely because they have demonstrated value in improving patient outcomes. Their usefulness, however, is greatly enhanced when other related parameters are measured. Examples of tests that help to define the nature of some primary evaluations are shown in Table 12.2.

Assessments of nutritional and toxicant status likewise are strengthened by reinforcing tests. Multiple web-like interactions between the effects of nutrients, toxicants and metabolic regulators generate the total picture of how focal issues impact patient outcomes. Thus, the impact of dietary iron deficiency is made worse by simultaneous hypochlorhydria, low vitamin C intake and toxic element exposures. Simultaneous determination of markers that reveal status of the related factors allows better prediction of responses to iron supplementation. In the previous chapters many references to related factors have been made in the discussions of individual nutrients, toxicants and cell regulators. Because of the number of factors and their interactions, the combinations that generate individual patient patterns are, for all practical purposes, endless. The general approach to multiple profile integration will be introduced in this chapter by considering related parameters in a specific example case.

Table 12.2 — Test Combinations to Guide Patient Management

Clinical Challenge	Primary evaluation	Reinforcing tests
Diabetes	Blood glucose	Glucose tolerance
		Insulin
		C-peptide
		Glycoproteins (HbAlc)
Heart disease risk	Cholesterol	Triglycerides
		LDL & HDL cholesterol
		Small, dense LDL
		Testosterone, insulin
		Lipid peroxides, Vitamin E
Peripheral neuropathy	Low serum vitamin B_{12}	Methylmalonate
		Homocysteine
		Complete blood count
Slow wound healing	Low serum zinc	RBC trace element profile
		Fatty acid profile (LA/DGLA)
Rapid aging	Elevated lipid peroxide	Serum vitamin E
		Serum Coenzyme Q_{10}
		RBC mineral profile

A Case Study —

Multiple Markers Within a Single Profile

Case History

Ms. B was described as a very bright workaholic who presented with a sense of brain fog, which had dramatically increased in recent weeks. She also reported greater difficulty with mood swings around her menstruation with severe depression, anxiety, and suicidal thoughts. She had chronic fatigue for two or three years. An overnight urine sample was sent for quantitative organic acid profiling. The results reveal corroborating evidence of underlying metabolic difficulty. The results are divided into 4 sets of analytes for discussion (Tables 12.3 to 12.6).

Energy Pathway Intermediates

In the first set, two features relate to nutrient requirements (Table 12.3). Elevated citrate and isocitrate are markers for arginine insufficiency (urea cycle inadequacy for ammonia clearance) and elevated malate and pyruvate are found in deficiencies of CoQ_{10} (Chapter 6, "Organic Acids"). This data suggests that arginine and CoQ_{10} supplementation can be helpful. Deficiency of the B-complex vitamins can also lead to elevated citrate, but there are usually multiple citric acid cycle intermediates elevated in such cases. In addition, there are other markers for the B-vitamins discussed below.

B-Vitamin Markers

The compounds shown in Table 12.4 are elevated in specific nutrient deficiencies as discussed in Chapter 6, "Organic Acids." Thiamin is the key nutrient required to clear the α-keto-acids. Methylmalonate is a specific marker for vitamin B_{12} inadequacy and hydroxymethylglutarate is the compound from which coenzyme Q_{10} is synthesized in the liver. Finding levels of hydroxymethylglutarate that are so low they can not be detected corroborates our conclusion from the first set regarding a relative coenzyme Q_{10} deficit and further suggests that mitochondrial inefficiency may be contributing to the brain fog reported by the patient.

Detoxification Markers

Abnormal values for three other compounds indicate that Ms. B is having difficulty with her elimination of toxic compounds (Table 12.5). Elevated orotate is a sensitive marker for arginine deficiency. Orotate is the

TABLE 12.3 — ENERGY PATHWAY MARKERS

Test	µg/mg creatinine		Limits
Citrate	2756	H	500–2300
Cis-aconitate	226		5–250
Isocitrate	988	H	50–800
α-Ketoglutarate	22.8		3–25
Succinate	24.5		5–35
Fumarate	0.82		0.2–1.2
Malate	7.8	H	≤ 6.0
Lactate	22		4 – 30
Pyruvate	0.97	H	≤ 0.7

TABLE 12.4 — VITAMIN MARKERS

Test	µg/mg creatinine		Limits
α-Ketoisovalerate	2.38	H	≤ 2.0
α-Keto-β-methylvalerate	0.85		≤ 1.2
Methylmalonate	4.1	H	≤ 3.0
β-Hydroxyisovalerate	9.2		≤ 20
Hydroxymethylglutarate	< 0.1	L	0.2 – 1.0

TABLE 12.5 — DETOXIFICATION MARKERS

Test	µg/mg creatinine		Limits
Orotate	224	H	≤ 180
Pyroglutamate	98.5	H	≤ 80
Sulfate	50.9	L	≤ 180

TABLE 12.6 — INTESTINAL DYSBIOSIS MARKERS

Test	µg/mg creatinine		Limits
p-Hydroxyphenylacetate	20		≤ 50
Tricarballylate	5.1	H	≤ 1.8
Dihydroxyphenylpropionate	0.05		≤ 0.9

product of an alternate pathway for clearing ammonia when the urea cycle is inefficient, and chronic subclinical ammonia exposure is a possibility. The decision to supplement with arginine because of high citrate is greatly strengthened. In addition, the clearance of orotate is a magnesium-dependent process, so there is evidence of need for this commonly deficient mineral.

A separate issue is raised by the rest of the pattern found in the third set. Glutathione and inorganic sulfate are critical for hepatic detoxification. Sulfate is the excretory form of sulfur from most sulfur-containing compounds in the body. Since glutathione makes up a major fraction of total body sulfur due to the presence of cysteine in its structure, it follows that depletion of glutathione lowers urinary sulfate. Pyroglutamate elevation signals loss of glutathione in the amino acid recovery pathway in the kidney (see Chapter 6, "Organic Acids"), which contributes to depletion of the total body glutathione pool. In this case, the sulfate is very low and the pyroglutamate is elevated. These two markers together strongly indicate glutathione depletion and gives extra cause for concern over the issue of detoxification capacity that may lead to endotoxemia. Since we now have evidence of impaired detoxification capacity, the question of toxin loads becomes more important. Indications of one significant source of toxins, intestinal microbial overgrowth, in this case are found in the bacterial overgrowth marker organic acids shown in Table 12.6.

INTESTINAL DYSBIOSIS MARKERS

The three compounds shown in shown in Table 12.6 are some of the metabolic markers of intestinal dysbiosis, all of which were within normal ranges in this case, except for tricarballylate. Growth of the bacteria that produce tricarballylate is favored by consuming diets high in carbohydrate, especially following treatments with wide-spectrum antibiotics. The compound does not seem to be highly toxic, but it avidly binds to magnesium, preventing absorption of the mineral. Animals with tricarballylic aciduria develop magnesium deficiency. Although the condition has been little investigated in humans, the finding of such greatly abnormal concentrations in this case adds to the suspicion of magnesium deficiency. Thus, we find additive signs pointing to magnesium inadequacy with high tricarballylate and the orotate accumulation noted above. If other microbial metabolites had been elevated in Ms. B's urine, concern about toxic loading from intestinal dysbiosis would have been greatly increased.

Although less than half of the markers from the organic acid profile have been reviewed at this point, the significant strengthening of conclusions by inspecting for patterns of related markers should be clear. One rule of thumb regarding efficient reading of complex marker profiles is to resist making final decisions about intervention focus until the entire set of analytes has been studied. Abnormalities found on the final page can easily shift the focus regarding the focal area most likely to be the current critical point needing aggressive support. Some patterns of organic acids and amino acids frequently found in specific conditions are shown in Tables 12.7 and 12.8. Most patients with complex degenerative disorders show various degrees of severity in multiple categories.

Table 12.7 — Organic Acid Analyte Clusters and Related Abnormalities on Other Test Profiles

Organic Acid	Health Threat				Other Related Tests	
	Mitochondrial Dysfunction	B-Complex Deficiency	Detoxification Challenge	Neuroendocrine Imbalance	Test*	Expected Result
Adipate, Suberate, Ethylmalonate	↑				FAP	Palmitic ↑ Stearic ↑
Citric Acid Cycle Intermediates	↑		↑		AAP bEle	EAA ↓, Gln ↓ Multiple ↓
HMG	↑				Fat-Sol	CoQ_{10} ↓
Pyruvate		↑			bEle	Mg ↓
α-Ketoglutarate		↑				
BCKA		↑			AAP	BCAA ↓
Methylmalonate		↑			FAP	Odd-chain ↑
β-Hydroxyisovalerate		↑			bEle	Cr ↓, V ↓
Xanthurenate		↑				
HVA, VMA				↓ or ↑	AAP	Tyr ↓, Phe ↓
5-HIA				↓ or ↑ (SSRI)	AAP	Trp ↓
Quinolinate, Kynurenate		↑		↑	bEle	Mg ↓
Pyroglutamate			↑		AAP	Met ↓, Cys ↓, Tau ↓
α-Hydroxybutyrate			↑		AAP	Met ↓, Tau, ↓
Sulfate			↓ or ↑		AAP	Met ↓, Tau ↓
Dysbiosis markers			↑		sMicro AAP IgG	Predominant ↓ Opportunists ↑ BCAA ↓ Many ↑
Phenyl compounds (p-hydroxyphenylacetate)			↑		AAP	Gly ↓, Ser ↓

** AAP = amino acids (plasma); FAP = fatty acids (plasma); bEle = elements in blood; Fat-Sol = vitamins A, E, β-Carotene, & CoQ_{10} (serum); sMicro = Microbial profile of stool*

Notes:

TABLE 12.8 — AMINO ACID ANALYTE CLUSTERS AND RELATED ABNORMALITIES ON OTHER TEST PROFILES

	Health Threat			Other Related Tests	
Amino Acid	**Dietary Deficiency/ Maldigestion**	**Chronic Inflammatory Disorder**	**Behavior Disorder**	**Test***	**Expected Result**
Arginine	—	↓		OAU	Citrate, Orotate ↑
BCAA	↓			AAP	Glutamine ↑
Phenylalanine	↓		↓	OAU	HVA, VMA ↓
Tryptophan	↓		↓	OAU	5HIA ↓
Methionine	↓			Detox	Hepatic capacities ↓
Threonine, Glycine, Serine	↓			OAU	Benzoate high ↑

** OAU = organic acids (urine); AAP = amino acids (plasma); Detox = detoxification capacity tests*

Advanced Study of the Case —

THE POWER OF SIMULTANEOUS MULTIPLE PROFILES

We can proceed to investigate the metabolic basis of Ms. B's brain fog, fatigue, and suicidal tendencies because the profile of organic acids in urine was not the only one that was performed. The other profiles will be discussed as an illustration of how the results can shed light on her metabolic status and allow us to determine the most critical areas of nutrient demand in this phase of Ms. B's life. The profiles do not include hormone, food allergy, or other immune system evaluations. Additional digestive system and detoxification assessments could also provide valuable insight. Simultaneous evaluation of multiple parameters can identify focal issues where aggressive correction frequently yields restoration of health. This way of approaching patient evaluations stems from an awareness that formation of hormones and other metabolic controls requires the chain of nutrients that serve as co-catalysts, antioxidants, and detoxification substrates. While the primary purpose of the profiles included in this case is evaluation of nutrient status, the nature of the analysis, especially in the case of organic acids in urine, affords insight into nutrient-related detoxification and digestive factors as well.

