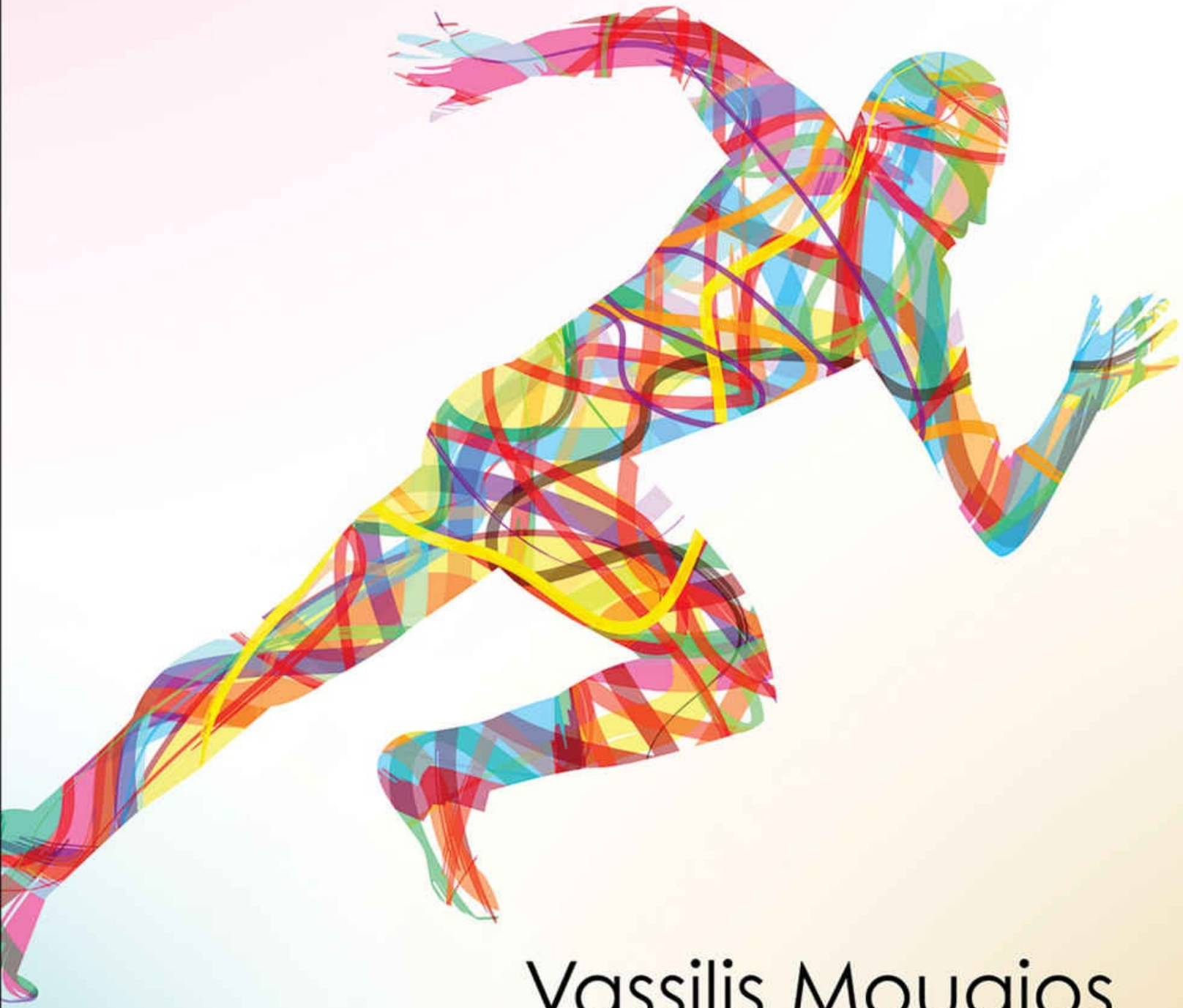
EXERCISE BIOCHEMISTRY

SECOND EDITION



Vassilis Mougios

SECOND EDITION

EXERCISE BIOCHEMISTRY

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HUMAN KINETICS

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To perseverance—turning the impossible into the possible

Contents

Preface

Exercise Biochemistry examines how exercise, in its many forms, changes the ways in which our bodies function at the molecular level. The primary tool for this endeavor can be none other than the principles of basic biochemistry, which are addressed in part I of the book. These principles are then applied, in part II, to the state of excitation of the nervous and muscular systems that results in movement. The ways in which exercise modifies metabolism, presented in part III, form the core of the book. Finally, part IV describes the use of simple (*routine*, as we call them) biochemical tests to assess an exercising person's health and performance.

New to This Edition

- A chapter on vitamins and minerals (chapter 6) as part of the extended presentation of how nutrition influences exercise metabolism
- A chapter on how exercise fights disease (chapter 15)
- Energy information (in the form of standard free-energy change) for almost all reactions, based on the most recent available data
- More explanatory figures and tables (35% more than in the previous edition)
- Enhanced pedagogical aids, including learning objectives for each chapter, more explanatory and informative notes, chapter summaries, key terms, more problems and critical-thinking questions, detailed answers to the problems and critical thinking questions, and an expanded glossary
- Introduction of systems biology, the various -omics (genomics, transcriptomics, proteomics, metabolomics) and bioinformatics

- A critical appraisal of ambiguous terminology and misconceptions surrounding exercise biochemistry and physiology
- An extensive discussion of the role of lactate in exercise metabolism and the controversies surrounding it
- A presentation of the latest findings on gene expression as they relate to training-induced adaptations
- A new look at fatigue and its causes
- Introduction of interval training and its effects on exercise metabolism
- Expanded presentation of how exercise perturbs the redox state and how we can evaluate it in exercisers
- Expanded presentation of designs and methods used to study exercise metabolism
- Expanded presentation of legal and illegal ergogenic aids
- Introduction of myokines and their roles in exercise

The discipline of exercise biochemistry is a rather young child of a rather young parent. The parent (biochemistry) is a little over one century old, during which period it has taken huge steps (even leaps) toward understanding the phenomenon of life in its most intricate details. As a result, it has offered invaluable service to the welfare of human beings. All health sciences today depend on biochemical findings for their own success; therefore, purely biochemical topics occupy a considerable portion of textbooks on subjects such as physiology, pathology, microbiology, and pharmacology. As a result, bright minds from other health sciences and chemistry have become biochemistry converts. In addition, biochemistry (having no Nobel Prize established for itself) has repeatedly looted the Nobel Prize in chemistry and physiology or medicine.

As for the child, exercise biochemistry possesses all the freshness, grace, and promise hidden in youth. It aspires to answer the countless hows and whys born of observation and experimentation on physical activity by means of the delicate, precise, and demanding language of molecular interactions. This language takes human knowledge—itself a product of immensely complex molecular interactions—through one of its hardest tests. As such, exercise biochemistry serves as an abutment to exercise physiology, sports

medicine, sport nutrition, and other branches of the health sciences focusing on exercise.

The main purpose of this book is to serve as a self-inclusive source of teaching material for undergraduate and graduate courses in exercise biochemistry. Given the introduction of more and more such courses in universities around the world—and the indispensability of this discipline for developing an in-depth understanding of how exercise changes bodily functions—I think now, as I did at the time of the first edition, that exercise biochemistry should have its own stand-alone textbooks. Furthermore, it is my firm belief that the publication of such textbooks will encourage even more institutions to introduce exercise biochemistry courses in their curricula. Putting into a single volume the necessary elements of basic biochemistry, along with extensive coverage of exercise biochemistry topics, should facilitate the work of both instructors and students.

Exercise Biochemistry may also prove useful as a reference to graduate students, doctoral candidates, and researchers in sport science who did not have the opportunity to be formally introduced to this discipline during their undergraduate years. In addition, it can supplement exercise physiology textbooks by covering the molecular basis of physiological processes described therein. The book is also addressed to physical education and sport professionals interested in how the human body operates during and after exercise. Finally, it is addressed to health scientists who have been impressed or wish to be impressed by how exercise transforms human metabolism.

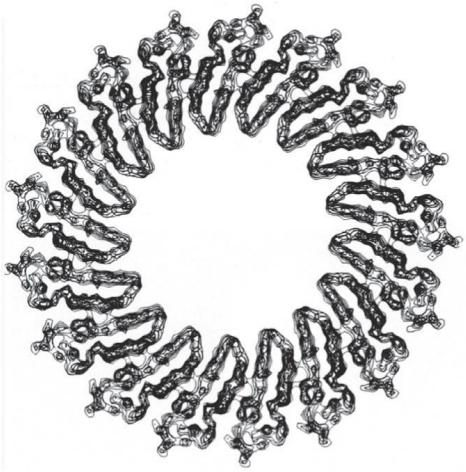
Considerable beauty can be found in modern biochemistry and exercise biochemistry. This beauty stems primarily from the revelation and appreciation of the ingenious ways in which animals have been solving—through how many failed efforts no one knows—the problems of self-conservation, reproduction, and response to the demands of movement on planet Earth for some hundred million years now. Beauty also exists in more tangible things, such as countless drawings and microscopic images (quite a few of which are presented here), as well as similarities with works of art such as the one presented below**.**

Interior of an ancient Greek pot of the sixth century BCE, filled with scenes of physical activity. In the perimeter, 17 young men dressed in women's gowns are dancing in worship of Hercules. In the middle, the demigod is wrestling with Triton, a sea monster, to force him to reveal where the golden apples of the Hesperides are kept.

Although books have been traditionally viewed as monologues delivered by their authors, I have tried, to the best of my abilities, to give this one the character of a conversation with the reader (dare I say, a long chat about exercise biochemistry). This is why I have interspersed the text with questions that sprang up in my head when I was a student (or later), questions that my students ask frequently during my teaching, and questions that I ask to prevent them from falling asleep. This is also why I have chosen to use mostly second person when addressing the reader.

I have written this book with the understanding that many of you may not feel comfortable with the laws of chemistry or, more generally, science. Therefore, I have used simple language, though without compromising scientific accuracy. Whether I have managed to maintain this delicate balance is up to you to decide. It is likewise up to you to determine whether this work has achieved its ultimate goal: to arm future sport scientists and physical education instructors with knowledge that will serve their professional, scientific, and personal needs while also protecting them from the ignorance, smatter, and misinformation that persist in many areas of exercise and sport. If it hasn't, I—and other hunters of the elusive trophy of excellence—may find consolation in the words that Peter Ustinov put in the mouth of Mr. Smith (the Devil) in one of his exquisite dialogues with the Old Man (God):

There is nothing in all of your
Creation as sterile, as lifeless, as
overwhelmingly negative as perfection.



Electron density map of a protein disc from tobacco mosaic virus (which attacks the leaves of the plant). Protein molecules in a helical array surround the genetic material of the virus; seventeen such molecules form a full circle. The similarity to the image on the left lies not only in the number but also in the angle and shape of the protein molecules, which look like people holding hands in a circular dance.

Courtesy of Dr. Aaron Klug.

A Guided Tour for the Student

This book is divided into 18 chapters organized in four parts. Part I presents basic biochemical information that is necessary for unhindered reading of the subsequent parts. It begins with an introduction to elementary bits of chemistry and biology (chapter 1), then continues with an overture to metabolism through general principles governing the exchange of mass and energy in living organisms, along with the biomolecules starring in this exchange (chapter 2). The next four chapters acquaint you with the major classes of biological compounds and nutrients. Chapter 3 belongs to proteins, which participate in almost every biochemical process. They are examined in terms of both structure and function, with special reference to the ones involved in oxygen transport and catalysis of reactions. Chapter 4 deals with nucleic acids and the flow of genetic information from DNA to RNA to protein. Chapter 5 describes the structure of carbohydrates and lipids (their metabolism is addressed in part III). Finally, chapter 6 considers vitamins and minerals in terms of utility, dietary sources, and consequences of deficiency.