SERUM VITAMINS AND ANTIOXIDANT STATUS

The array of markers for functional vitamin adequacy in the organic acid profile covers most vitamins except the fat-soluble group. A separate profile of fat-soluble antioxidant vitamins extracted from serum was performed on Ms. B (Table 12.9).

Here is evidence from an independent assay that supports the previous assessment of functional CoQ_{10} inadequacy by showing that the circulating levels are below normal. In addition to its electron transport

Notes:

role, CoQ_{10} performs a general membrane antioxidant function in concert with the other antioxidants in this profile. We see that β-carotene is undetectable and, although vitamins E and A are not below their respective limits, the levels are just over the stated lower limits. The pattern of inadequate antioxidant protection found here leads us to suspect that the rate of oxidant damage to tissue components is too high. The suspicion is confirmed by finding elevated levels of lipid peroxides in serum (Table 12.10). With these results, we have a point of reference for the current rate of oxidative damage against which future testing may be compared. Any interventions that improve antioxidant capacity should cause a lowering of the serum lipid peroxide level. Lipid peroxide levels are found elevated in some cases even though the fat-soluble antioxidant vitamins are all normal. In such cases, the next most likely area of insufficiency is that of trace elements.

Elemental Status

Elemental status assessment was performed by measuring concentrations in erythrocytes (Table 12.11). Functional deficiency of magnesium was suggested by the first set of organic acids discussed above, and there is an additional functional deficiency of zinc revealed below under fatty acids (Table 12.12) . An independent assay of circulating levels of these elements in erythrocytes (shown above) gives significant weight to conclusions about deficiencies indicated by metabolic blocks. The important findings of low circulating levels of essential nutrients help to identify nutrient deficiency. Erythrocyte magnesium of 25 ppm is enough to explain the molecular basis of symptoms of mood swings and depression in this patient. Look closely here to see how many trace elements are near the low limit. We find that, in addition to low levels of selenium and zinc, chromium, molybdenum, and vanadium are within 10% of their low limits, indicating a potential problem with trace element absorption and assimilation. Low stomach acid output is the most frequently occurring factor that leads to general trace element malabsorption.

Fatty Acid Status

Pertinent features of the fatty acid profile are shown in Table 12.12. The pattern displays the frequently found deficiency of alpha linolenic acid for which flax seed oil is recommended. Note the relationship of the two omega 6 fatty acids, linoleic (C18:2) and gamma

Table 12.9 — The Serum Vitamin Profile

Antioxidants	µg/L		Limits
Coenzyme Q_{10}	0.53	L	0.8 – 1.5
Vitamin E	13.1		12 – 40
Vitamin A	0.59		0.5 – 1.2
β-Carotene	< 0.2	L	0.4 – 3.5

Table 12.10 — Serum Lipid Peroxide Test Results

Serum Lipid Peroxides	µmol/ml		Limits
Lipid peroxides	1.8	H	≤ 0.9

Table 12.11 — The Erythrocyte Element Profile

Element	ppm packed cells		Limits
Chromium	0.72		0.7–2.7
Copper	0.88		0.5–2.0
Magnesium	25	L	40–70
Manganese	0.09L		0.1–0.4
Molybdenum	0.03		0.03–0.16
Potassium	479		200–1000
Selenium	0.31	L	0.4–2.0
Vanadium	2.06		2.0–7.0
Zinc	7.9	L	10–40

Table 12.12 — Erythrocyte Fatty Acid Profile Data

Fatty Acids in Erythrocytes	µM		Limits
Alpha linolenic	0.6	L	1–3
Eicosapentaenoic	6.4		3.5–8
Docosapentaenoic	14		10–30
Docosahexaenoic	28		15–35
Linoleic	42		40–70
Gamma linolenic	0.8	L	1–3
Eicosadienoic	10.4		7.5–25
Dihomo-gamma-linolenic	3.1	L	5–9

linolenic acid (C18:3). The former is converted to the latter by the action of the zinc-dependent enzyme, delta-6-desaturase (see Chapter 5, "Fatty Acids"). Failure of adequate conversion is frequently due to zinc deficiency and results in such a pattern of normal linoleic with low gamma linolenic. The pattern is strengthened by simultaneously finding low dihomogamma linoleic acid (DGLA). This is the pattern mentioned in the "Elemental Status" section above. Long chain PUFAs need to be supplied as fish and black currant oils while correcting the zinc deficiency in such cases. The adequate EPA and DHA levels found in the current case, however, mean that a balance of flax and fish oils is the best choice to restore fatty acid balance.

Since we know that aggressive supplementation of polyunsaturated fatty acids tends to increase the rate of lipid peroxide formation, it is important to remember that this patient already has elevated lipid peroxide results. That means that levels of β-carotene, CoQ_{10} and vitamin E should be raised before the addition of flax and fish oils. In most patients, a two week course of aggressive fat-soluble vitamins will produce significant improvements in membrane antioxidant status. Then the supplemental oils are unlikely to cause further perturbation of the oxidative damage products. These cases call for more short term follow up to assure that the lipid peroxide levels do not rise excessively as the fatty acid status improves.

Amino Acid Status

The amino acid profile for Ms. B shows all amino acids within normal limits except for slightly low values for leucine and lysine (Table 12.13). Note especially the arginine level. Here we find a value within normal limits, while multiple metabolic markers indicated a functional deficit of the nutrient. In addition, arginine is not considered an essential nutrient for adults. This example illustrates how functional markers assist identification of critical nutrient status. The simultaneous elevations of citrate and orotate described above override the fact that the circulating arginine concentration is within the limits that the laboratory has approximated as "normal." A clinical trial of free form amino acids may produce a favorable response when patterns of low and low-normal plasma amino acids are found as in this case. This is especially worth considering with Ms. B who presented with multiple symptoms (fatigue, loss of mental clarity, and depression) that are known to respond to amino acid replacement. Another reason to initiate free form amino acid support is that supplemental vitamins, elements and essential fatty acids can easily cause a fall of fasting essential amino acids due to stimulation of utilization for protein synthesis or detoxification.

This case presents an example of the integration of data from amino acid and organic acid profiles to establish a plan of metabolic management. When multiple indicators of functional amino acid inadequacies are present, a combination amino acid supplementation formula could be useful for restoration of optimal nutritional status although most amino acids in plasma were in the "normal" range. Such interventions to restore optimal function rather than simply assure normal concentrations are a common theme in nutritional medicine. The clinician is challenged to use laboratory limits in new ways because nutrient concentration values that are normal may still be inadequate under the conditions of other nutrient therapy.

The frequently observed patterns found in this case cover all essential nutrient categories and illustrate the general concept of strengthening conclusions based on clinical laboratory data by using multiple markers within and between different metabolic profiles. The varieties of patterns for abnormal results are endless, but the experienced practitioner develops a keen sense of where to look for reinforcing results.

Table 12.13 — Amino Acids in Plasma

Plasma Amino Acids	mM		Limits
Arginine	37		35–160
Histidine	84		65–150
Isoleucine	56		50–160
Leucine	100	L	105–250
Lysine	125	L	140–250
Methionine	22		20–60
Phenylalanine	46		45–140
Threonine	120		85–250
Tryptophan	50		45–120
Valine	178		180–480

Fatty Acids and Organic Acids in a Case of Chronic Fatigue

The data shown in Table 12.14 is from a 63-year-old female with long-standing chronic fatigue. A special pattern of omega-3 dominance is found in which the major imbalance is lack of the series 1 eicosanoid precursor, DGLA. In addition, severe (water-soluble) oxidative stress is shown by the greatly elevated 8-hydroxy-2'-deoxyguanosine. The patient demonstrated a rapid increase in her energy and ability to function normally when aggressive vitamin C, glycine (to assist glutathione formation), Zn and evening primrose oil was implemented. Her improvement indicates favorable effects from the antioxidant support that can reduce inflammatory cycles. An unusual aspect is the indication of improvement from enhancing series 1 eicosanoids by addition of GLA. Although the more likely protocol-driven intervention would have been to use fish oils, the laboratory data clearly points to the much more effective use of primrose oil in this case.

Table 12.14 — Blood Spot Fatty Acids and Related Urinary Organic Acids

Dried Blood Spot Fatty Acids	% Total		Limits
Eicosapentaenoic	3.88	**H**	0.19–1.84
Docosahaxaenoic	2.66	**H**	0.61–2.95
Linoleic	11.0		9.4–15.4
Gamma Linolenic	.031	**L**	.027–.257
Dihomogamma Linolenic	0.37	**L**	0.38–1.23
Arachidonic	5.4		2.8–7.3
Total C:18 Trans Fatty Acids	0.26		<= 1.15
LA/GLA	356	**H**	49–397
AA/EPA	10.51	**H**	0.20–3.62
Index of Omega-3 Fatty Acids	6.54		> 0.93
Organic Acids in Urine	**µg/mg creatine**		**Limits**
p-Hydroxyphenyllactate	1.2	**H**	<= 2.0
8-Hydroxy-2-deoxyguanosine	10.4	**H**	<= 7.6

Notes:

INTEGRATING CLINICAL IMPRESSIONS WITH MULTIPLE LABORATORY PROFILES

Impressions:

- 4.5 decades of potential nutritional deficit
- Essential fatty acids?
- Magnesium?
- Deficiencies of B_6?, B_{12}?
- Essential fatty acid status?
- Familial metabolic lesions?
- Dysbiosis endotoxins?
- Delayed food reactions?
- Challenged immunoregulation?
- Malabsorption of fat soluble vitamins (A, D, E, K) and minerals?

MR. L'S AMINO ACIDS

History and Observations

Mr. L was a forty-seven-year-old man who presented with a history of sinus congestion and depression. He noted a family history of depression (father and brother) and related that his first bout of severe depression, which required a 6-month course of standard medications, occurred after he had put himself on a low calorie weight loss diet. As a child, he had a history of "hay fever" and recurrent ear infections, which were treated with multiple antibiotics. Asthma, which seemed to be associated with environmental and food changes, was being controlled with occasional use of a steroid inhaler.

Additional family history included attention deficit disorder and chronic sinusitis in both of Mr. L's sons, ages 6 and 12. His parents' deaths were due to coronary artery disease and cancer. Mr. L experienced lactose intolerance with symptoms of bloating and diarrhea when he consumed milk or ice cream. He had hemorrhoids about every six months with rectal bleeding. He also had a recurrence of numbness and weakness in the extremities and leg cramps at night, which were successfully treated with calcium. He complained of increasing symptoms of joint and muscle pain and headaches. The most annoying symptoms were associated with a more recent degeneration of nasal membrane integrity. Symptoms of excessive mucus formation, stuffy nose, hay fever, and sinus problems were all reported with severe effects. He had been on antibiotics five or six times in the past year.

Impressions

As the running list of impressions listed in the text box shows, multiple nutrient insufficiencies with toxin loads exceeding detoxification capacities are initially suspected. Initial laboratory evaluations included standard serum chemistries, food-specific IgG antibody levels, plasma amino acids, urinary organic acids, and erythrocyte minerals. Foods showing significantly elevated IgG levels were egg, kidney bean, navy bean, and peanut. Moderate levels were found for milk and string bean, and almond, pineapple, and lima bean showed low levels. The amino acids, tyrosine, taurine, glycine, and serine were low in fasting plasma and the sulfate to creatinine ratio was low, along with multiple elevations of bacterial products in urine. Mineral insufficiencies were indicated by low erythrocyte magnesium and zinc. His serum cholesterol was slightly elevated.

Interventions

Mr. L was instructed about the use of an elimination/rotation diet in which all foods with severe and moderate IgG reactions were eliminated. He was placed on supplementation of a high-potency multivitamin-mineral, additional B-complex vitamins, and a combination mineral product containing calcium, magnesium, and zinc. A rice-based hypoallergenic medical food was used to support detoxification function and a broad-spectrum *Lactobacillus* formula, berberine, and

grapefruit seed extract with calcium undecylenate were used to reestablish orthobiosis.

Four Week Follow-up

At a four-week follow up visit, Mr. L reported much improvement in GI symptoms with some improvement of sinus symptoms. The rice-based formula was causing some reactions, so it was replaced with fructo-oligosaccharide and glutamine to assist intestinal healing. His health assessment questionnaire score had dropped from 34 to 11, indicating significant improvement. At this time he was also advised to start a custom amino acid powder that was produced according to the description in Chapter 4, "Amino Acids." At a twelve-week follow up, Mr. L reported that he is "feeling great'" The sinus congestion was relieved within a few days of starting the amino acids. His mood was steady and his energy level was greatly impoved.

Long-term Follow-up

Long-term follow up visits were done to check for responses to additional, normal life stressors. At six months, all previous areas of complaint were still much improved and his serum cholesterol was within normal limits. The health questionnaire score increased slightly due to symptoms of seasonal allergies. At nine months, Mr. L reported feeling "much, much better." He says, "Many problems are gone for good." All IgG reaction are markedly decreased and urinary sulfate and plasma amino acids have all increased to normal or near-normal limits.

Discussion

This patient is typical of individuals who have been described as "the walking wounded" who attempt to live with annoying and mildly debilitating symptoms. None of the symptoms would lead to a conclusion of overt nutrient deficiency. Laboratory evaluation revealed the multiple molecular nature of the problem. This "walking wounded" patient can now be described as walking with intestinal hyperpermeability and small intestinal bacterial overgrowth accompanied by an immune system burden of IgG-antigen complexes. Nutrient insufficiencies are overlaid in this situation impeding the return of wellness by restricting hepatic detoxification capacity. The elimination of IgG-reactive foods and the apparent restoration of the intestinal barrier integrity helped reduce the chronic challenge to the immune system. The main presenting symptom involving the mucosal membranes was not completely resolved, however, until the introduction of an extra ten grams of free form amino acids and glutamine daily. This step may be considered to have two critical effects. First, the repair of the tall columnar epithelial cells of the gut is enhanced by the improved availability of non-essential amino acids, especially glutamine, which is used for energy and regeneration by those cells. Glutamine sparing is accomplished by supplying essential amino acids with cofactors, αKG, and pyridoxal-5-phosphate. The second effect is that of increased growth hormone activity with increasing plasma amino acid levels. Mucosal membrane repair is expected to accelerate under the action of growth hormone.

The stages of healing in this case may be diagrammed as Gut → Nose → Brain. The insomnia and depression were the final symptoms to clear. The origin of mental improvement likely involved the reduction of toxins flowing from the gut and the improvement of neurotransmitter precursor availability because of improved amino acid intake and assimilation with lowered detoxification demands.

Notes:

MR. F'S STAGES OF HEALTH

Impressions:

- Chronic inflammatory response?
- B-complex and Mg depletion?
- Food allergic responses?
- Dysbiosis endotoxins?
- Delayed food reactions identified
- Reduced endotoxins
- Normal immune response restored
- Now nutrient related metabolic effects manifest.
- B-vit's and AA's low
- Insidious poison detected
- Nutrient levels raised to meet individual requirements
- New opportunist growing

Stage I

At the age of 34, Mr. F had experienced soft tissue pain for 12 years and joint pain for the past two years. The pain was being controlled with Flexeril and NSAIDs. For many years, he had experienced difficulty with sleep and multiple symptoms of inflammatory bowel syndrome. The onset coincided with the start of a stressful business after graduating from college.

A lactose/mannitol test showed normal carbohydrate assimilation with hyperpermeability. Hydrogen breath test and stool cultures revealed bacterial overgrowth. Very high levels of IgG class antibodies to milk, eggs, and chicken, all of which were frequently consumed, were found.

The bowel syptoms were improved with regular consumption of steamed vegetables, supplementing antioxidant vitamins, and avoidance of challenging foods. The favorable organisms, *Lactobacilli* and *Bifidobacteria*, were also supplemented and within ten weeks the joint and soft tissue pain was virtually absent without medication, and bowel and sleep habits became normal. Over the next year he also noticed fewer upper respiratory infections.

Stage II

After several months of decreased pain, Mr. F started experiencing increasing bouts of profound fatigue and persistent lethargy. Testing of organic acids in urine revealed very low levels of citrate and fumarate with simultaneous low VMA, the neurotransmitter catabolite, and elevated 3,4-dihydroxyphenylpropionate the *Clostridium* metabolic marker. Simultaneous profiling of trace elements in hair showed elevation of mercury, while mercury was undetected in 24-hour urine. The organic acid profile also revealed a low sulfate, indicating depletion of hepatic conjugation pathways. A fasting plasma amino acid analysis revealed multiple low levels of the essential amino acids plus low tyrosine and taurine.

The supplement program was shifted to include a customized free form amino acid powder fortified with tyrosine and taurine. N-acetyl cysteine (NAC) was added to boost glutathione production and assist in the mobilization of mercury. This routine, with careful control of meal size, reduced his fatigue. A final effort to identify endotoxin sources with fungal testing led to the finding of greatly elevated IgG levels to *Pullularia pullalans*. Treatment with the antifungal agent, Lamisil, while maintaining probiotic organisms, resulted in remission of persistent musculoskeletal pain.

Notes:

Mrs. O's Fibromyalgia

Chief Complaints — Medical History

A 50-year-old white female, Mrs. O, presented at the clinic with postmenopausal symptoms of severe hot flashes and depression. She had been diagnosed 10 years prior with fibromyalgia, a debilitating illness characterized by severe muscle aches and pain, fatigue and depression. The only treatment that had seemed to help her was Prozac, which controlled her pain although, with the advent of menopause, depression was still a factor. She had been taking hormone replacement, Premarin and progesterone for 2 years. This helped with the hot flashes but also made her very irritable. At the time she first visited the clinic, the fibromyalgia pain had returned to a significant degree, and she was tired all the time, depressed, retaining water and wanted to get off her medications as she felt they were affecting her adversely. Her health assessment questionaire score showed a very high self-assessment of adverse symptoms. She commented to one of the doctors, "I give myself 8 months. If this treatment doesn't work, then I want to end it all, as life was not worth living this way.

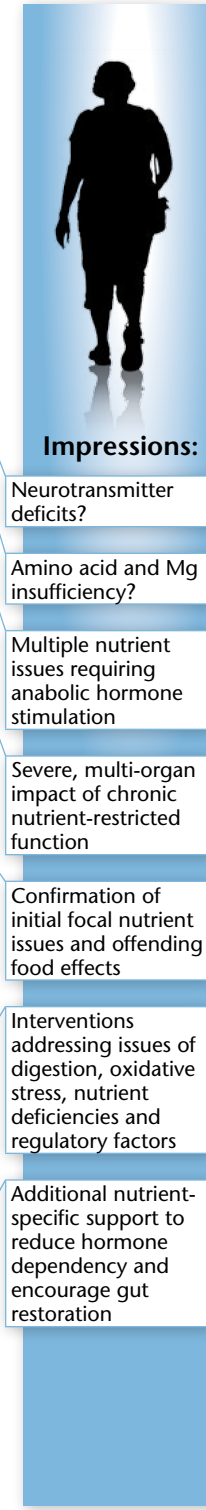
Test Results

Blood chemistries and complete blood count were normal except for elevated cholesterol. The organic acid analysis indicated multiple functional B vitamin deficiencies that can significantly affect her body's ability to generate energy. The amino acid panel revealed significant essential amino acid deficiencies (8 out of 10). The IgG1&4 food allergy panel indicated adverse reactions to milk and wheat.