Part II explores the biochemical basis of the neural (chapter 7) and muscular (chapter 8) processes that enable, for example, your lungs to fill with air, your heart to beat, and your eyes to move from left to right and back again as you read these lines. You will discover an amazing sequence of delicate, precise, and coordinated processes that endow us with the synonym of life: movement.

How does our metabolism change with exercise? This question is answered in part III. Its first four chapters examine the effects of exercise on the metabolism of the four classes of compounds that supply energy—specifically, compounds of high phosphoryl-transfer potential (chapter 9),

carbohydrates (chapter 10), lipids (chapter 11), and proteins (chapter 12). Chapter 13 describes how exercise alters the expression of our genes, which is a prerequisite for adaptations to training. Chapter 14 integrates all previous chapters of part III by examining the interaction and interdependence of energy sources during exercise; the factors governing their choice; the adaptations to the various types of training; and matters related to fatigue, recovery, and training. Finally, chapter 15 explores how exercise fights an impressive variety of chronic diseases.

Part IV shows how biochemical testing can aid an athlete or, more generally, an exercising person. The three chapters in this part examine two dozen parameters that provide useful information about health and performance. These parameters are divided into groups that indicate iron status (chapter 16), metabolites (chapter 17), and enzymes and hormones (chapter 18). Each parameter has its own value and contributes to forming the picture of an exerciser's health and performance.

Throughout the book, I use extensive cross-referencing to help you locate relevant information given elsewhere. To facilitate cross-referencing, I number sections, figures, tables, and equations in independent sequences within each chapter. Most of the equations are chemical; that is, they describe reactions. Hence, I use the term *reaction* when I refer to a chemical equation.

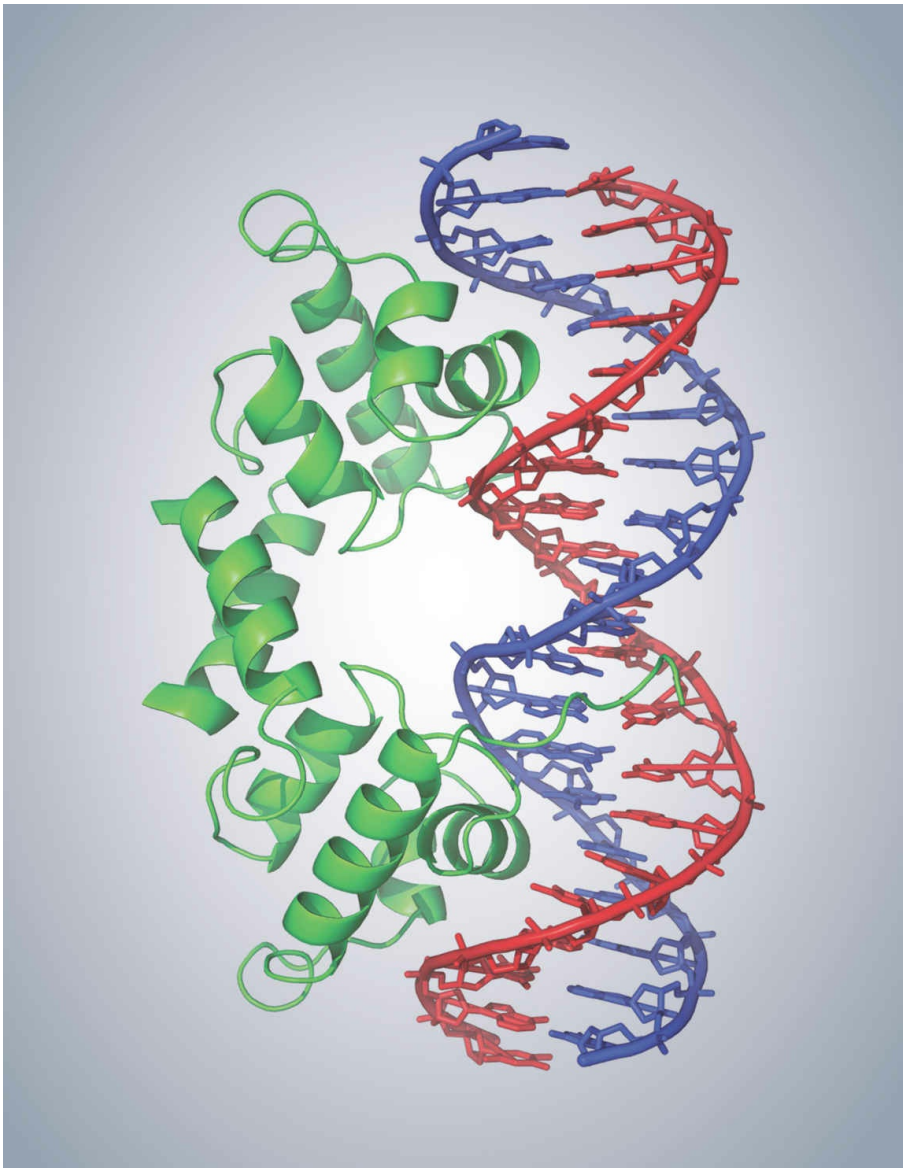
In the wide page margins, you will find explanatory notes, bits of nonbiochemical information to aid in understanding the text, brief descriptions of complicated processes (e.g., biochemical pathways), and trivia. Naturally, the margins are for your own notes too.

Each chapter begins with learning outcomes and closes with a summary, a collection of key terms, references and suggested readings, and problems and critical thinking questions. I recommend returning to the learning outcomes after you have finished a chapter in order to make sure that you have mastered them. Key terms are marked in bold throughout the text; terms that are also rendered in color are included in the glossary at the end of the book. There you will also find answers to the problems and questions, as well as an index.

Acknowledgments

Many people have helped bring this book to completion. My wife, Maria, created a warm and supporting environment at home, where I did most of my writing. Amy Tocco, my acquisitions editor, walked me through the first steps in this edition. Judy Park, my developmental editor, assisted me in greatly improving the manuscript. Joanne Brummett took good care of the artwork. Tom Tiller did fine copyediting work. Dalene Reeder managed permissions, Anne Mrozek assisted throughout the publication and proofing process, and Denise Lowry skillfully laid out the book.

I am thankful to David Bishop, Gregory Bogdanis, Claude Bouchard, Petros Dinas, Robert Fitts, Paul Gastin, Martin Gibala, Athanasios Kabasakalis, Spyridon Methenitis, Anatoli Petridou, and Russell Richardson for stimulating discussions and helpful suggestions. Finally, I owe many thanks to the numerous students (undergraduate, graduate, and doctoral) who have attended my classes and worked with me in the lab over the past 30 years. Through their love, inquisitiveness, encouragement, and demand, they became the catalysts (biochemically speaking, the enzymes) of this book.



The introductory figure for part I (facing page) depicts a protein (mostly in the form of α helices on the left) interacting with a DNA double helix on the right as if in a dance. It is this kind of interaction between our most versatile components (the proteins) and the repository of our genetic information (DNA) that enables DNA to dictate how an organism is built and how it functions. See chapters 3 and 4 for protein and DNA structure and function.

PART I

Biochemistry Basics

Organic chemistry is the chemistry of carbon compounds.
Biochemistry is the study of carbon compounds that crawl.

—Mike Adams

Biochemistry is one of the sciences dealing with the phenomenon of life. Along with biology, physiology, medicine, pharmacology, and other related sciences, it examines living organisms and their components with the aim of deciphering their structure and function. Humans exploit the knowledge produced by biochemical research to improve their quality of life and protect the environment.

What distinguishes biochemistry from other, cognate sciences is that it moves at the fundamental level of life's expression: the level of atoms, molecules, and their interactions within living systems. The major tool for such a science can be none other than the laws of chemistry. This is why it is called *biochemistry*—that is, the chemistry of life.

If one were to further explore the term *chemistry of life*, one would have no difficulty defining its first part. Indeed, chemistry can be easily defined as the science that deals with the structure and properties of substances, along with how they interact to produce other substances. Defining life, however, has proven challenging, to the degree that many resort to the stance expressed in the phrase "I know it when I see it." Of the many attempts made to

encapsulate the features of life that distinguish it from inanimate matter in a succinct definition, I would single out the one proposed by NASA: “Life is a self-sustaining chemical system capable of Darwinian evolution” (Joyce GF [1994]. Foreword. In Deamer DW, Fleischaker G, eds., *Origins of Life: The Central Concepts* [Jones and Bartlett, Boston], pp. xi-xii.).

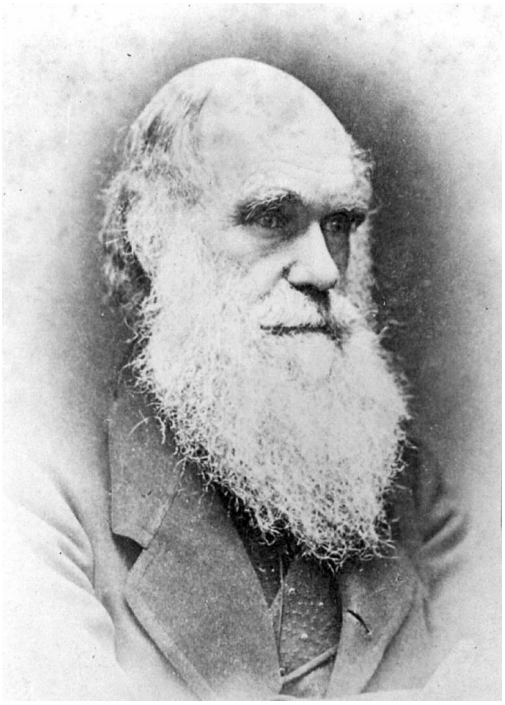


Figure I.1 **Charles Darwin.**
Courtesy of the Library of Congress, www.loc.gov/item/20027251182/.

Although an exhaustive analysis of this definition lies beyond our scope, one may note the following elements: the description of life as a *chemical* system; the assignment of self-sustainability, a feature that will be particularly evident throughout most of the chapters; and the capability for Darwinian evolution, which of course refers to the theory of evolution put forth by the British naturalist Charles Darwin (figure I.1) in the mid-19th century. Darwin changed the way we humans think of life and ourselves by proposing (jointly with Alfred Russel Wallace, also a British naturalist) not only evolution as the universal process of descent of living species (including *Homo sapiens*) from common ancestors but also natural selection as evolution’s driving force.

Here is an illustration of the variety of people's notions about the descent of living organisms on Earth. The Shuar, an indigenous people living in the tropical rain forest between the Andes and the Amazon Basin in Ecuador and Peru, believe in a kind of reverse evolution: Initially, all living things were human, but, depending on their good or bad behavior, they were transformed by the mighty spirit Arútam into different animals and plants. That world view may explain this people's (and many indigenous peoples') respect for nature: They consider animals and plants their siblings.

Let me close this brief account of what life is with an example pertinent to exercise biochemistry. One result of natural selection has been the appearance and perfection of biochemical processes ensuring the evolution of animals that are ever stronger, faster, and more resilient during their approximately one billion years of existence. For example, we may envisage that the chance appearance of phosphocreatine (a fast energy source for vertebrates) and creatine kinase (the enzyme that unleashes the energy of phosphocreatine)—both of which are addressed in detail in chapter 9—endowed animals with increased speed. In the case of an herbivore, this capability would boost its chance of outrunning a predator and, hence, surviving, reproducing, and bequeathing this advantage to its offspring. In the case of a carnivore, the same capability would boost its chance of outrunning its prey, with the same results as for the herbivore. By contrast, an herbivore or carnivore devoid of the advantage of speed would stand a smaller chance of surviving, reproducing, and passing on this trait to the next generation. Over time, and generation after generation, fast individuals in a population will outnumber and, finally, wipe out the slow ones; at the same time, high levels of phosphocreatine and creatine kinase will be naturally selected over low levels.

Part I of the book you are reading offers an acquaintance with the building blocks of living organisms (with a bias toward humans); the principles governing their functioning; and certain basic expressions of life, such as the catalysis of biochemical reactions and the flow of genetic information. In short, part I presents biochemical knowledge that is a prerequisite for entry into the world of exercise biochemistry addressed in parts II, III, and IV.