Treatment Protocol

At the clinic that treated Mrs. O, part of the routine treatment protocol for all diagnosed fibromyalgia patients is a series of vitamin and mineral intravenous (IV) infusions, two per week for one month. They have found this to significantly accelerate the recovery of these patients. In addition, the patient is put on a regimen of oral nutrients designed to correct the biochemical imbalances detected by the testing.

Mrs. O's treatment included a high potency multiple vitamin and mineral, extra B-complex, chromium, omega 3 fatty acids, extra magnesium in liquid form, betaine HCl and pepsin as a digestive/absorptive aid, black cohosh and soy isoflavones to help with the hot flash symptoms. She also used a custom blended essential amino acid mixture formulated according to amino acid levels found in her test. A medical food product designed to treat systemic inflammatory processes and a probiotic supplement to help normalize bowel microflora was also included. 5-Hydroxytryptophan (5-HTP) was recommended before bed. Since the patient was also taking Prozac, this was slowly tapered off as the 5-HTP was increased. Methylsulfonyl methane (MSM) was recommended for its anti-inflammatory properties. Milk and wheat were removed from her diet for four weeks to determine potential adverse immunologic consequences of elevated IgG levels found for these foods.



Follow-up: 2 Weeks

The IV infusions were painful for her and took longer than normal. She developed an itchy rash that went away upon stopping the MSM. She had stopped all hormone replacement and feels worse than ever. However, she understood that she may feel worse before she feels better due to the changes her body must go through to get healthy.

Follow-up: 6 Weeks

The IV infusions were no longer painful and she was experiencing much more energy and was pain free. She had not taken Prozac for four weeks and felt very upbeat emotionally. Her hot flashes had stabilized and were infrequent and mild now. She felt the supplements had helped a lot with that. She had lost several pounds and was no longer bloated. An additional intravenous magnesium solution was started. She reported that it relaxes her and makes her feel "happy."

Follow-up: 12 Weeks

The patient continued feeling well. IVs were reduced to once a month for maintenance. Her supplement schedule was reduced to maintenance levels. Her health assessment questionaire score was down from 83 to 12, reflecting greatly reduced pain and discomfort.

Discussion

While this patient had a particularly successful outcome, most fibromyalgia patients notice significant improvement in their symptoms with this therapeutic approach. The aggressive IV nutrient protocol increased the percentage of successful outcomes at this clinic.

More on Pattern Recognition and Illustrative Scenarios

All clinical assessment is a matter of pattern recognition. Patterns of signs and symptoms make syndromes and lead to pathognomonic disease definition. In molecular medicine, patterns can lead to greater specificity of nutrient deficiency and aggressiveness of intervention for a given case. Sometimes multiple profiles of apparent disparate functions are found to form links that direct the clinician's attention to the more pertinent system failure. Concurrent findings of multiple food allergies, along with metabolic markers of intestinal bacterial overgrowth and elevated markers of free radical pathology are a good example of combining multiple profiles to reach a diagnostic conclusion. Any one of the three testing areas might lead to correct interventions, but together, they point to the nature of the problem as an inflammatory bowel with degeneration of both immune and physical barriers and resulting increased antioxidant demand.

Tables 12.15 to 12.20 illustrate clusters of data that amplify clinical insight about nutritional interventions. The following section presents an illustration of how such clusters are used with patient history and physical information in specific patients. Each one presents a different scenario with differing starting point observations. The tables should be read from top to bottom as a temporal sequence of tests that were performed.

TABLE 12.15 — A COMMON PHENOTYPE OF AUTISM

Test	Finding	Nutrient Regimen Indication
Plasma Amino Acids	Low methionine, glycine, taurine and homocysteine	Methionine-enhanced essential amino acid formula, N-acetylcysteine, taurine and glycine
Serum Vitamins	Low Vitamins A and E and high lipid peroxides	Fat soluble vitamins (cod liver oil) and dietary polyphenol sources
Erythrocyte Elements	Low magnesium, zinc, and selenium	Magnesium and trace elements
Plasma or Erythrocyte Fatty Acids	Low EPA and DHA with high AA	Fish oils (after antioxidant improvement)
Urinary Organic Acids	1. High succinate, fumarate and malate	Coenzyme Q_{10}
	2. High methylmalonate and formiminoglutamate	Oral cyanocobalamin or sub-cutaneous methyl cobalamin and folate
	3. High quinolinate and kynurenate (normal xanthurenate)	Magnesium, taurine and glycine
	4. High tricarballylate, D-lactate and D-arabinitol	Eliminate simple sugars and starch and implement anti-fungals

Table 12.16 — Heal the Gut

Test	Finding	Nutrient Regimen Indication
IgG Food Profile	Multiple high antibody levels	Specific food avoidance or rotation
Organic Acids (urine)	Multiple bacterial metabolic markers	Probiotic and bacteriostatic agents
Lipid Peroxides	Elevated	Strict hypoallergenic diet with probiotics, glutamine. Zn, pantothenic acid, and full spectrum antioxidant support

Table 12.17 — Improve Digestion

Test	Finding	Nutrient Regimen Indication	Clinical Impression Development
Erythrocyte Elements	Multiple low trace elements	Betaine-HCl and mineral aspartates	Hypochlorhydria
Organic Acids (urine)	Multiple bacterial markers	Restore orthobiosis	Hypochlorhydria reinforced; protein maldigestion encouraging bacterial growth
Amino acids (plasma)	Multiple low essential amino acids	Free-form, essential amino acid supplement	Chronic hypochlorhydria leading to amino acid depletion, but amino acids should be started slowly to assure assimilation and prevent further stimulation of intestinal bacterial growth

Table 12.18 — Oxidative Challenge With Trace Element Involvement

Test	Finding	Nutrient Indications	Clinical Impression Development
Erythrocyte Elements	Low-normal zinc with low selenium	Zinc not profoundly depleted	Possible need for selenium
Fatty acid profile	High LA/DGLA	n-6 fatty acids needed	Functional zinc deficiency
Antioxidant profile	Normal	Fat soluble vitamins not profoundly depleted	
Lipid peroxide	Elevated	Some part of antioxidant protection lacking	Aggressive repletion of both selenium and zinc is needed to overcome functional insufficiency of both elements and interim GLA needed

Table 12.19 — Detoxification and Mitochondrial Inefficiency

Test	Finding	Nutrient Regimen Indication	Clinical Impression Development
Organic acids (urine)	High benzoate and p-hydroxyphenylacetate and tricarballylate	Bacterial overgrowth	Challenged hepatic detoxification
Amino acids (plasma)	Low glycine and serine	Support with glycine or essential amino acid mixture	Limited glycine conjugation capacity with increased demand for excretion of microbial toxins
Organic acids (urine)	High β-hydroxybutyrate	Impaired insulin function	Poor gluconeogenic support of plasma glucose
Organic acids (urine)	Elevated adipate and suberate	Supplement carnitine	Simultaneous mitochondrial, dysbiotic, and detoxification challenges

Table 12.20 — Toxic Elements and Glutathione Depletion

Test	Finding	Nutrient Regimen Indication	Clinical Impression Development
DMSA challenge 24 hr. urine	Elevated cadmium and mercury	Reduce toxic metal exposure	Possible oral or IV chelation and amalgam removal
Amino acids (plasma)	Low methionine and taurine	Need for sulfur amino acids	Restore cellular metal complexing ability
Organic acids (urine)	Elevated pyroglutamate	Glutathione depletion	Aggressive N-acetylcysteine needed with repeat testing of sulfur compound status before implementing aggressive chelation or amalgam removal

ALGORITHMS FOR SUPPLEMENTATION FROM METABOLIC PROFILES

The design of a customized diet supplementation plan that will help restore a patient's normal metabolic function is the goal of nutrient use in molecular medicine. Laboratory results can be used to guide variations in nutrient supplementation levels. An algorithmic framework that may serve to translate test results into nutrient supplementation regimes is presented in this section (Table 12.21). Each nutrient is shown with relevant laboratory evaluation. The supplementation levels range from basic "insurance formula" to therapeutic intervention amounts for adults. Testing can reveal circumstances where certain nutrients should be explicitly excluded. These cases show a "0" intervention level in the tables. If all test results for a given nutrient are negative, default amounts (listed for "No abnormalities" in the tables) will prevent deficiencies during periods of dietary inadequacy. The permutations that are shown illustrate the use of coincident abnormalities to signal

TABLE 12.21 — CUSTOMIZING DIETARY SUPPLEMENT INTERVENTIONS BASED ON LABORATORY EVALUATIONS*

Vitamin A

	Test		I.U./d
1	Vitamin A (s)	**H**	0
2	No abnormalities		0–3,000
3	Lipid peroxides (s, u)	**H**	3,000–5,000
4	Vitamin A (s)	**L**	5,000–7,000
	3 AND 4 True		7,000–10,000

β-Carotene

	Test		mg/d
1	Vitamin A (s)	**H**	0
2	β-Carotene (s)	**L**	—
	No abnormalities		0–5,000
	1 AND 2 True		5,000–10,000
	2 True AND 1 NOT True		10,000–25,0000

Vitamin E

	Test		mg/d
1	Vitamin E (s)	**H**	0
2	No abnormalities		50–100
3	Arachidonic acid (p)	**H**	100–300
	Vitamin E (s)	**L**	300–600
4	Lipid peroxides (s, u)	**H**	300–600
5	3 AND 4 True		500–1,000

Vitamin B_1 (Thiamin)

	Test		mg/d
	No abnormalities		0–5
1	α–Keto-β–methylvalerate (u)	**H**	5–10
2	α–Ketoisovalerate (u)	**H**	5–10
3	α–Ketoisocaproate (u)	**H**	5–10
4	Pyruvate (u)	**H**	5–10
5	αKG (u)	**H**	5–10
	Any three or more True		30–50

Vitamin B_2 (Riboflavin)

	Test		mg/d
	No abnormalities		0–5
	1, 2, OR 3 for Vitamin B_1 (adjacent)		5–10
1	Succinate (u)	**H**	5–10
2	Pyruvate (u)	**H**	5–10
3	αKG (u)	**H**	5–10
4	Oleic acid (p, e)	**H**	10–20
	1, 2 AND 3 True		30–50

Vitamin B_3 (Niacin)

	Test		mg/d
1	No abnormalities		0–5
2	Keto acids (u)	**H**	20–70
3	Lactate (u)	**H**	20–70
4	2 AND 3		70–120
5	Total cholesterol (s)	**H**	200–400
6	LDL cholesterol (s)	**H**	200–400
7	Total/HDL cholesterol	**H**	200–400
8	6 AND 7 True		400–600
9	3, 5 AND 7 True		600–800

Vitamin B_6 (Pyridoxine)

	Test		mg/d
1	Homocysteine (s)	**H**	30–50
2	Leucine (p)	**H**	10–30
3	Isoleucine (p)	**H**	10–30
4	Glutamine (p)	**H**	10–30
	No abnormalities		0–10
	2, 3 AND 4 True		30–50

**The specimen tested is indicated by the letter in parentheses following the test, where s = serum; p = plasma; u = urine; e = erythrocyte, wb = whole blood.*

Iron			
	Test		mg/d
1	Iron (s)	H	0
2	% Saturation (s)	H	0
3	Iron (s)	L	—
4	No abnormalities		0–12
5	3 & Iron-binding capacity (s)	H	5–10
6	3 True AND 2 NOT True		15–30

Zinc			
	Test		mg/d
1	Zinc (e)	H	0
2	No abnormalities		0–20
3	Zinc (e)	L	20–40
4	LA/DGLA	H	20–40
5	2 True AND Lipid peroxides (s)	H	40–60
6	5 True AND Zinc (e)	L	50–100

N-Acetylcysteine			
	Test		mg/d
1	No abnormalities		0
2	Sulfate (u)	L	200–300
3	Pyroglutamate (u)	H	200–300
4	2 and 3 True		300–400
5	4 True AND Taurine (p)	L	400–600
6	4 True AND Methionine (p)	L	400–600

Coenzyme Q_{10}			
	Test		mg/d
1	No abnormalities		0
2	Serum Q10 (s)	L	30
3	Hydroxymethylglutarate (u)	H	30
4	Succinate (u)	H	60
5	2 AND Lipid peroxides (s)	H	120
6	2, 3, AND 5 True		300

Flax Oil (Alpha Linolenic Acid)			
	Test		gm/d
1	ALA (p, e)	H	0
2	ALA (p, e)	L	3
3	2 True and EPA (p, e)	H	3
4	2 True and EPA (p, e)	L	6

Tryptophan			
	Test		gm/d
1	Trp (p, u)	H	0
2	Trp (p, u)	L	20–50
3	5-HIA (u)	L	50–100**
4	2 AND 3 True		100–200**

*** Add carefully if SSRI is used*

Biotin			
	Test		µg/d
1	No abnormalities		0
2	Vaccenic (p, r)	H	1,000–2,000
3	β-Hydroxyisovalerate (u)	H	2,000–5,000

Selenium			
	Test		µg/d
1	No abnormalities		0–50
2	Lipid peroxides (s)	H	50–100
3	Selenium (r)	L	100–200
4	Mercury (wb)	H	200–400
5	2 AND 3 True		200–400

Carnitine			
	Test		mg/d
1	No abnormalities		0
2	Adipate (u)	H	300–600
3	Suberate (u)	H	300–600
4	Ethylmalonate (u)	H	300–600
5	2 AND 3 True		600–900

varying supplementation. Not all of the permutations can be shown in these tables, but an attempt was made to list those most relevant. These algorithms include the most directly relevant tests, but they exclude the impact of other factors such as an increased body burden of toxic metals.

Notes:

CONCLUSION

The clinical situations and cases presented in this chapter serve to illustrate the following:

1. Modern degenerative diseases are of complex origin and the progression of symptoms is dependent on each patient's history. Complex cases that defy classical diagnosis and therapy may be amenable to molecular medicine approaches that depend on identification of nutrients, toxins, or metabolic controls that are at the root of the problem.
2. Complex cases must be approached in a way that restores normal function to cells, tissues, and organs. Simple, safe means such as nutrient supplements, food derivatives, and probiotic organisms, when used in the appropriate levels for a given patient, can restore normality to biochemical processes that control cell function.
3. Laboratory evaluations can identify the interventions that are required for restoration of normal function. The laboratory results allow focused interventions of specific nutrients in doses adjusted according to the severity of the depletion and related metabolic impairment.

Notes:

Appendix A

Comprehensive Cardiovascular Health Risk Assessment

Richard S. Lord and J. Alexander Bralley

Introduction

This book is organized by category of tests and how they can be applied to different disease processes. A reverse approach is to focus on a particular disease and see how various tests can be combined to guide therapeutic decisions. Cardiovascular disease (CVD) presents a good opportunity to demonstrate this approach since its etiology is mutifactorial and can include inflammation, oxidant stress, hormone function, and other factors, all of which are influenced by nutrient status. The exclusive use of serum cholesterol as the primary laboratory measure of CVD risk should be evaluated in light of the fact that 50% of all those who experience a coronary event have normal cholesterol levels. Clearly there are other factors that should be evaluated that may have even greater impact on cardiovascular health and disease.

The following tables list the different analytes that can be measured to assess CVD risk and various nutritional interventions that can be used to modify these risk factors. Literature references are provided for further information on the value of each analyte in assessment of CVD risk and treatment.

Table A.1 — Factors Which May Be Assessed To Evaluate CVD Risk

Test Category	Analyte (Measured in Serum, Plasma or Erythrocyte)	Reference
Blood Lipid Components	Total cholesterol	1
	HDL, LDL cholesterol	2
	Triglycerides	3
	Lipoprotein(a)	4, 5, 6
Ratios	Total cholesterol/HDL cholesterol	3
	LDL/HDL	3
Cholesterol Fractionation	Lipoprotein particle and sizes	7, 8, 9
Inflammatory Indicators	High sensitivity C-reactive protein	10, 11, 12
	Myeloperoxidase	13, 14
Antioxidant Status	Vitamin E isomers	15, 16, 17, 22
	Coenzyme Q_{10}	18, 19, 20, 21, 23
Oxidant Stress Indicators	Lipid peroxides	24
	Ferritin	25, 26
	Isoprostanes	74
	Oxidized LDL	27, 29
Hormonal Influences	Insulin	28, 30, 31
	Testosterone	32, 33, 34, 35, 36
Other	RBC magnesium	37, 38, 39, 40
	Homocysteine	41, 42
	ADMA	43, 44, 45
	L-Carnitine	46, 47, 48
	Fibrinogen	49, 50

Notes:

Table A.2 — Potential Nutritional Interventions for Abnormal Laboratory Results

Analyte	Intervention	Metabolic Association
Total Cholesterol	Garlic [51]	Inhibits HMG-CoA reductase, increases bile acid excretion
	Niacin (B_3) [52]	Decreases synthesis in liver, favorably alters subfractions of LDL
	Vitamin C [53]	Anti-inflammatory, antioxidant, decreases cholesterol
	Vitamin E [54]	Paraoxonase prevents oxidation of LDL and HDL, vitamin E scavenges free radical oxidants which can inhibit paraoxonase activity
	Fish oil [55, 56]	Activation of peroxisome proliferator-activated receptors (PPARs), stimulates fatty acid oxidation
Low HDL Cholesterol	Niacin (B_3) [57]	Reduces atherogenic apolipoprotein (Apo) B, increases anti-atherogenic Apo A1
	Fish oil	Pro-inflammatory eicosanoids decrease HDL, fish oil increases anti-inflammatory eicosanoids
	Vitamin C [58]	Raises HDL and lowers triglycerides
High LDL	Garlic, Niacin (B_3), Vitamins C & E	As above
High Triglycerides	Carnitine [59]	Increases oxidation of fatty acids
	Chromium	Potentiates insulin response lowering triglyceride rich particles
	Fish oil	Activates PPARs increasing fatty acid oxidation
	Vitamin C [58]	Increases uptake into cells
Lipoprotein(a)	Niacin [52]	Decreases synthesis
Ferritin	Black, green tea [60]	Inhibits iron absorption
Fibrinogen	Fish oil [61]	Reduces thrombin formation
	Garlic [62]	Inhibits platelet aggregation
hs-C-Reactive Protein	Fish oil [63]	Produces anti-inflammatory eicosanoids
Insulin	Chromium [64]	Improves insulin sensitivity
	Vanadium [65]	
	Niacin (B_3) [66]	
Testosterone	DHEA [67]	Testosterone precursor
	Boron [68]	Decreases elimination
	Zinc [69]	Inhibits conversion to dihydrotestosterone
Homocysteine	Folate, B_{12}, B_6 [70,71]	Improves homocysteine catabolism
Lipid Peroxides	Fat soluable antioxidants [72, 16]	Protects lipids from oxidation
Asymmetric Dimethylarginine	Arginine [73]	Nitric oxide precusor
	Antioxidants [75, 76]	Protects enzyme which catabolizes ADMA

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Appendix B

Nutrient Evaluations Related to Standard Serum Chemistries

Richard S. Lord and J. Alexander Bralley

Nutrient Evaluations Related to Standard Serum Chemistries

Standard serum chemistry test panels are designed largely to assist with the diagnosis of diseases, such as heart disease, liver failure or kidney failure. The abnormality limits for this type of test are usually set to show abnormality only when results are outside 95% of reference population values. The limits may also be determined by measurements performed on cohorts of subjects and controls that are defined by independent criteria. These procedures produce high sensitivity and specificity for the tests so that the rate of correct identification of the presence or absence of diseases is high.

Two exceptions to the rule of 95% limits are serum cholesterol and serum thyroid stimulating hormone (TSH). These tests are used for disease risk assessment in addition to diagnosis of frank hypercholesterolemia and primary hypothyroidism. Their limits are in the range of 80% of broadly defined reference populations. Thus, mildly elevated cholesterol or parathyroid hormone levels are assessed in ways similar to essential nutrient abnormalities. Interventions are planned to avoid or slow the progression to frank diseases. For tests that assess essential nutrient status, identifying individuals who are outside of 80% limits (first or fifth quintiles) was discussed in Chapter 1, "Basic Concepts."