CHAPTER 1

Introduction

Learning Objectives

After reading this chapter, you should be able to do the following:

- List the six most abundant elements in living matter, along with their symbols, atomic numbers, and atomic masses, as well as the numbers of bonds their atoms form with other atoms.
- Distinguish a molecular formula from a structural one and attribute at least one advantage to each.
- Calculate molecular mass from a molecular formula and construct the molecular and structural formulas of some simple biological compounds.
- Distinguish anions from cations and recognize the structural formulas of an amino acid in its neutral and zwitterionic forms.
- Define radicals and provide an example.
- Define polar (or hydrophilic) compounds and nonpolar (or hydrophobic) compounds, explain the rules of miscibility of substances based on their polarity, and ascribe a substance to one of the two categories on the basis of its miscibility with water.
- Describe the terminology regarding solutions and concentration.
- Distinguish chemical reactions from physical processes and

provide examples of each.

- Describe the terminology concerning chemical reactions, state the principles that govern them, and apply these principles to balance a chemical equation and write down its equilibrium constant.
- Define pH; use pH value to recognize whether a solution is neutral, acidic, or alkaline; and calculate changes in pH from changes in proton concentration and vice versa.
- Calculate the concentrations of an acid and its conjugate base in solution based on knowledge of its dissociation constant.
- Discuss how buffer systems protect biological fluids from perturbations of the pH as part of the homeostasis of organisms.
- Name the classes of biological substances in living organisms and the classes of nutrients in the human diet.
- Distinguish prokaryotic from eukaryotic cells and identify the main components of an animal cell.

This introductory chapter of part I provides knowledge that is indispensable for unhindered reading of the remainder of the book. In particular, this chapter contains elementary concepts of chemistry, a presentation of the classes of biological substances and nutrients, and a brief description of the cell and its components.

1.1 Chemical Elements

The matter that surrounds us, and the matter of which we are made, is composed of **chemical elements**, such as hydrogen, oxygen, and carbon. The smallest unit of an element that maintains its properties is the **atom**. Every atom consists of a **nucleus** and surrounding **electrons**. The nucleus itself contains **protons**, which carry positive electric charge, and **neutrons**, which carry no electric charge, except for the hydrogen nucleus, which consists of a single proton (figure 1.1). Each electron, on the other hand, carries a negative

electric charge of equal absolute value to that of a proton. Thus, an atom—in which the number of electrons equals the number of protons—is electrically neutral.

In contrast to the widely held notion that electrons move around a nucleus in definite orbits, experimental data from 90 years ago have shown that electron movement is more sophisticated and can be better explained in terms of **atomic orbitals**. An atomic orbital is described by a complex mathematical equation, from which chemists can calculate the probability of finding an electron in a specific position relative to the nucleus. Schematically, the position of electrons is depicted by **electron clouds**, which are denser where it is more likely to encounter electrons and thinner where it is less likely to do so (figure 1.1).

Ninety-four elements exist in nature; of these, twenty-six are known with certainty to make up living organisms, and six occupy more than 97% of the mass of biological substances. These six elements are **hydrogen** (symbolized as H), **carbon** (C), **nitrogen** (N), **oxygen** (O), **phosphorus** (P), and **sulfur** (S). Some features of these elements are presented in table 1.1.

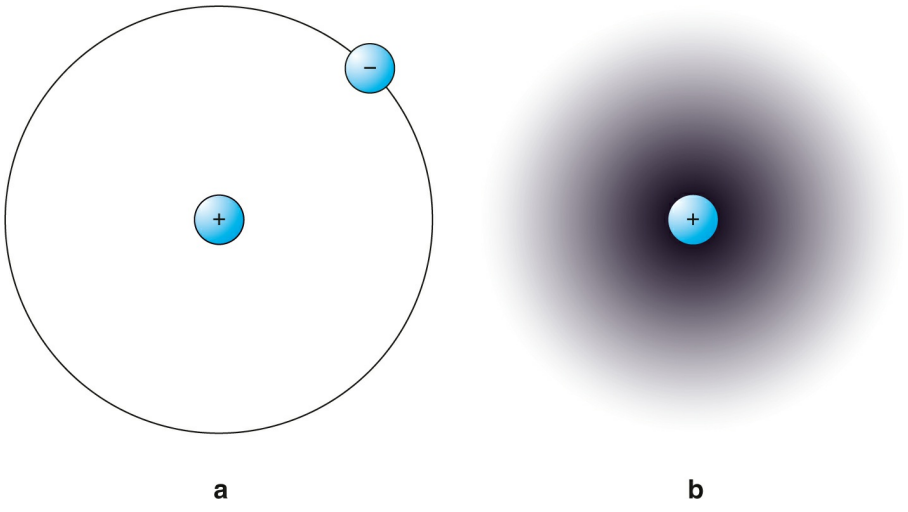


Figure 1.1 Atomic structure. The simplest of atoms, that of hydrogen (consisting of a positively charged proton forming the nucleus and a negatively charged electron), serves as an example of two ways to depict atomic structure. The so-called planetary model *(a)*, which suggests that electrons move around the nucleus in stable orbits, does not explain experimental findings that the atomic orbital model *(b)* does explain. According to the latter model, electrons move in a chaotic way that can be represented only by clouds, the thickness of which illustrates how much of its time an electron spends in a given place. In the case of hydrogen, the atomic orbital model predicts that the electron spends most of its time at the

nucleus (a picture quite different from that given by the planetary model) and infinitely less time as one moves away from the nucleus.

Table 1.1 The Most Abundant Elements in Living Organisms

Name	Symbol	Atomic number	Atomic mass (Da)a	Bonds with other atoms
Hydrogen	H	1	1	1
Carbon	C	6	12	4
Nitrogen	N	7	14	3
Oxygen	O	8	16	2
Phosphorus	P	15	31	5
Sulfur	S	16	32	2

[a]Rounded to the nearest integer and expressed in daltons (Da).

Atomic Number

The **atomic number** of an element indicates the number of protons in its nucleus; for the elements listed in table 1.1, the atomic number ranges from 1 to 16. Atomic number is characteristic of and unique to a given element, which means that two atoms of the same element must have the same number of protons. This is not the case with neutrons, which are protons' "roommates" in the nucleus. That is, two atoms of the same element may differ in their numbers of neutrons. Such atoms are called **isotopes**. To distinguish isotopes, we place a superscript number, equal to the sum of protons and neutrons, before the element's symbol. For example, though the vast majority of carbon atoms found in nature have six protons and six neutrons (and are thus symbolized as ^{12}C), a small percentage (1.1%) possess seven neutrons (and are thus symbolized as ^{13}C). In addition, a negligible proportion possess eight neutrons. (How would you symbolize them?)

Atomic Mass

The **atomic mass** (also known as **atomic weight**) of an element is the mass of one of its atoms. The unit of measure for atomic mass is the **dalton**. It is symbolized as Da and defined as one twelfth of the mass of a ^{12}C atom. Thus, one dalton is an inconceivably small mass, just $1.66 \cdot 10^{-24}$ g.

For the lighter elements, such as those listed in table 1.1, when the atomic mass is rounded up to the nearest integer, it coincides with the sum of protons and neutrons in the nucleus of the element's most abundant isotope. For instance, the atomic mass of hydrogen is approximately 1 Da, and its main isotope has only one proton in the nucleus, whereas the atomic mass of phosphorus is almost 31 Da, and its predominant isotope has 15 protons and 16 neutrons.

Size and Units of Measure

Having described the mass of atoms, it is now worth completing the picture by addressing their size, which is similarly infinitesimal. As a unit of measure, we use the **angstrom (Å)**, which equals 10^{-10} m. The atomic diameters of the six most abundant elements in living organisms range from 0.7 to 2.2 Å.

One may wonder: If the planetary model of atomic structure (in which the diameter of an atom is sharply defined by the orbit of its outermost electrons) is wrong, then how is the atomic diameter defined in the atomic orbital model, in which there are no definite boundaries of the electron clouds? Scientists address this question in a variety of ways, one of which is to measure the distance between nuclei when two atoms of the same element are bound together. It is assumed that the greatest possible closeness for two atoms is limited by their sizes.

Table 1.2 Base Units of the International System Used in the Book

Unit	Symbol	Quantity
Meter	m	Length
Kilogram	kg (not kgr)	Mass
Second	s (not sec)	Time
Mole	mol	Amount of substance

Before proceeding further, and since we have already considered some units of measure, it is useful to list, in table 1.2, the base units of the International System (SI, or, in French, *Système international*) that I use throughout this book (I also use several non-SI units, such as the angstrom).

In addition, to express multiples and submultiples of units, we frequently add prefixes to their symbols; some prefixes are presented in table 1.3.

Table 1.3 The Most Common Prefixes of Units

Symbol	Name	Equivalent to
M	mega	10^{6}
k	kilo	10^{3}
d	deci	10^{-1}
c	centi	10^{-2}
m	milli	10^{-3}
μ	micro	10^{-6}
n	nano	10^{-9}
p	pico	10^{-12}

1.2 Chemical Bonds

Atoms can form **chemical bonds** with atoms of the same element or of different elements. A chemical bond requires at least two electrons, which in most cases are contributed mutually by the atoms participating in the bond. According to the current scientific view, the movement of these electrons is constrained. Thus, whereas before bond formation that movement was described by isolated atomic orbitals, after bond formation it obeys the equations of new orbitals, termed **molecular orbitals**, because this is how molecules (described in the next section) form.

The bond formed when two atoms share electrons is called **covalent**. If each atom contributes one electron, then a single bond forms; it is symbolized by a thin line between the atoms. Two atoms may be linked by a double bond or even a triple bond, which are, respectively, formed by two or three electron pairs and symbolized by a double or triple line.

The number of bonds that an atom can form is dictated by the distribution of electrons in its atomic orbitals; this distribution is called the *electronic structure*. As we will see, knowing this number is essential for building the structural formulas of molecules. The numbers of covalent bonds formed by the six most abundant elements in living organisms are shown in the last column of table 1.1. Thus, the atoms of the elements that compose biological

substances can be joined covalently to one, two, three, or four atoms. (Although table 1.1 shows that phosphorus can form five bonds, two of them are directed toward one atom as a double bond; therefore, P bonds with four atoms.) Figure 1.2 presents the atoms of these elements and their bonds.

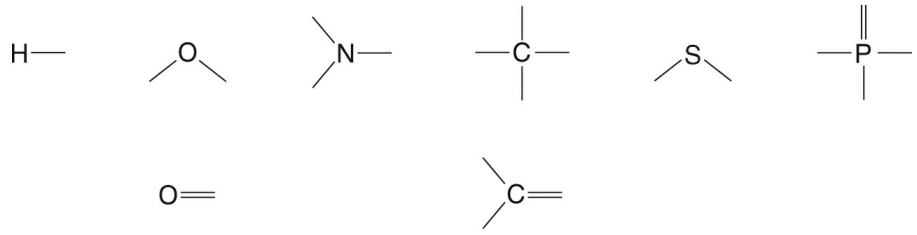


Figure 1.2 **Atoms with their bonds.** Because of the nature of the atomic orbitals in which their electrons fit, the atoms of the six main elements of life form specific numbers of bonds with other atoms, as shown here. The bonds are projected onto the plane of the paper, but those of N, C (when surrounded by four single bonds), and P do *not* all lie on the same plane.

1.3 Molecules

Atoms are joined by covalent bonds to form **molecules**. For example, two hydrogen atoms connected by a single bond form a hydrogen molecule. If, in contrast, the molecules of a substance are composed of atoms belonging to different elements, then the substance is called a **compound**. Water, consisting of two hydrogens linked to an oxygen, is the most abundant compound in our bodies. It is an **inorganic** compound, because it contains no carbon; however, most biological compounds are **organic** (i.e., they contain carbon).