The previous chapters have discussed many ways in which nutrient insufficiencies and toxicant effects can be part of the underlying origin of metabolic diseases. Generally, the chronic, degenerative diseases that are diagnosed with the aid of standard serum chemistries are tied to earlier events that may be observed in the special nutrient and toxicant assessments. The table in this appendix is intended to help identify specific relationships that might be investigated to improve patient outcomes.

Notes:

Table B.1 — Nutrient Evaluations Related to Standard Serum Chemistries

	Test		Potential Clinical Indications	Related Nutrient Evaluations
Electrolyte Panel	Sodium	L	Metabolic acidosis Adrenal dysfunction	Urinary organic acids, adrenal stress profile
		H	Dehydration Renal dysfunction	Elemental profile
	Potassium	L	Adrenal hyperactivity Magnesium deficiency	Adrenal stress profile, elemental profile
		H	Adrenal dysfunction	Adrenal stress profile, elemental profiles
	Chloride	L	Adrenal dysfunction Hypochlorhydria	Adrenal stress profile, gastrointestinal function profile
		H	Renal failure Excessive salt or aspirin intake	Urinary elemental profile
	Carbon dioxide	L	Metabolic acidosis B-vitamin deficiency Sleep apnea Breathing abnormality	Urinary organic acids, gastrointestinal function profile
		H	Hypochlorhydria Alkalosis	Gastrointestinal function profile
	Calcium	L	Osteoporosis Thyroid dysfunction Parathyroid dysfunction HPA axis dysfunction Heavy metal toxicity	Bone loss marker, elemental profiles, detoxification status
		H	Thyroid or parathyroid dysfunction Chemical or heavy metal toxicity Excess vitamin D	Urinary iodide, adrenal stress profile, urinary organic acids for markers of oxidative stress and detoxification
	Phosphorus	L	Hypochlorhydria Insufficient protein assimilation	Plasma amino acids, gastrointestinal function profile
		H	Renal or parathyroid dysfunction Excessive phosphate intake	Elemental profiles
	Test		**Potential Clinical Indications**	**Related Nutrient Evaluations**
Metabolic Panel	Blood urea nitrogen	L	Hypochlorhydria Dietary protein deficiency Maldigestion Malabsorption Liver failure	Gastrointestinal function profile, plasma amino acids, urinary organic acids, detoxification profiles
		H	Renal failure	
	Uric acid	L	Low hematopoiesis Copper deficiency	Elemental profiles
		H	Gout Rheumatoid arthritis Atherosclerosis Hepatic failure Hyperhomocysteinemia	Urinary organic acids, immune profiles, cardiac health profile, detoxification profiles
	Glucose	L	Hypoglycemia	Organic acid profile, amino acid profile
		H	Diabetes	Trace element profile, fatty acid profile
	Bilirubin, total (Conjugated + unconjugated)	H	Gilbert's syndrome Chemical or heavy metal toxicity Liver failure Enhanced erythrocyte turnover Congestive heart failure Sickle cell anemia	Detoxification capacity profile, cardiac health profile

Table B.1 continued on following page...

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	Test		Potential Clinical Indications	Related Nutrient Evaluations
Metabolic Panel	Albumin	L	Liver failure Alcoholism Malnutrition Chemical or heavy metal toxicity Inflammation Insulin insensitivity Obesity	Porphyrin profile, urinary organic acids, elemental profile, fatty acid profile, cardiac health profile
		H	Dehydration	—
	Globulin	L	Inflammation Immune deficiency	Food sensitivity profile, gastrointestinal function profile
		H	Hypochlorhydria Chemical or heavy metal toxicity Liver damage Autoimmune disease	Gastrointestinal function profile, porphyrin profile, urinary organic acids, elemental profiles, plasma amino acids
	A/G Ratio	L	Protein deficiency	Plasma amino acids
		H	Myeloma	Fatty acid profile, Serum antioxidants and oxidative stress profile
	Test		**Potential Clinical Indications**	**Related Nutrient Evaluations**
Enzyme Panel	AST (SGOT)	L	Vitamin B_6 or protein deficiency Alcoholism Liver disease	Urinary organic acids, plasma amino acids, plasma fatty acids, gastrointestinal function profile
		H	Acute myocardial infarction Liver disease Skeletal muscle breakdown Metastatic cancer	Plasma amino acids, cardiac health profile, elemental profiles, serum adma, urinary organic acids, hepatic detoxification capacity profile
	ALT (SGPT)	L	Vitamin B_6 or protein deficiency Alcoholism Liver disease	Urinary organic acids, plasma amino acids, plasma fatty acids, gastrointestinal function profile
		H	Liver disease Fatty liver Congestive heart failure Salicylate toxicity	Detoxification capacity profile, urinary organic acids, cardiac health profile, plasma fatty acids
	Alkaline phosphatase	L	Hypothyroidism Pernicious anemia Scurvy Low fat or low protein diet Zinc deficiency Excessive vitamin D intake	Plasma fatty acids, plasma amino acids, elemental profile, urinary iodine, serum vitamin d, urinary organic acids
		H	Elevated bone turnover/loss Hypothyroidism Paget's disease Rickets Bile acid deficiency Excessive dietary fat or protein	Bone turnover marker, plasma fatty acids, gastrointestinal function profile, plasma amino acids, urinary iodine, serum vitamin D

Table B.1 continued on following page...

Notes:

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	Test		Potential Clinical Indications	Related Nutrient Evaluations
Lipid Panel	Triglycerides	L	Oxidative stress Chemical/metal toxicity Liver dysfunction Low dietary carbohydrates	Oxidative damage markers, porphyrins, toxic elements, organic acids, fatty acids
		H	Insulin resistance Diabetes Fatty liver Hypothyroidism	Cardiac health profile, organic acids, fatty acids, iodine, ADMA, hormones
	Cholesterol	L	Oxidative stress Chemical/metal toxicity Liver dysfunction Low dietary carbohydrates Viral hepatitis Hyperthyroidism	Oxidative damage markers, porphyrins, toxic elements, organic acids, fatty acids, hormones
		H	Insulin resistance Diabetes; Fatty liver Hypothyroidism Acute biliary obstruction; Pancreatitis	Cardiac health profile, oorganic acids, fatty acids, ADMA, hormones
	HDL Cholesterol	L	Oxidative stress Chemical/metal toxicity Sedentary lifestyle Obesity Insulin resistance Fatty liver Starvation Diabetes Hypothyroidism Uremia	Oxidative damage markers, porphyrins, organic acids, fatty acids, amino acids, elements, cardiac health profile, iodine
	Test		**Potential Clinical Indications**	**Related Nutrient Evaluations**
Iron Panel	Iron, TIBC, Iron saturation	L	Iron deficiency	Trace element profile
			Iron excess	Antioxidants
		H	Inflammation Hemochromatosis Iron excess Oxidative damage	Cardiac health profile, elements, oxidative damage markers, immune profiles, hepatic detoxification capacity
	Test		**Potential Clinical Indications**	**Related Nutrient Evaluations**
Thyroid Panel	Total T3, T3 Uptake, T4, T7, TSH	L	Primary hypothyroidism	Urinary iodine, plasma amino acids, organic acids
		H	Primary hyperthyroidism	

Appendix C

Interactions of Drugs, Nutritional Supplements and Dietary Components

Richard S. Lord, J. Alexander Bralley, and Cass Nelson-Dooley

For mechanisms of action, drug-nutrient contraindications, antagonisms, and synergies, please see the sources from which the table has been adapted.[1, 2]

For information on the symptoms of vitamin and mineral deficiencies induced by a given medication, see the corresponding chapters.

On-line resources are:

- *NIH Clinical Center*
  http://clinicalcenter.nih.gov/ccc/patient_education/drug_nutrient/
  (Accessed on 03/03/08.)
- *National Guideline Clearinghouse*
  http://www.guideline.gov/resources/summaryarchive.aspx#3631
  (Accessed on 03/03/08.)

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Table C.1 — Drug-Nutrient Interactions

Category	Drug Type	Affected Nutrients	Mechanism
Analgesic	Aspirin, salicylates	Vitamin C, Folic Acid, Glycine, Histidine, Potassium, Zinc, Vitamin K	Drug most likely to produce vitamin C deficiency in normal individuals. Chronic use can cause iron depletion due to blood loss in GI tract. Aspirin depletes folic acid by displacing bound serum folate. Causes urinary loss of potassium. ***Depletion-related symptoms:*** weakness & low energy from anemia. Salicylates in high doses can reduce vitamin K epoxide reductase, resulting in vitamin K deficiency. ***Detoxification effects:*** Salicylates can decrease histidine levels. Aspirin overdose depletes plasma glycine.
Female Hormone	Estrogens	Vitamin B_6, Folic Acid	Estrogen metabolism interferes with absorption of both folic acid and vitamin B_6. Since B_6 is involved with synthesis of serotonin, depletion can cause anxiety, depression, sleep disturbances, and irritability. Anemia from folate depletion causes weakness and low energy. Low levels of folic acid are associated with increased incidence of birth defects, cervical dysplasia, and elevated homocysteine, which is a major risk factor for cardiovascular disease.
	Oral Contraceptives	Vitamins A, B_2, B_6,B_{12}, C, Folic Acid, Zinc, Magnesium	Studies show reduced serum levels of nutrients listed with use of OCs. Depletions of vitamins B_6, B_{12}, and C are not as frequently seen with the use of newer low dose estrogen OCs. Zinc deficiency can lead to depressed growth, poor immune function, and alopecia. OCs may increase serum retinol.
Diuretic	Hydralazine (Apresoline)	Vitamin B_6, Magnesium	These antihypertensives also are diuretics and block an enzyme which can cause vitamin depletion. B_6 deficiency can cause depression and/or nerve damage causing numbness or tingling of hands or feet.
	Loop Diuretics: Furosemide (Lasix), Bumetamide (Bumex), Torsemide (Demadex), Ethacrynic acid (Edecrin)	Magnesium, Vitamin B_1, Vitamin B_6, Potassium, Zinc	Diuretic-induced magnesium and potassium deficiencies can cause increased irregularities in heartbeat blood pressure. Increased urination may also cause depletion of vitamins B_1 and B_6. Chronic use of furosemide can cause B_1 deficiency.
	Thiazide Diuretics: Hydrochlorothiazide (Esidrix, HydroDIURIL), Indapamide (Lozol), and Metolazone (Zaroxolyn)	Magnesium, Potassium, Sodium, Zinc	Urinary depletion of magnesium and potassium can exacerbate irregular blood pressure and cardiac function. Zinc depletion can suppress wound healing and immune function. Hyponatremia could also develop.
	Potassium-Sparing Diuretics: Amiloride (Midamor, Moduretic), Triamterene (Dyazide, Dyrenium, Maxzide), Spironolactone (Aldactazide, Aldactone)	Magnesium, Potassium, Sodium, Zinc	Inhibits enzyme necessary for folic acid synthesis. Chronic use can lead to folic acid depletion. Diuresis can also cause calcium depletion. **CAUTION:** ***taking potassium with potassium-sparing diuretics could cause hyperkalemia.*** ***(Symptoms = weakness, impairment of speech cognition)***
Anti-hyperlipidemia	***HMG-CoA Reductase Inhibitors:*** Lovastatin (Mevacor), Simvastatin (Zocor), Pravastatin (Pravachol), Fluvastatin (Lescol)	CoEnzyme Q_{10}	These drugs block a liver enzyme necessary for synthesis of cholesterol and CoQ_{10}. CoQ_{10} depletion can affect cellular energy production, regulation of blood pressure, and cardiac function.
	Bile Acid Sequestrant: Cholestyramine (Questran) and Colestipol (Colestid)	Vitamins A, D, E, K, B_2, B_3, and B_{12}, β-Carotene, Folic Acid, Iron, Fat	Nutrient depletions caused by poor absorption. Long time intervals are required between nutritional supplements and pharmaceutical dosing. Fat absorption is also inhibited. May decrease enterohepatic resorption of vitamin B_{12}.

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Category	Drug Type	Affected Nutrients	Mechanism
Anti-seizure	***Barbiturates:*** Phenobarbital (Luminal Sodium), Pentobarbital (Nembutal Sodium), Thiopental, Secobarbital (Seconal), Methohexital	Vitamin D, Calcium, Folic Acid	Long-term use interferes with vitamin D metabolism and may reduce the absorption of calcium. Phenobarbital may reduce plasma levels of vitamins D and E. Folic acid levels are lowered in both plasma and erythrocytes.
	Phenytoin (Dilantin), Carbmazepine (Tegretol), Primidone (Mysoline)	Vitamins D, E, Calcium, Folic Acid, Vitamin B_{12}, Biotin	Decreases vitamin D availability, reducing absorption of calcium. Decreases serum folate and Phenytoin may decrease vitamins D and E in plasma. Vitamin B_{12} and folic acid absorption are also decreased. Can increase biotin metabolism, decreasing biotin plasma levels. Carbamazepine inhibits folate absorption; long-term use could create anemia. Carbamazepine decreases plasma levels of vitamin E and pyridoxal 5′-phosphate. These depletions can result in bone disease, anemia, neurological problems, as well as gum and periodontal disease.
Anti-Inflammatory	***Corticosteroids:*** Prednisone (Meticorten), Dexamethasone (Decadron), Methylprednisolone (Medrol)	Calcium, Vitamin D, Potassium, Selenium	These drugs reduce levels of vitamin D and decrease the absorption of calcium, resulting in bone loss and skeletal problems. Long-term use may also deplete potassium, selenium, and zinc.
	Gout Medications: Colchicine (ColBENEMID), Probenecid (Benemid)	β-Carotene, Folate, Vitamin B_2 and D, Potassium, Sodium	Colchicine inhibits the absorption of all these nutrients. Changes in pH cause GI symptoms and B_{12} malabsorption. Decreases folate blood levels. Probenecid may inhibit absorption of B_2 and renal tubular secretion. Symptoms from these nutrient depletions include weakness and peripheral neuritis.
	Nonselective NSAIDs: Indomethacin, Indocin	Vitamin C, Folic Acid, Amino Acids, Iron	Decreases absorption of both vitamin C and folic acid. Increases rate of gastric emptying which decreases absorption of amino acids. Indocin can cause iron deficiency due to blood loss.
	Other Nonselective NSAIDs: Ibuprofen, Naproxen (Naprosyn), Sulindac (Clinoril)	Folic Acid	These anti-inflammatory drugs competitively inhibit the enzymatic synthesis of folic acid. Long-term use could lead to anemia. Low levels of folic acid are associated with increased incidence of birth defects, cervical dysplasia, and elevated homocysteine, a major risk factor for cardiovascular disease.
Anti-rheumatic	Sulfasalazine (Azulfidine)	Folic Acid	Intestinal absorption of folic acid is inhibited, which can lead to anemia-related weakness and low energy.
	Metal-binding: Penicillamine	Copper, Vitamin B_6, Zinc	When taken together, both these nutrients and penicillamine are poorly absorbed. By binding P5P, can result in functional vitamin B_6 deficiency.

Table C.1 continued on following page...

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Category	Drug Type	Affected Nutrients	Mechanism
Anti-bacterial	***Broad-spectrum:*** Amoxicillin / Clavulanic Acid (Augmentin), Vancomycin ***Combination anti-fungal & anti-bacterial:*** Amphotericin B, nonlipid (Fungizone)	All B-Vitamins, Biotin, Vitamin C, Vitamin K	Antibiotics kill pathogenic and beneficial bacteria such as Acidophilus and Bifidus. The "friendly bacteria" produce vitamins B_2, B_3, B_6, B_{12}, K, biotin, folic acid, pantothenic acid, and a number of natural antibiotics in our intestines. Destroying acidophilus & bifidus bacteria can cause nutrient depletions and impair the immune system.
	Tetracycline Antibiotics: Demeclocycline, Doxycycline, Methacycline, etc.	Calcium, Magnesium, Manganese, Zinc, Vitamin B_6, Vitamin B_{12}	Tetracyclines chelate calcium, magnesium, and zinc. Long term use can cause mineral depletions. Take manganese separately from antibiotic. Tetracyclines also interfere with the absorption of vitamins B_6 and B_{12}.
	Cycloserine, Ethionamide, Isoniazid (INH)	Vitamin B_6	Can cause functional vitamin B_6 deficiency by binding P5P. Ethionamide increases vitamin B_6 requirements.
	Cephalosporins: Cefoperazone, Cefotetan, Cefamandole, Latamoxef, Cefazolin	Vitamin K	Inhibit a liver enzyme that can result in vitamin K deficiency and hypoprothrombinemia.
	Fluoroquinolones: Ciprofloxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, etc.	Zinc	When taken together, both Zn and these antibiotics have decreased absorption.
	Bactrim (also Septra, Trimpex)	Folate	Mild folate antagonists with only minimal risk. However, long-term use and/or high dose usage may create a deficiency, especially in compromised patients.
Anti-fungal	Ketoconazole (Nizoral)	Vitamin D	This antifungal may inhibit biosynthesis and breakdown of 1,25-dihydroxy-Vitamin D.
Anti-Diabetic Drugs	***Sulfonylureas:*** Diabeta (Glynase, Micronase), Tolinase	Coenzyme Q_{10}	Inhibition of the NADH-oxidase enzyme can lead to a coenzyme Q_{10} deficiency
	Biguanides: Metformin (Glucophage)	Vitamin B_{12}	Competitive inhibition of vitamin B_{12} absorption could cause depletion in some individuals.
	Diabetic gastroparesis and heartburn: Metaclopramide HCl (Reglan)	Vitamin B_2	May inhibit absorption and renal tubular secretion of vitamin B_2.
Antacid	Aluminum-containing (Gaviscon, Maalox, Mylanta), Calcium-containing (Mylanta, Rolaids, Tums), Magnesium-containing (Gaviscon, Maalox, Mylanta), Sodium Bicarbonate (Alka Seltzer)	Calcium, Phosphorus, Copper, Iron, Magnesium, Manganese, Potassium, Zinc, Protein, Folic acid, Vanadium	An alkaline pH inhibits the absorption of these nutrients. Chronic use can lead to skeletal problems due to calcium & phosphate depletion. The digestion of protein is also diminished. Sodium bicarbonate-altered intestinal pH specifically inhibits the absorption of folic acid. Magnesium-containing antacids can decrease manganese absorption if taken concomitantly. Aluminum hydroxide may decrease vanadium absorption.
	H-2-Receptor Antagonists: Cimetidine (Tagamet), Famotidine (Pepcid), Nizatidine (Axid), Ranitidine (Zantac)	Vitamin B_{12}, Calcium, Folic Acid, Vitamin D, Iron, Zinc, Protein	Malabsorption of dietary B_{12}, iron, and folic acid by H-2 antagonists may contribute to nutrient depletions. Altered pH may also reduce absorption of calcium, vitamin D, and zinc. Altering gastric pH also interferes with digestion of protein. Folic acid depletion is associated with increased incidence of birth defects, cervical dysplasia, and elevated homocysteine, a major risk factor for cardiovascular disease.

Table C.1 continued on following page...