Molecular Formula

What a compound is made of is depicted by its **molecular formula**, which contains the symbols of the elements present in a molecule of the compound, written in a row, and the number of atoms rendered as a subscript numeral to the right of the relevant symbol if it exceeds one. Thus, the molecular formula of water is H_2O. From a molecular formula, we can calculate the **molecular mass** (or **molecular weight**)—that is, the sum of the atomic masses (or atomic weights) of the elements constituting the molecule.

Naturally, we must first multiply the atomic mass of each element by the number of its atoms in the compound. Like atomic mass, molecular mass is measured in daltons; thus the molecular mass of water is 18 Da (1 · 2 + 16).

By convention, the elements of an organic compound are lined up in the following order in its molecular formula: C, H, and then the rest in alphabetical order.

If we substitute grams for daltons in the expression of molecular mass, we get one **mole** of the compound, which is symbolized as mol (see table 1.2). Thus, 1 mol of water is 18 g. One mole of any compound contains the same number of molecules, which is $6.022 \cdot 10^{23}$. This is **Avogadro's number**, named after the Italian scientist Amedeo Avogadro, in honor of his contribution to molecular theory in the 19th century.

Given the definition of a mole, it follows that in order to convert grams of a substance into moles one divides by the molecular mass, whereas in order to convert moles of a substance into grams one multiplies by the molecular mass.

Structural Formula

In addition to the kind and number of atoms in a molecule of a compound, chemists are interested in the way in which atoms are connected. Such information is provided by **structural** (or **constitutional**) **formulas**, which we can build by knowing the number of bonds that each atom can form. This is where the last column of table 1.1 comes into play. The rule is that each atom must be surrounded by as many bonds as that column dictates. Verify the rule by examining the compound in figure 1.3*a*, which is the amino acid alanine. (Amino acids are dealt with in chapter 3.)

Structural formulas can be either detailed (showing all bonds among atoms) or abbreviated. For example, we can simplify the formula of alanine by substituting —COOH for the group of atoms at the right-hand side of the molecule, known in organic chemistry as the **carboxyl group** (figure 1.3*b*). Likewise, we can substitute $—NH_2$ for the group of atoms on the left, known as the **amino group**, and $—CH_3$ for the group of atoms at the top, known as the **methyl group**. Two compounds may have the same molecular formula

but different structural formulas because of different configuration. Such compounds are called **isomers**.

Groups (such as the carboxyl, amino, and methyl groups) are assemblies of atoms that cannot stand alone but form parts of molecules or ions. As a result, they are depicted with at least one bond extending from them.

Two compounds differ in **configuration** if one cannot be converted into the other without breaking and reforming certain covalent bonds. If, to the contrary, one can be converted into the other by merely twisting part of it, then we say that they differ in **conformation**.

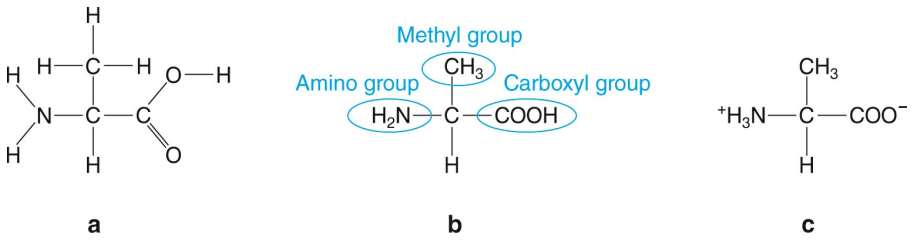


Figure 1.3 **Structural formulas.** Chemical compounds arise by the linking of atoms of different elements with covalent bonds. Pictured here are structural formulas for a relatively simple biological compound known as alanine. All bonds are shown in *(a)*, whereas for the sake of brevity only the bonds around the central carbon atom are shown in *(b)*, in which the bonds within the amino, carboxyl, and methyl groups have been removed. This form of representation (omitting the most common bonds) is more usual. In aqueous (watery) solutions, such as biological fluids, alanine is ionized *(c)*.

1.4 Ions

Molecules are electrically neutral, because they are composed of neutral atoms. However, most compounds in biological fluids take the form of **ions**; that is, they carry electric charges, because some atoms are more stable if they have more or fewer electrons than protons. In the former case, the ion carries a negative charge, is termed an **anion**, and is symbolized by the addition of a superscript minus sign to the right of its molecular formula (say, X^-). In the latter case, the ion carries a positive charge, is termed a **cation**, and is symbolized by the addition of a superscript plus sign to the right of its molecular formula (say, Y^+). An ion may carry more than one charge, in which case the number of charges is placed in front of the plus or minus sign

(say, Z^{2+}).

In biochemistry, for the sake of simplicity we often take the liberty of referring to ions as molecules. For example, we refer to the ATP molecule or to a protein molecule, although, as we will see, they are predominantly ionic in biological fluids.

When a charge is introduced, the number of bonds formed by an atom changes: Atoms bearing one negative charge form one bond fewer than the number indicated in table 1.1, whereas atoms bearing one positive charge form one bond more.

A molecule in a biological fluid can be easily converted into an ion through the exchange of one or more hydrogen cations (H^+) with its surroundings. H^+ is nothing more than a proton, since this is what is left of a hydrogen atom when we strip it of its sole electron. Being that small, H^+ is extremely mobile. Where does H^+ come from? Water (the main constituent of biological fluids) dissociates to a small degree into H^+ and hydroxyl anion (OH^-), thus supplying material for the formation of ions.

Because of their atomic makeup, some groups, such as the carboxyl group, tend to release a proton, thus acquiring a negative charge (—COO^-). In contrast, other groups, such as the amino group, tend to attract a proton, thus acquiring a positive charge (—NH_3^+). An example of an ion (in fact, an ion bearing two charges) is presented in figure 1.3*c*. Note that an ion, such as that of alanine, may be neutral as a whole if the positive charges equal and cancel out the negative ones. Ions of this kind are termed **zwitterions** (*zwitter* is German for “both at the same time”).

1.5 Radicals

Most molecules and ions feature electrons in pairs, but some have one or more unpaired electrons. Such chemical entities are called **radicals** (or **free radicals**, which is a redundant term), and we may denote the unpaired electron by a superscript dot next to the molecular formula. A dot is not to be confused with a negative charge; it is just there to raise awareness of an unusual feature.

One example of a radical is nitric oxide, or NO (it is usually depicted

without a dot). Since it carries an odd number of electrons (15, to be exact—7 from N and 8 from O), it is inevitably left with one unpaired electron; nevertheless, it carries no charge. A similar example is the hydroxyl radical, or HO. (not to be confused with the hydroxyl ion), which, as shown, is usually depicted with a dot. One example of a charged radical is superoxide, which is produced by the addition of an electron to an oxygen molecule (O_2) and is symbolized as $O_2^{\cdot -}$. This symbolism carries both the (unnecessary) dot and the (necessary) minus sign.

As we will see in section 9.6, radicals are produced naturally in the body and increase during exercise. In fact, their role in mediating many effects of exercise on the body is increasingly appreciated, as we will see in chapter 14.

1.6 Polarity and Miscibility

Although the positive and negative charges are equal in the neutral molecules of any compound, they may not be evenly distributed. The reason is that the nuclei of some atoms (notably N and O) attract bonding electrons more strongly than do the nuclei of other atoms (notably H and C). Thus, atoms of the former kind acquire a **partial negative charge** (symbolized as δ^- and pronounced delta minus), whereas atoms of the latter kind acquire a **partial positive charge** (δ^+). In such molecules, we can discern one negative and one positive electric pole, and we call the compound **polar** (figure 1.4). On the other hand, if charges are evenly distributed within the molecules of a compound, we call it **nonpolar**.

Molecules of an element (for example, O_2) are nonpolar, because they are composed of identical atoms whose nuclei have the same strength to attract electrons.

In organic chemistry and biochemistry, molecules or groups that contain only C and H are nonpolar because the nuclei of the two elements attract bonding electrons to a similar degree. In contrast, molecules or groups that contain N, O, or S connected to C or H are usually polar because the nuclei of the former elements attract bonding electrons more strongly than do the nuclei of the latter elements.

The **polarity** of a substance (that is, whether and to what degree it is polar) affects an important physical property: its **miscibility** (that is, its ability to mix) with other substances. Here's how:

- Polar substances tend to mix with polar substances.
- Nonpolar substances tend to mix with nonpolar substances.
- A polar substance and a nonpolar substance do not mix readily with each other.

Thus these interactions follow the saying "Birds of a feather flock together."

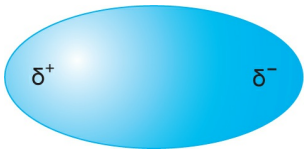


Figure 1.4 **Polar compound.** An uneven distribution of charges within the neutral molecule of a compound results in the appearance of two poles having partial (less than one) positive and negative charges.

1.7 Solutions

When the mixing of two or more substances results in a homogeneous mixture—that is, a mixture having the same composition all over its mass—we call that a **solution**. In a solution, we usually distinguish the **solvent**, which is the substance present in the highest proportion, from the **solute(s)**, which consists of the dissolved substance(s). We define the **concentration** of a solute as the amount of it that is contained in a certain amount of solution or solvent. A common unit of concentration is mole per liter of solution (mol/L, or $\text{mol} \cdot \text{L}^{-1}$, or M), or molar concentration.

A liter (symbolized as L or l, not lt) is a non-SI unit of volume equal to one thousandth of a cubic meter ($1\ \text{L} = 10^{-3}\ \text{m}^3$).

The term *concentration* is often used loosely for mixtures, such as many biological fluids, which do not qualify as solutions because they are not homogeneous. The concentrations of substances dissolved in biological fluids

are relatively low. The highest ones are on the order of 10^{-2} mol · L^{-1}, whereas the lowest ones go as far down as 10^{-12} mol · L^{-1}.

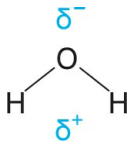


Figure 1.5 **The polarity of water.** The molecule of water is polar for two reasons. First, its two bonds are not aligned; rather, they form a 105° angle. Second, the nucleus of oxygen attracts the bonding electrons more strongly than do the nuclei of hydrogen. Thus, oxygen carries a partial negative charge, whereas the area between the hydrogens presents a partial positive charge.

The solvent in biological systems is water. It is a polar compound (figure 1.5) and, as such, mixes readily with other polar compounds, which are thus called **hydrophilic** (meaning “water loving” in Greek). One example of a hydrophilic compound is sugar. In contrast, nonpolar compounds do not mix readily with water and are thus called **hydrophobic** (“water fearing”). Examples of hydrophobic compounds include oils. Naturally, nonpolar compounds dissolve in nonpolar solvents, and one common nonpolar solvent is paint thinner.

Polar is hydrophilic; nonpolar is hydrophobic.

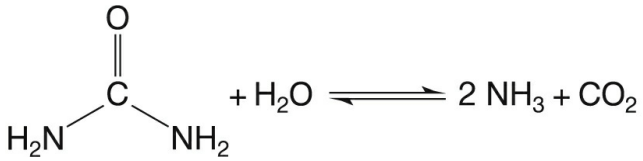
1.8 Chemical Reactions

Often the atoms, molecules, or ions present in a mixture do not stay inert but instead interact to form new chemical entities. These interactions are called **chemical reactions**, and they differ fundamentally from physical processes, in which no new chemical entities are produced. For example, the dissolution of sugar in a cup of coffee is a physical process, because it forms nothing new—the sugar molecules just go from being embedded in a crystal to being surrounded by water molecules. In contrast, the burning of a piece of wood involves several chemical reactions, one of which is the conversion of cellulose into carbon dioxide and water.

Is life the outcome of chemical reactions or physical processes? Both!

Thousands of chemical reactions take place within living organisms to enable them to produce energy and build their components. On the other hand, numerous physical processes, such as the dissolution or diffusion of an ion into a biological fluid or the binding together of two molecules, let biological molecules interact and convey messages. In fact, most biological processes involve both chemical and physical interactions in the aqueous environment of biological fluids.

The substances consumed during a chemical reaction are termed **reactants**, whereas the substances produced are the **products**. To represent a reaction, chemists write its equation, which includes the reactants on the left side and the products on the right. The two sides are usually divided by a rightward-pointing arrow or by two opposite-facing harpoons. Here is an example of a chemical equation.



(equation 1.1)

Equation 1.1 represents the **hydrolysis**, or breakdown by water, of urea (a simple biological compound addressed in sections 12.9 and 17.10) into ammonia and carbon dioxide. The bidirectional harpoons signify that the reaction is **reversible**, meaning that it can also go the other way under certain conditions. The reverse reaction represents the synthesis of urea from ammonia and carbon dioxide, in which case the latter two become the reactants while urea and water become the products. The way in which a reversible reaction will go depends on energy factors explored in section 2.1.

Chemical reactions are governed by the **principle of mass conservation**, which dictates that atoms neither form nor vanish (they are only rearranged). Because of this principle, the two sides of a chemical equation must have the same kind and number of atoms. To ensure the principle, one may have to add numbers in front of some reactants or products. In the case of equation 1.1, the number 2 is inserted in front of ammonia.

Unlike chemical reactions, nuclear reactions involve the vanishing of existing atoms and the formation of new ones.

Reactions involving ions are governed additionally by the **principle of charge conservation**, which requires that the algebraic sum of charges, or net charge, be equal on the two sides of the equation. To achieve this equality, one may have to add one or more H^+ to one of the two sides.

A **balanced chemical equation** is one that complies with the two principles just laid out. As compared with an unbalanced equation, it provides the advantage of offering quantitative as well as qualitative information about the reaction it depicts. In other words, it shows not only which compounds react and what products result but also what the proportions of molecules and moles are. Thus, equation 1.1 informs us that one mole(cule) of urea and one mole(cule) of water produce two mole(cule)s of ammonia and one mole(cule) of carbon dioxide. All chemical equations in this book are balanced.

1.9 Chemical Equilibrium

If a reaction is left to proceed far enough, it reaches a state in which no further change is detected in the concentration of any participating substance. This state is called **equilibrium** and is described by the **equilibrium constant (K_{eq})**, which is the ratio of the (mathematical) product of the molar concentrations of the reaction products to the product of the molar concentrations of the reactants at equilibrium. Thus, for equation 1.1, K_{eq} is:

$$K_{eq} = \frac{[NH_3]^2[CO_2]}{[H_2NCONH_2][H_2O]} \qquad \text{(equation 1.2)}$$

The brackets around the formula or name of a substance denote its concentration. Thus, $[NH_3]$ denotes the concentration of ammonia. If a substance participates in a reaction by more than one molecule (as is the case with ammonia in our example), then the K_{eq} expression must contain its concentration raised to its number of molecules—hence $[NH_3]^2$.

The value of K_{eq} depends on the nature of the reactants and products, the temperature, the pressure, and, occasionally, the presence of other substances in the reaction medium. K_{eq} shows the direction and degree to which a reaction proceeds, since the higher it is the farther to the right the reaction will go, resulting in the presence of high concentrations of products and low

concentrations of reactants at equilibrium. Certain reactions go almost completely (quantitatively, as we say) in one direction under certain circumstances and are characterized as **irreversible**. In such cases, we can use a unidirectional arrow. The K_{eq} of an irreversible reaction is infinite, since at equilibrium there are no reactants, which results in the denominator of the K_{eq} expression being zero.

1.10 pH

The ease with which protons are detached from or added to chemical compounds (as in the formation of ions) endows them with an important role in chemical processes, such that the outcome of many reactions depends on the concentration of protons, or $[H^+]$. Because $[H^+]$ is usually very low (for example, 10^{-7} mol · L^{-1} in pure water as a result of its small-scale dissociation into H^+ and OH^-) and, hence, a bit difficult to express, chemists have devised a convenient index of it. That index, **pH**, is defined as the negative decimal logarithm of the molar concentration of protons.

$$\mathrm{pH} = -\log_{10}\left[\mathrm{H}^+\right], \text{ or } \left[\mathrm{H}^+\right] = 10^{-\mathrm{pH}} \qquad \text{(equation 1.3)}$$

pH is a dimensionless quantity (it has no units). The pH of pure water is 7. (Just compare the information provided in the previous paragraph, $[H^+] = 10^{-7}$, with the second form of equation 1.3, $[H^+] = 10^{-pH}$, and you will see that pH = 7.) When the pH of a solution is 7, the solution is **neutral** (figure 1.6). If an acid (defined here as a proton donor) is added to water, then the $[H^+]$ increases and the pH drops below 7 (because of the negative sign on the right-hand side in equation 1.3). The solution then becomes **acidic**; examples of acidic solutions include lemon juice and vinegar. If, on the other hand, a base (defined here as a proton acceptor) is added to water, then the $[H^+]$ decreases and the pH rises above 7. The solution then becomes **alkaline**, or **basic**; examples of alkaline solutions include whitewash and soapy water. The pH of most biological fluids is nearly neutral and is referred to as **physiological**. As we will see in subsequent chapters, this pH can change with exercise. The pH of blood is 7.4.

Do not confuse the electric neutrality of a molecule with the pH neutrality of a solution; the two concepts are not related.

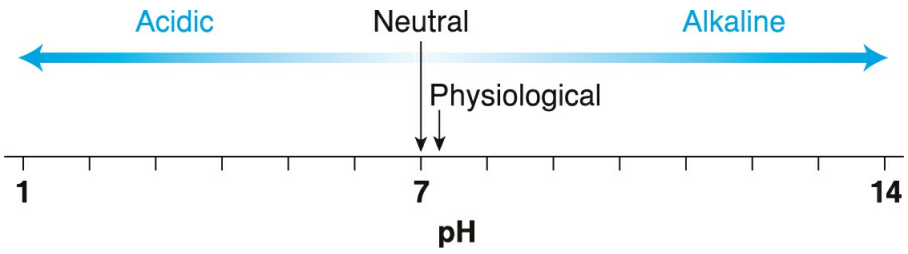
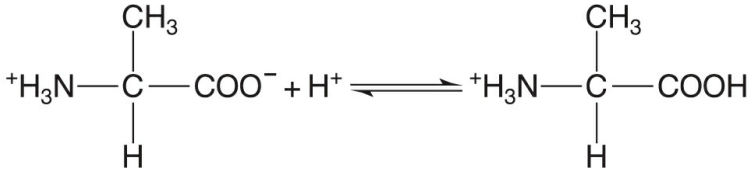


Figure 1.6 **The pH scale.** pH is an index of a solution's acidity (that is, how acidic it is) and plays an important role in biochemical processes. Shown here is its most useful range (1 to 14).

1.11 Acid–Base Interconversions

If a compound in aqueous solution can exchange protons with its surroundings, then its form is not fixed but depends on the pH of the solution. Such a compound binds protons when the pH decreases (because the $[H^+]$ increases and there are more H^+ to bind) and loses protons when the pH increases (because the $[H^+]$ decreases and some H^+ tend to dissociate).

Let's explore these transitions by using alanine as an example. The predominant form of alanine in a neutral solution is the one shown in figure 1.3*c*. If a strong acid (such as hydrochloric acid, HCl, dissociating fully into H^+ and Cl^-) is added, its H^+ will tend to associate with the negatively charged carboxyl group of alanine (because of electrostatic attraction between opposite charges) and neutralize it.



(equation 1.4)

or $A + H^+ \rightleftharpoons AH^+$ if we substitute A for alanine. We refer to the K_{eq} of the reverse reaction (that is, the dissociation of AH^+ into A and H^+) as the **dissociation constant** (***K***, omitting for the sake of simplicity the subscript *a* that is added next to *K* in chemical texts).

$$K = \frac{[A][H^+]}{[AH^+]} \qquad \text{(equation 1.5)}$$

We further define pK as –log K (in accordance with the definition of pH). We can now introduce pK and pH into equation 1.5 by taking the logarithms of its two sides.

Remember that log (x · y) = log x + log y.

$$\log K = \log \frac{[A]}{[AH^+]} + \log [H^+] \qquad \text{(equation 1.6)}$$

Therefore,

$$-\log [H^+] = -\log K + \log \frac{[A]}{[AH^+]} \qquad \text{(equation 1.7)}$$

Therefore,

$$\text{pH} = \text{p}K + \log \frac{[A]}{[AH^+]} \qquad \text{(equation 1.8)}$$

which is known as the **Henderson-Hasselbalch equation**. This equation establishes a relationship between pH, pK, and the concentration ratio of a base to its conjugate acid, thus permitting the calculation of any of the three when the other two are known.

The Henderson-Hasselbalch equation dates to 1916 and owes its formulation to the American biochemist Lawrence Joseph Henderson and the Danish physician Karl Albert Hasselbalch.

Two compounds differing by one H^+ are known as conjugate acid (the protonated one) and conjugate base.

An interesting relationship arises when pH = pK. According to the Henderson-Hasselbalch equation,

$$\log \frac{[A]}{[AH^+]} = 0$$

Therefore,

$$\frac{[A]}{[AH^+]} = 1$$

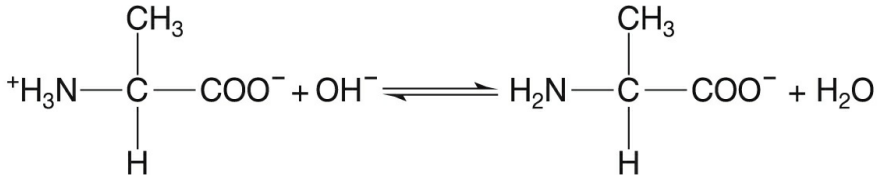
or

$$[A] = [AH^+]$$

Thus, *equal concentrations of a conjugate acid and base are present when pH = p*K. For example, the p*K* of alanine (regarding the dissociation of its carboxyl group) is 2.3. Hence, at pH 2.3, half of it carries an ionized carboxyl group and half carries a nonionized carboxyl group.

It also follows from the Henderson-Hasselbalch equation that *the conjugate acid predominates when pH < p*K, *whereas the conjugate base predominates when pH > p*K. This is why the carboxyl group of alanine is mostly deprotonated in a neutral solution.

Let's consider now what happens when we add a strong base (such as sodium hydroxide, NaOH, dissociating fully into Na^+ and OH^-) to a neutral alanine solution. The predominance of OH^- over H^+ will result in the dissociation of some H^+ from the positively charged amino group of alanine to combine with OH^- and form H_2O.



(equation 1.9)

The p*K* of alanine with regard to the dissociation of its amino group is 9.9. By analogy to the previous discussion, at pH 9.9, half of alanine will carry an ionized amino group

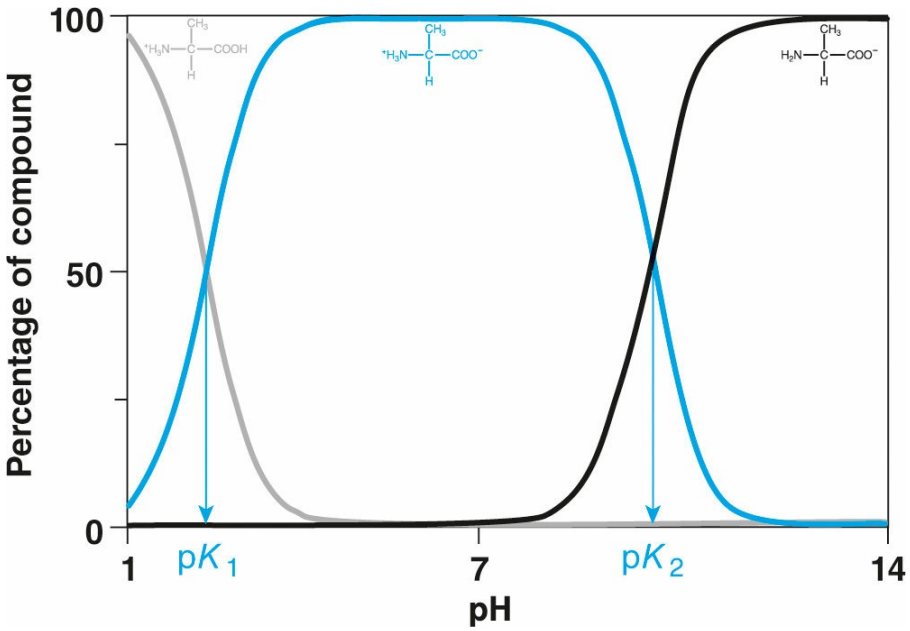


Figure 1.7 **The importance of pH.** Alanine, having two groups that can serve as proton donors and acceptors (the amino and carboxyl groups), serves as a telltale example of the role played by pH in determining the predominant form of a compound in solution. Starting at pH 1 and moving on to higher values, the cationic form gives way to the zwitterionic one, which gives way to the anionic one at very high pH values. The cationic and zwitterionic forms coexist in equal amounts at pH = pK_1 = 2.3, whereas the zwitterionic and anionic forms coexist in equal amounts at pH = pK_2 = 9.9.

and half of it will carry a nonionized amino group, while the amino group will be mostly protonated in a neutral solution. Figure 1.7 summarizes, in graphical form, the dependence of the form of alanine on pH.