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Category	Drug Type	Affected Nutrients	Mechanism
Antacid	***Proton Pump Inhibitors:*** Omeprazole (Prilosec), Lansoprazole (Prevacid), Rabeprazole (Aciphex)	Calcium, Vitamin B_{12}, Protein	By altering the gastric pH, these drugs may cause malabsorption of vitamin B_{12}. Probable interference with protein digestion. Concomitant use of these medications with calcium may reduce calcium absorption.
Anti-arrythmia	Digoxin (Lanoxin)	Calcium, Magnesium	Increased urinary excretion of both calcium and magnesium can lead to deficiencies. Magnesium deficiencies increase likelihood of cardiac dysrhythmias and atrial fibrillation.
	Beta Blockers: Propanolol (Inderal), Metoprolol (Lopressor), etc.	Coenzyme Q_{10}	These drugs antagonize the activity of the enzymes involved in the synthesis of coenzyme Q_{10}. Deficiency can cause heart, blood pressure, and immune system-related problems.
Psychiatric Medications	***Tricylic Antidepressants:*** Amitriptyline (Elavil), Nortriptyline (Pamelor), Imipramine (Tofranil), Desipramine (Norpramin), Doxepin (Sinequan), etc.	Coenzyme Q_{10} , Vitamin B_2	Tricyclics inhibit enzymes necessary for production of coenzyme Q_{10}. Deficiency can cause cardiovascular symptoms. Both Elavil & Tofranil deplete vitamin B_2 by interfering with absorption. Deficiency can cause skin, neurological and energy problems.
	Antipsychotic Agents: Chlorpromazine (Thorazine), Thiothexane (Navane), Thioridazine (Mellaril), Fluphenazine esters (Prolixin), etc.	Vitamin B_2, Coenzyme Q_{10}	These drugs inhibit the absorption of vitamin B_2 & coenzyme Q_{10}. May inhibit conversion of riboflavin (to FMN and FAD). Depletion of these vitamins can cause skin, neurological, and energy-related problems.
	Mood Stabilizer: Valproic acid	Vitamin B_6	Can reduce plasma P5P.
Weight management	Orlistat (Xenical)	Vitamins A, D, E, K	May decrease exocrine output and reduces fat absorption.
	Sibutramine (Meridia)	Tyrosine, Tryptophan	Simultaneous inhibition of serotonin (by 53%), norepinephrine (by 54%), and dopamine (by 16%)
Laxative	Mineral Oil, Sennosides (Agoral, Haley's M-O)	Vitamins A, D, E, and K, β-Carotene	Inhibits absorption—fat soluble nutrients dissolve in the mineral oil and are lost when the oil is excreted.
	Docusate/ Phenolphthalein (Feen-a-Mint)	Potassium	Causes decreased nutrient absorption due to increased intestinal motility and mucosal permeability.
	Bisacodyl (Correctol, Dulcolax)	Potassium	Intense peristalsis and rapid bowel emptying can cause hypokalemia.
Anti-proliferative (Chemotherapy)	Chemotherapy drugs	Most Nutrients	Many chemotherapy drugs cause nausea, vomiting, and significant damage to gastric and intestinal mucosa. These factors cause decreased appetite and malabsorption leading to a wide variety of nutrient depletions.
Anti-asthmatic	Theophylline (Theo-Dur)	Vitamin B_6	Inhibits enzyme pyridoxal kinase causing vitamin B_6 depletion.
Anti-clotting	Warfarin sodium (Coumadin)	Vitamin K	Interferes with the enzyme responsible for the synthesis of vitamin K.
Anti-viral	Zidovadine, Retrovir (AZT)	Copper, Zinc	Drug causes specific depletion of both copper and zinc.
Anti-bone resorptive	Bisphosphonates: Etidronate (Didronel), Pamidronate (Aredia), Alendronate (Fosamax), Risedronate (Actonel), Tiludronate (Skelid)	Zinc	When taken together, both zinc and the bisphosphonate have reduced absorption.

Table C.2 — Nutrient Supplements

Supplement Component	Affected Nutrient(s)	Mechanism
Boron, boric acid	Vitamin B_2	Displaces riboflavin binding and increases excretion.
Calcium	Iron, Magnesium, Manganese, Zinc	May depress zinc absorption in postmenopausal women. Calcium (over 2g) can decrease absorption of magnesium. Calcium and manganese taken together result in decreased absorption of manganese.
Chromium	Vanadium	May decrease vanadium absorption.
Copper	Zinc	Taking these essential minerals together may decrease copper absorption.
Iron	Copper, Manganese, Vanadium, Zinc	When taken together, absorption of both iron and these essential minerals can be reduced. High-dose nonheme iron can decrease copper status. Ferrous ion can decrease absorption of vanadium.
Magnesium	Manganese	Concomitant intake of these nutrients can reduce absorption of manganese.
Molybdenum	Copper	High intake of molybdenum can decrease copper status.
Pantothenic acid (high-dose)	Biotin	Can decrease absorption of biotin by competing for the same uptake mechanism in colonocytes.
Phosphate salts	Magnesium, Zinc	When taken together, mineral absorption can be inhibited.
Phytosterols and phytostanols	Vitamin E	May lower plasma vitamin E.
***Potassium** (Chloride):* Kaon-CL, Klor-Con, K-Dur, K-Tab, Slow-K, etc.	Vitamin B_{12}	Slow release of potassium chloride salts alters intestinal pH, which decreases absorption of vitamin B_{12}. Depletion can cause weakness and tiredness associated with anemia.
Psyllium	Vitamin B_2	Decreases absorption of riboflavin when taken together.
Sodium alginate	Calcium, Magnesium	Decreases absorption of these minerals.
Squalene	Vitamin K	May decrease absorption of vitamin K if taken together.
Vitamin A (high-dose)	Vitamin K	High doses of vitamin A may decrease vitamin K.
Vitamin C	Copper	1500 mg vitamin C has been shown to decrease copper transporting protein.
Vitamin E (high-dose)	Vitamin K	A vitamin E metabolite can inhibit vitamin K-dependent gamma-glutamyl carboxylase activity.

Table C.3 — Dietary Components

Food Component	Affected Nutrient	Mechanism
Alcohol	Vitamin B_6	High alcohol intake increases P5P catabolism.
Chloride	Vanadium	May decrease absorption of vanadium.
EDTA	Vanadium	May decrease absorption of vanadium.
Fructose	Copper	High-fructose diets can decrease copper.
Phytic Acid or Inositol hexaphosphate	Calcium, Chromium, Copper, Manganese, Magnesium, Zinc	Foods high in phytic acid can reduce absorption of these minerals. Inositol hexaphosphate may depress absorption of calcium, magnesium, and zinc.
Olestra	Vitamins A, D, E, K	Inhibits absorption of vitamins.
Oxalic acid	Calcium, Magnesium, Manganese, Zinc	Foods high in oxalic acid can reduce absorption of these minerals.
Sulfites, Tea, coffee, and decaf coffee	Vitamin B_1 (Thiamin), Zinc	Taken together with vitamin B_1, these foods can inactivate the vitamin. Caffeine and tannins can decrease zinc absorption.

Index

Note. Figures are indicated by *f* after the page number. Tables are indicated by *t* after the page number. Pages that contain figures and/or tables as well as discussion in the text are indicated by *f* or *t* in parentheses.

A

B

C

D

E

F

G

H

J

K

L

M

N

O

P

T

U

V

W

X

Y

Z

Common Abbreviations

AA	arachidonic acid
αAAA	alpha-aminoadipic acid
AANB	alpha-amino-n-butyric acid
AHB	alpha-hydroxybutyrate
AHBD	alpha-hydroxybutyrate dehydrogenase
ADMA	asymmetric dimethylarginine
AI	adequate intake
Ala	alanine
ALA	alpha lipoic acid, alpha linolenic acid, 5-alpha levulinic acid
ALT	alanine transaminase
Arg	arginine
Asn	asparagine
Asp	aspartic acid
BCAA	branched-chain amino acid
BH4	tetrahydrobiopterin
Ca	calcium
CAC	citric acid cycle
CHO	carbohydrate
Cit	citrulline
CLA	conjugated linoleic acid
CNS	central nervous system
CoA-SH	coenzyme A
CoQ10	coenzyme Q10
Cr	chromium
Creat.	creatinine
Cu	copper
CYP	cytochrome P450
Cys	cystine
DFO	desferrioxamine
DGLA	dihomogammalinolenic acid
DHA	docosahexaenoic acid
DHEA	dehydroepiandosterone

DMPA	N-(2,3-dimercaptopropyl) phthalamidic acid
DMSA	meso-2,3-dimercaptosuccinic acid
DMPS	2,3-dimercapto-1-propanesulfonic acid
EAA	essential amino acid
EDTA	ethylenediamine tetraacetic acid
EFA	essential fatty acids
EGOT	erythrocyte glutamate-oxaloacetate transaminase
EGPT	erythrocyte glutamate pyruvate transaminase
EGR	erythrocyte glutathione reductase
EMI	estrogen metabolite index
EPA	eicosapentaenoic acid
ER	estrogen receptor
FA	fatty acid
FAD	flavin adenine dinucleotide
Fe	iron
FIGLU	formiminoglutamic acid
FMN	flavin mononucleotide
GABA	gamma-aminobutyric acid
GALT	gut-associated lymphoid tissue
GH	growth hormone
GI	gastrointestinal
GLA	gamma linolenic acid
Gla	gamma-carboxyglutamate
GH	growth hormone
Glu	glutamine
Gly	glycine
GTPCH	GTP cyclohydrolase
HCl	hydrochloric acid
HCys	homocysteine
5-HIA	5-hydroxyindoleacetic acid
His	histidine
HLys	hydroxylysine
HMG	hydroxymethylglutarate

Notes:

HNE	4-hydroxy-2-nonenal
HPA	hyperphenylalaninemia
HPLA	p-hydroxyphenyllactate
HPro	hydroxyproline
HVA	homovanillic acid
I	iodine
I-3-C	indole-3-carbinol
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ID	iron deficiency
IDA	iron deficiency anemia
IgE	immunoglobulin E
IGF-1	insulin-like growth factor 1
IgG	immunoglobulin G
Ile	isoleucine
K	potassium
α-KG	alpha-ketoglutarate
LA	linoleic acid
LDL	low-density lipoprotein
Leu	leucine
Lys	lysine
MBC	mononuclear blood cell
Met	methionine
MetSO	methionine sulfoxide
Mg	magnesium
MMA	methylmalonic acid
µM	micromolar ((µg/l) / m.w.)
Mn	manganese
5-MTHF	5-methyltetrahydrofolate
MTHFR	methylene-THF reductase
NAC	N-acetylcysteine
NAD	nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate

NEAA	non-essential amino acid
NO	nitric oxide
NOS	nitric oxide synthetase
OAU	organic acids in urine
Orn	ornithine
P-5-P	pyridoxal-5′-phosphate
PE	phosphoethanolamine
Phe	phenylalanine
PKU	phenylketonuria
ppm	parts per million (mg/L)
Pro	proline
PTH	parathyroid hormone
RBC	red blood cell
RDA	recommended daily allowance
ROS	reactive oxygen species
SAM	S-adenosylmethionine
Se	selenium
Ser	serine
SN1-SN2	amino acid transporter system
SOD	superoxide dismutase
SSRI	serotonin-specific reuptake inhibitor
Tau	taurine
TBA	thiobarbituric acid
TBARS	thiobarbituric acid reactive substances
THF	tetrahydrofolate
Thr	threonine
TIBC	total iron-binding capacity
T/T	Triene/Tetraene (Mead/Arachidonate)
Tyr	tyrosine
UC	ulcerative colitis
Val	valine
VMA	vanilmandelic acid
Zn	zinc