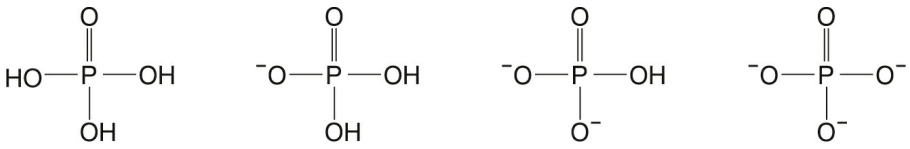


Figure 1.8 **The many faces of an acid.** For an extreme example of the different forms that an ionizable compound can take, consider phosphoric acid (H_3PO_4). Its three hydrogens are detachable, which gives rise to three ions in addition to the nonionized molecule. As the pH rises, there is a gradual transition from phosphoric acid to phosphate. Phosphoric acid has three pK: 2.1, 7.2, and 12.3.

Throughout this book, molecular and structural formulas of ionizable

compounds depict the predominant form at physiological pH. In addition, the names of acids reflect the fact that they are ionized at physiological pH. Thus I refer to phosphate rather than phosphoric acid (figure 1.8 and section 2.3), aspartate rather than aspartic acid (section 3.1), palmitate rather than palmitic acid (section 5.7), lactate rather than lactic acid (section 10.19), and so on. See also problem 10 on lactic acid and lactate at the end of this chapter.

1.12 Buffer Systems

Because changes in the pH of a solution affect the form of the ionizable solutes, which in turn affects interactions and reactions among them, cells and multicellular organisms have evolved ways to maintain constancy in the pH of their fluids. This is just one expression of **homeostasis**, defined as the maintenance of a biological parameter at a relatively stable level within an organism despite temporary fluctuations. The biological mechanisms accountable for homeostasis, or *homeostatic* mechanisms, protect organisms against instability that may prove threatening or even lethal.

A buffer system is analogous to the water-absorbing gel that is sometimes added to the soil in gardening. Thanks to its chemical structure, the gel can absorb amounts of water many times its weight. Thus, when a plant is overly watered, the gel swells and retains much of the water. In contrast, when the plant is not watered and the soil runs dry, the gel releases its water for the roots to absorb. If you substitute a buffer system for the gel and protons for the water, then you will get the analogy.

To protect the pH of their fluids against perturbations caused by the production of acids or bases, living organisms contain compounds of high **buffer capacity**, which is defined as the amount of strong acid or strong base that needs to be added to one liter of a solution in order to change the pH by one unit. Such compounds typically come in pairs of conjugate acid and base, which are interconverted upon absorbing or releasing H^+ (as in reactions 1.4 and 1.9). These pairs are called **buffer systems** and constitute homeostatic mechanisms that prevent acids and bases from changing the pH.

A buffer system is not efficient at just any pH value. Rather, its buffer capacity is maximal at pH = p*K*, where the concentrations of the conjugate

acid and base are equal. The buffer capacity gradually decreases as the pH draws away from the p*K*. We will consider three important buffer systems—based on proteins, bicarbonate, and phosphate—later in this book (sections 3.5, 3.12, and 9.4, respectively).

1.13 Classes of Biological Substances

Studying biochemistry is facilitated by dividing biological substances into classes. More versatile and, hence, more interesting are the organic compounds, for which the term **biomolecules** has been reserved. There are four major classes of biomolecules (there are also biomolecules that do not belong to any of these classes). In order of their presentation in chapters 3 through 5, the classes are **proteins**, **nucleic acids**, **carbohydrates**, and **lipids**.

These classes differ greatly in structure and function. Most of their molecules are large and are characterized as **macromolecules**. How large are they? They can contain millions of atoms. To study them would be despairingly difficult if they did not consist of smaller units that are identical or similar to each other. These building blocks, which are easier to study, are called **monomers** (meaning "single parts" in Greek), whereas the macromolecules that result from joining monomers together are **polymers** ("multiple parts").

Apart from organic substances, living organisms also contain inorganic substances. Water alone constitutes more than half of the mass of most living organisms. In addition, a multitude of inorganic ions are dissolved in biological fluids or bound to organic compounds. Most abundant among these ions are sodium (Na^+, deriving from *natrium*, the name of the element in Latin and many other languages); potassium (K^+, deriving from *kalium*); chloride (Cl^-); calcium (Ca^{2+}); magnesium (Mg^{2+}); hydrogen carbonate, or bicarbonate (HCO_3^-); and hydrogen phosphate (HPO_4^{2-}). These seven expand the number of elements we have met in living organisms to eleven.

1.14 Classes of Nutrients

Human biochemistry and exercise biochemistry are intimately linked to

nutrition for a variety of reasons:

- We are unable to grow, maintain our tissues, and perform bodily functions unless we regularly receive food.
- Exercise performance and many of the biological responses to exercise depend on the amounts and kinds of food and dietary supplements that one takes.
- Diet affects several biochemical parameters that exercise biochemists monitor, both in athletes and in exercisers more generally, in order to evaluate health and sport performance.

Thus, although this is not a book about nutrition or sport nutrition, your study of its subject matter will be greatly facilitated by some knowledge of human nutrition. As a start, let me define and list the nutrients in the human diet.

A **nutrient** is any food component that serves one or more of the following three purposes: provision of energy, provision of raw materials for the building of tissues, and regulation of bodily functions. These uses may seem a bit vague, but they will become clearer as we dwell on each one in subsequent chapters, starting with chapter 2.

As with biological substances, the study of nutrients is facilitated by grouping them into classes. In this case, the classes are carbohydrates, **fats**, proteins, **vitamins**, and **minerals**; there are also nutrients, such as water, that do not belong to any class.

It is important to distinguish between nutrients and foods. Nutrients are food components; foods, on the other hand, are complex mixtures of many nutrients. Understanding this difference enables us to avoid the common misconception of identifying a food with a nutrient, which can result in overlooking certain foods as sources of certain nutrients. For example, it is wrong to say that pasta *is* carbohydrate or, worse, pure carbohydrate. It also contains considerable amounts of proteins, vitamins, and minerals, not to mention that more than 50% of cooked pasta is water. The right thing to say, then, is that pasta is a good source of carbohydrate. Likewise, it is wrong to say that chicken *is* protein or, worse, pure protein. Chicken meat also contains considerable amounts of fats, vitamins, and minerals, and it too consists of more than 50% water. With this makeup in mind, what would be

the right thing to say about chicken?

> Excellent information about the nutrient content of foods can be found in the U.S. Department of Agriculture Food Composition Databases, which are available at https://ndb.nal.usda.gov/ndb/search/list.

You may have noticed some overlap between the classes of biological substances and the classes of nutrients—specifically, carbohydrates and proteins, as well as fats, which are just lipids by another name (the one preferred by nutritionists). Thus, when we run into these three classes of substances in subsequent chapters, we will examine them as components of both our bodies and our food. The remaining class of organic biological compounds—nucleic acids—are not considered nutrients in nutrition science (though they serve the regulation of bodily functions). Vitamins, on the other hand, do not qualify as a major class of biological compounds because of their minute amounts in tissues. Finally, the nutrient class of minerals coincides with the class of inorganic substances mentioned in the previous section.

Carbohydrates, fats, and proteins are collectively referred to as **macronutrients**, meaning nutrients that we consume in large quantities. Indeed, a regular diet contains tens to hundreds of grams of each of these classes daily. In contrast, vitamins and minerals are known as **micronutrients** because we consume only a few grams or even less than a gram of each of these classes daily.

1.15 Cell Structure

The **cell** is the building block of living organisms. Primitive organisms, such as bacteria, are unicellular, whereas organisms that appeared later in the course of evolution are multicellular. The human body is thought to contain approximately 10^{13} to 10^{14} human cells, while a systematic calculation by Eva Bianconi and coworkers narrowed the number down to $3.72 \cdot 10^{13}$ (that is, 37.2 trillion) for a 30-year old person weighing 70 kg and measuring 1.72 m in height.

Referring to *human* cells in the human body may sound redundant, but it isn't. The reason is that we host a vast variety of microbes, located mainly in the skin and along the alimentary tract, which are believed to outnumber our own cells by a factor of ten.

Cells vary in size. A bacterial cell measures about 1 μm in diameter, whereas the cells of multicellular organisms are usually 10 to 100 times larger. An average human cell measures about 25 μm in diameter.

Cells of multicellular organisms also vary by type. For example, cells in the skin are different from those in muscle, which are different from those in the brain, in terms of appearance, content, and function. Some 200 cell types have been identified in the human body, mainly with the aid of microscopy.

Despite this great diversity of cells—both within an organism and from species to species—they also have many features in common (figure 1.9). Beginning with their boundaries, all cells are enclosed in a membrane called the **plasma membrane**, which separates cell contents from extracellular space. The plasma membrane is just about 50 Å thick and consists of lipids and proteins, to which carbohydrates may be attached.

The interior of a cell is called the **cytoplasm**. Its main component is water, which surrounds a multitude of molecules, ions, and molecular complexes. The cytoplasm is fairly uniform in the simplest of cells, the **prokaryotic cells**. Prokaryotes were the first form of life to appear on Earth some 3.5 billion years ago and comprise two large groups: bacteria and archaea. On the other hand, multicellular organisms (and many unicellular organisms), which appeared on Earth during the past two billion years, contain cells that are more complex and are called **eukaryotic cells**. Eukaryotes comprise four kingdoms: protists, fungi, plants, and animals.

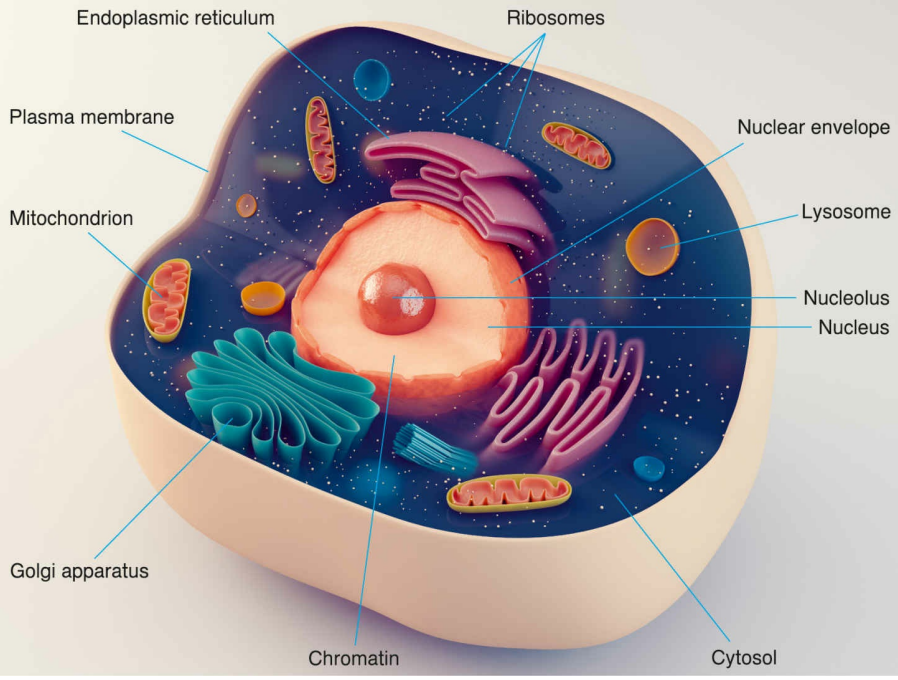


Figure 1.9 **Animal cell.** A typical animal cell contains a nucleus and nucleolus. The nucleus harbors most of the cell's DNA, whereas the nucleolus hosts the synthesis of rRNA. Other compartments include the endoplasmic reticulum, where many substances are made; the Golgi apparatus, which packages proteins for export; mitochondria, where most of our energy is produced; and lysosomes, which are demolition centers. All of these organelles bathe in the cytosol and are segregated from it by membranes. The entire cell is wrapped in the plasma membrane. Other components shown here will be presented in upcoming chapters.

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Eukaryotic cells include internal compartments, or organelles, enclosed in membranes similar to the plasma membrane. The largest, densest, and most conspicuous intracellular organelle is the **nucleus**. The nucleus contains the main genetic material of the cell, which is a nucleic acid known universally by the initials DNA (short for deoxyribonucleic acid). The nucleus is not considered part of the cytoplasm, which is thus defined as the interior of a cell except the nucleus in eukaryotic cells. A spherical area within the nucleus, called the **nucleolus**, is the site of synthesis of another nucleic acid, rRNA (short for ribosomal ribonucleic acid). The nucleolus is akin to the innermost and smallest of three nested dolls (the outer ones being the nucleus and the cell).

The nucleus is surrounded by an extensive network of tubules and flattened sacs, which is called the **endoplasmic reticulum**. This system is the

site of synthesis, storage, and transport of substances to other parts of the cell or outside the cell. It ends in a similar, though more distinct, system of flattened sacs, stacked one on top of another and called the **Golgi apparatus** or **Golgi complex**. This organelle is where proteins to be secreted outside the cell are packaged and occasionally processed.

The cytoplasm of eukaryotic cells is filled with organelles named **mitochondria** (singular: *mitochondrion*), which constitute the power plants of the cell. Specifically, they are the main sites where nutrients are burned by oxygen and energy is produced for cellular functions. Other organelles include **lysosomes** and **peroxisomes**, which are specialized sites where cellular components are broken down. What is left of the cytoplasm outside all of the intracellular organelles is the **cytosol**.

Summary

Living matter is made up of at least 26 elements, 6 of which (H, C, N, O, P, and S) constitute more than 97% of it. Elements differ primarily in atomic number—that is, in the number of protons (positively charged particles) in the nucleus of each of their atoms. Protons share the nucleus with neutrons (neutral particles), while electrons (negatively charged particles) float around in atomic orbitals. Atomic mass (expressed in Da) approximates the sum of protons and neutrons.

Atoms tend to link with each other through chemical bonds to form molecules. The number of bonds that an atom can form is dictated by its electronic structure and ranges from one to five for the six main elements of life. Substances whose molecules contain atoms of different elements are called compounds, which are depicted by both molecular formulas (showing the elements that make up a molecule of the compound along with their proportions) and structural formulas (showing the bonds between atoms). Molecular and structural formulas help us calculate the molecular mass of a compound as the sum of the atomic masses of its constituent elements.

Ions are chemical entities that carry one or more charges. Radicals are molecules or ions that carry unpaired electrons. The neutral molecules of a compound may have uneven distribution of charges internally, which results in the appearance of two electric poles. Such compounds are termed *polar*, as opposed to *nonpolar* compounds, in which charges are evenly distributed. A polar compound does not mix readily with a nonpolar compound. Hence, one needs compounds of the same character in order to make a solution (a mixture in which all components are evenly distributed). A key parameter that describes the composition of solutions is the concentration, which is the amount of a solute in an amount of solution or solvent. Because water is polar, it mixes readily with polar compounds, which are therefore referred to as *hydrophilic*. In contrast, nonpolar compounds do not mix readily with water and are thus called *hydrophobic*.

Atoms, molecules, and ions react to form new chemical entities. Chemical reactions, in conjunction with physical processes, are the essence of life. Reactions are depicted by equations, in which the atoms and net charge on the side of the reactants must equal the atoms and net charge on the side of the products. For reversible reactions—that is, reactions that can go both ways—a key measure of the direction in and degree to which they proceed is the equilibrium constant.

An important parameter of biological fluids is their pH. On the pH scale, 7 marks a neutral solution; < 7 marks an acidic one; and > 7 marks one that is alkaline, or basic. pH affects which form of a conjugate acid–base pair predominates in solution, which in turn affects a multitude of biological processes. For this reason, living organisms have evolved buffer systems, which are pairs of conjugate acids and bases acting as proton donors and acceptors, respectively, in order to protect their homeostasis (stability) in terms of pH.

Living organisms contain both organic and inorganic

substances. Four major classes of organic substances are proteins, nucleic acids, carbohydrates, and lipids. Inorganic substances comprise water and an assortment of ions. A similar categorization recognizes five classes of nutrients in the human diet: carbohydrates, fats, proteins, vitamins, and minerals. The first three are known collectively as macronutrients and the latter two as micronutrients.

The building blocks of living organisms are cells. The simplest of cells, the prokaryotic, consist of a plasma membrane surrounding a relatively unvarying cytoplasm. Eukaryotic cells, in contrast, enclose a variety of organelles in their plasma membranes. The most conspicuous organelles in an animal cell are the nucleus, endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, and peroxisomes, all of which are bathed in cytosol.

Problems and Critical Thinking Questions

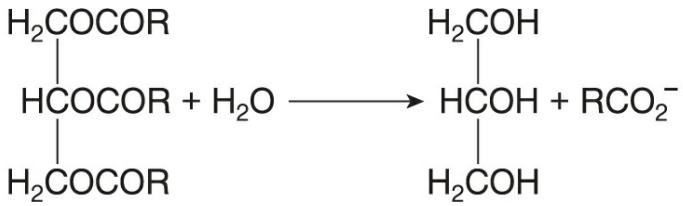
1. What is the difference between a molecular formula and a structural formula? What are the advantages of each?
2. Write down the molecular formula of alanine and calculate its molecular mass.
3. Glycine is an amino acid that differs from alanine in that it has a hydrogen in place of the methyl group.
    a. Construct its structural formula and write down its molecular formula.
    b. Calculate its molecular mass.
    c. Construct the structural formula of its zwitterionic form.
4. Based on the information provided in section 1.6, decide whether the following chemical entities, all containing 2 C, are polar or nonpolar.
    a. Ethane, CH_3CH_3

b. Ethanol, CH_3CH_2OH

c. Ethylamine, $CH_3CH_2NH_2$

d. Ethanoic acid (acetic acid), CH_3COOH

5. Detergents are used widely, both in the household and in industry, to remove dirt from clothes, dishware, and other surfaces. Judging from their miscibility with other substances, would you consider them hydrophilic or hydrophobic?
6. If the concentration of alanine in blood is 0.4 mmol · L^{-1}, what would it be in mg · dL^{-1}? Conversely, if the concentration of glycine in blood is 1.5 mg · dL^{-1}, what would it be in mmol · L^{-1}?
7. Is the evaporation of a liquid a physical process or a chemical reaction? What about the rusting of a metal?
8. The following equation depicts a hydrolysis that will be described in detail in chapter 11.



(*R* represents a so-called aliphatic chain, whose structure you do not need to know to solve the problem.)

a. Balance the equation.

b. Write down the equilibrium constant by using molecular formulas.

9. As we will see in chapter 10, the pH of the cytosol in a muscle cell may drop by nearly one unit after hard exercise. Suppose that it drops from 7.2 to 6.4.

a. Express the transition qualitatively (using two of the terms *neutral, acidic,* and *alkaline*).

b. How does the $[H^+]$ change?

10. Lactic acid has the molecular formula $C_3H_6O_3$ and dissociates into the lactate anion ($C_3H_5O_3^-$) and H^+ with a p*K* of

approximately 4. Calculate which of the two forms predominates in a neutral solution and by what factor over the other.

11. As we will see in a systematic manner in chapter 10, hard-working muscles increase their production of lactate, which exits to blood in equal amounts with H^+.
    a. Assume that the amount of H^+ entering blood is 15 times the amount already present. What effect would this have on the blood pH if no other factors were at work?
    b. In reality, the pH of blood does not change. Explain why.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

chemical element
atom
nucleus (of the atom)
electron
proton
neutron
atomic orbital
electron cloud
hydrogen
carbon
nitrogen
oxygen
phosphorus
sulfur

atomic number
isotope
atomic mass, atomic weight
dalton
angstrom, Å
chemical bond
molecular orbital
covalent bond
molecule
compound
inorganic compound
organic compound
molecular formula
molecular mass, molecular weight
mole
Avogadro’s number
structural formula, constitutional formula
carboxyl group
amino group
methyl group
isomer
configuration
conformation
ion
anion
cation
zwitterion
radical, free radical
partial negative charge
partial positive charge

polar
nonpolar
polarity
miscibility
solution
solvent
solute
concentration
hydrophilic
hydrophobic
chemical reaction
reactant
product
hydrolysis
reversible reaction
principle of mass conservation
principle of charge conservation
balanced chemical equation
equilibrium
equilibrium constant, K_{eq}
irreversible reaction
pH
neutral solution
acidic solution
alkaline solution, basic solution
physiological pH
dissociation constant, K
Henderson-Hasselbalch equation
homeostasis
buffer capacity

buffer system
biomolecule
protein
nucleic acid
carbohydrate
lipid
macromolecule
monomer
polymer
nutrient
fat
vitamin
mineral
macronutrient
micronutrient
cell
plasma membrane
cytoplasm
prokaryotic cell
eukaryotic cell
nucleus (of the cell)
nucleolus
endoplasmic reticulum
Golgi apparatus, Golgi complex
mitochondrion
lysosome
peroxisome
cytosol

References and Suggested Readings

Readers who wish to delve further into the topics presented in part I of this book can avail themselves of a number of recent texts addressing general biochemistry. Of these, I recommend the appropriate pages here and at the end of the remaining chapters included in this part of the book.

Bianconi E, Piovesan A, Facchin F, Beraudi A, Casadei R, Frabetti F, Vitale L, Pelleri MC, Tassani S, Piva F, Perez-Amodio S, Strippoli P, Canaider S (2013). An estimation of the number of cells in the human body. *Annals of Human Biology* 40: 463-471.

Garrett RH, Grisham CM (2012). *Biochemistry* (Cengage, Boston), pp. 1-50.

Mathews CK, van Holde KE, Appling DR, Anthony-Cahill SJ (2013). *Biochemistry* (Pearson, New York), pp. 2-56.

Nelson DL, Cox MM (2017). *Lehninger Principles of Biochemistry* (Freeman, New York), pp. 1-74.

CHAPTER 2

Metabolism

Learning Objectives

After reading this chapter, you should be able to do the following:

- Define metabolism and outline the process of reactions in a metabolic pathway.
- Classify metabolic reactions based on their free-energy changes.
- Describe the variants of free-energy change, explain how they are linked to the equilibrium constant, and calculate any one of these parameters when the rest are known.
- Explain the high energy content of ATP based on its structure and illustrate its conversion into ADP and AMP.
- Distinguish the phases of metabolism in terms of changes in metabolite size, accompanying ATP-ADP interconversion, and prevailing redox reactions.
- Distinguish biological oxidations from reductions in terms of oxygen and hydrogen transfers between metabolites.
- Name the three pairs of compounds that facilitate biological redox reactions, and identify which member of each pair serves as the oxidant and which as the reductant.
- State the ultimate oxidant in animals and explain how it works.

- Draw a diagram of catabolism to show its three stages and differentiate the stages in terms of where they take place and how much energy they produce.

The origin of the term *metabolism* (the Greek word *metabolé* meaning "change") is indicative of what it denotes: **Metabolism** is the sum of the chemical reactions occurring in a living organism or in part of it. These reactions are termed **metabolic reactions**, and the compounds that participate in them are referred to as **metabolites**. Thousands of reactions are believed to take place in even the simplest of organisms. Nevertheless, the picture of metabolism is not a picture of chaos; instead, it is that of an organized and coordinated hive (figure 2.1). This understanding greatly facilitates the study of metabolism. Some of its features are as follows.

- Although the number of metabolic reactions is huge, many are similar in terms of reactants, products, and reaction mechanisms (that is, the rearrangements of chemical groups through which they occur). Thus, there are many recurring themes in metabolism.
- Some reactions can be placed in a row such that a product of the first is a reactant in the second, a product of the second is a reactant in the third, and so on (figure 2.2). A reaction sequence of this kind is called a **metabolic pathway**.
- A relatively small number of compounds play central roles in the metabolism of almost all living organisms.
- The speed at which metabolic reactions proceed is controlled by a multitude of factors through which some common patterns emerge. Several of these patterns are discussed in the context of exercise in part III.

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Figure 2.1 **Metabolism through the eyes of an architect.** Built in 1972 by Kisho Kurokawa, the Nakagin Capsule Tower in Tokyo is considered the icon of Metabolism, a vanguard Japanese architectural movement that infused architectural design with analogs of biological processes (such as growth, tissue renewal, and exchange of materials and energy between organisms and their surroundings).

In this chapter, you will be acquainted with some principles of metabolism. This acquaintance will enable you to easily follow the processes described in subsequent chapters.

Figure 2.2 **Metabolic relay.** Reactions form metabolic pathways if they are linked in such a way that a product of one is a reactant in another.

2.1 Free-Energy Changes of Metabolic Reactions

Let's begin our examination of the principles governing metabolism by picking up what we left pending in section 1.8—that is, by exploring the factors that determine which way a reversible reaction will go. These factors are the subject of **chemical thermodynamics**, a branch of chemistry and physics dealing with energy changes in chemical systems. Its application to living organisms constitutes the field of **bioenergetics**.

Δ (Greek capital delta) before the symbol of a function denotes change; it is used for this purpose because it is the first letter of the Greek word for *difference (diaphorá)*. Δ of a function is defined as the value at the final state minus the value at the initial state. For example, if you lost 4 kg by dieting, then your Δweight would be –4 kg. For more uses of Δ, see section 3.15.

When a chemical reaction takes place in a system, such as a cell, the energy of the system usually changes because the products have different energy content from that of the reactants. In biological systems operating at constant temperature and pressure (conditions that more or less prevail in the human body), energy change is described in terms of **enthalpy change**, which is symbolized as ΔH. Another useful thermodynamic parameter is **entropy change**, or ΔS, where entropy is a measure of disorder in a system. These two terms are combined to produce a third term, **free-energy change**, or ΔG, as follows:

$$\Delta G = \Delta H - T\Delta S \qquad \text{(equation 2.1)}$$

where T is the absolute temperature.

T is measured in degrees Kelvin (K) and equals the degrees Celsius plus 273. T is always positive.

Of the terms just presented, ΔG is the most useful one for biochemists because it provides a single, clear criterion as to which way a reaction—or physical process, for that matter—will go. Considering that the ΔG of a reaction is the free energy of the products (the final state) minus that of the reactants (the initial state), one can discern three possibilities:

- $\mathbf{\Delta} G < 0$. In this scenario, the free energy of the products is lower than that of the reactants. The reaction, termed **exergonic**, can proceed by itself (spontaneously, as we say), because it runs "downhill" (figure 2.3). Therefore, it is favored.
- $\mathbf{\Delta} G > 0$. The free energy of the products is higher than that of the reactants. This reaction, termed **endergonic**, cannot proceed spontaneously, because it runs "uphill"; therefore, it is not favored. It is understood, however, that if a reaction is not favored in one direction, it is favored in the reverse direction. Reverse reactions have opposite $\mathbf{\Delta} G$, because the reactants of one are the products of the other.
- $\mathbf{\Delta} G = 0$. The free energy of the products equals that of the reactants, and the reaction is at equilibrium. Note that equilibrium is dynamic, not static. That is, the reaction does not stop, but while certain amounts of reactants are converted into products, equal amounts of products are converted back into reactants.

Think about this the next time you are told that you need positive energy in your life in order to succeed!

Thus, the desirable metabolic reactions are the exergonic ones—those with negative $\mathbf{\Delta} G$. In such reactions, $\mathbf{\Delta} G$ shows the amount of free energy released when the reaction moves from its initial state to equilibrium. The $\mathbf{\Delta} G$ of a reaction can be negative through a proper combination of the values of $\mathbf{\Delta} H$ and $\mathbf{\Delta} S$ according to equation 2.1. Specifically, $\mathbf{\Delta} G$ will be negative if

- $\mathbf{\Delta} H$ is negative and $\mathbf{\Delta} S$ is positive, or
- both $\mathbf{\Delta} H$ and $\mathbf{\Delta} S$ are negative but the absolute value of $\mathbf{\Delta} H$ is higher than that of $T\,\mathbf{\Delta} S$, or
- both $\mathbf{\Delta} H$ and $\mathbf{\Delta} S$ are positive but the absolute value of $\mathbf{\Delta} H$ is lower than

that of $T\,\Delta S$.

However, there is no way in which ΔG can be negative if ΔH is positive and ΔS is negative.

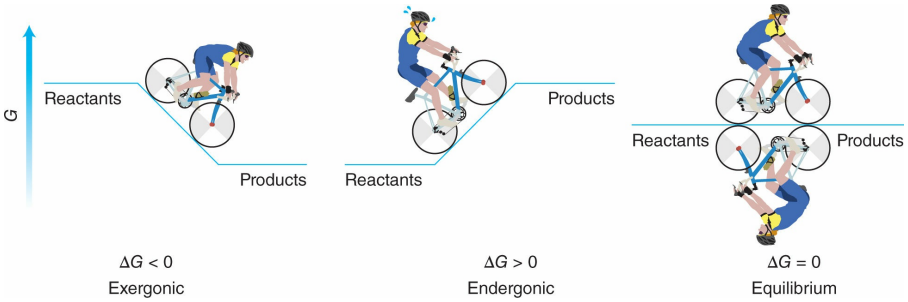


Figure 2.3 **Riding up and down the free-energy slopes.** The difference in free energy ***(G)*** between the reactants and the products of a metabolic reaction determines how feasible the reaction is. If the products lie at a lower level than the reactants (left), then the reaction is favored and proceeds spontaneously. If the products lie at a higher level than the reactants (middle), then the reaction is not favored and requires the input of free energy to proceed. Finally, if products and reactants lie at the same level (right), the reaction is at equilibrium, meaning that the reactants are converted into products and back at the same rate.

In addition to showing whether a reaction is favored, ΔG serves another function. In exergonic reactions, ΔG is the amount of energy that can produce work (defined in physics as the product of the force applied to a body by its displacement) at constant temperature and pressure. In fact, the terms *exergonic* and *endergonic* derive from the Greek word for *work* (*érgon*); more specifically, they mean "releasing work" and "absorbing work," respectively. Work production is vital to a multitude of biological functions, such as muscle activity and transport of solutes. This importance is another reason that exergonic reactions are desirable (apart from being able to proceed spontaneously).

2.2 Determinants of Free-Energy Change

The value of a reaction's ΔG is determined by the nature and concentrations of the participating substances. To make this general statement specific, let's consider the reaction

$$A \rightleftharpoons B \qquad \text{(equation 2.2)}$$

in which A and B are not necessarily single substances; rather, they symbolize the reactants and products in a collective fashion. The ΔG of this reaction is given by the equation

$$\Delta G = \Delta G^\circ + RT \ln \frac{[B]}{[A]} \qquad \text{(equation 2.3)}$$

where

- ΔG° (pronounced "delta G naught") is the **standard free-energy change** (to be defined in a moment);
- R is the so-called gas constant, equal to $1.987 \cdot 10^{-3}$ kilocalories (a unit of energy to be defined later in this section) per mole per Kelvin degree (that is, $1.987 \cdot 10^{-3}$ kcal $\cdot$ mol^{-1} $\cdot$ K^{-1});
- T is the absolute temperature (see previous section);
- ln is the natural logarithm; and
- [B]/[A] is the ratio of the (mathematical) product of the molar concentrations of the reaction products to the product of the molar concentrations of the reactants.

ΔG° is defined as the free-energy change of the reaction when the concentration of every participating substance in solution is 1 mol $\cdot$ L^{-1} (called the *standard state*). In this case, the concentration ratio in equation 2.3 becomes 1, whose natural logarithm is 0; therefore, $\Delta G = \Delta G^\circ$. Being based on a standard state, ΔG° is independent of concentrations and permits a fair comparison of different reactions.

Equation 2.3 enables us to establish a relationship between ΔG° and K_{eq} (the equilibrium constant introduced in section 1.9). Since, at equilibrium, $\Delta G = 0$ (see previous section) and $K_{eq} = [B]/[A]$, it follows that

$$0 = \Delta G^\circ + RT \ln K_{eq}, \text{ or } \Delta G^\circ = -RT \ln K_{eq} \qquad \text{(equation 2.4)}$$

Equation 2.4 shows that the higher the K_{eq}, the more negative the ΔG°. In other words, the farther a reaction is shifted toward the products at equilibrium (resulting in a high [B]/[A]), the more negative the free-energy change in the standard state will be. Note, however, that ΔG can be quite different from ΔG° depending on the actual concentrations of reactants and products. For example, the ΔG of a reaction with a positive ΔG° can be

negative if [B] is much lower than [A]. (Look at equation 2.3 and remember that numbers smaller than 1 have negative logarithms.) In general, a reaction can be favored (shifted to the right) if the concentrations of the reactants are much higher than those of the products.

The previous statement points to a way of affecting the outcomes of metabolic reactions that is frequently employed by organisms: changing the concentrations of reactants, products, or both. For example, the concentration of a reactant may increase in a cell as a result of increased entry from the extracellular space. This increase will decrease the value of [B]/[A] in equation 2.3 and, hence, ΔG, thus making the reaction more favored. The same end will be reached if the concentration of a product decreases because of increased exit from the cell or use in another reaction. We will encounter such cases when we examine exercise metabolism in part III.

For metabolic reactions, which usually take place in aqueous solutions of nearly neutral pH, we use a modified $\Delta G°$ that is denoted by $\Delta G°'$ (delta G naught prime) and defined as the standard free-energy change at pH 7. I will report this function of metabolic reactions in the remainder of the book.

Free-energy changes are measured in energy units. The most commonly used energy unit in biochemistry is the **kilocalorie (kcal)**. One kilocalorie is the amount of energy required to raise the temperature of one kilogram of water by one degree—in particular, from 14.5 to 15.5 °C. The kilocalorie is connected to the **joule (J)**, the SI unit of energy, through the equation 1 kcal = 4,184 J, or 1 kcal = 4.184 kJ.

2.3 ATP, the Energy Currency of Cells

Living organisms are in a state of continuous exchange of mass and energy with their environment. As far as energy is concerned, plants obtain it from sunlight and harness it through photosynthesis. Animals, including we humans, obtain it by burning foodstuffs. Part of the energy (about one fourth) produced through this process is captured in the synthesis of **adenosine triphosphate**, or **ATP**, whereas the rest is released as heat.

We refer to ATP as an energy currency because it is the primary compound used by cells in their energy transactions. As we will see later in

detail, cells exploit the energy from sunlight or foodstuffs to synthesize ATP, and they spend energy by breaking down ATP. Thus, living organisms use ATP the way we use money in everyday life—working to earn it and spending it to meet our needs.

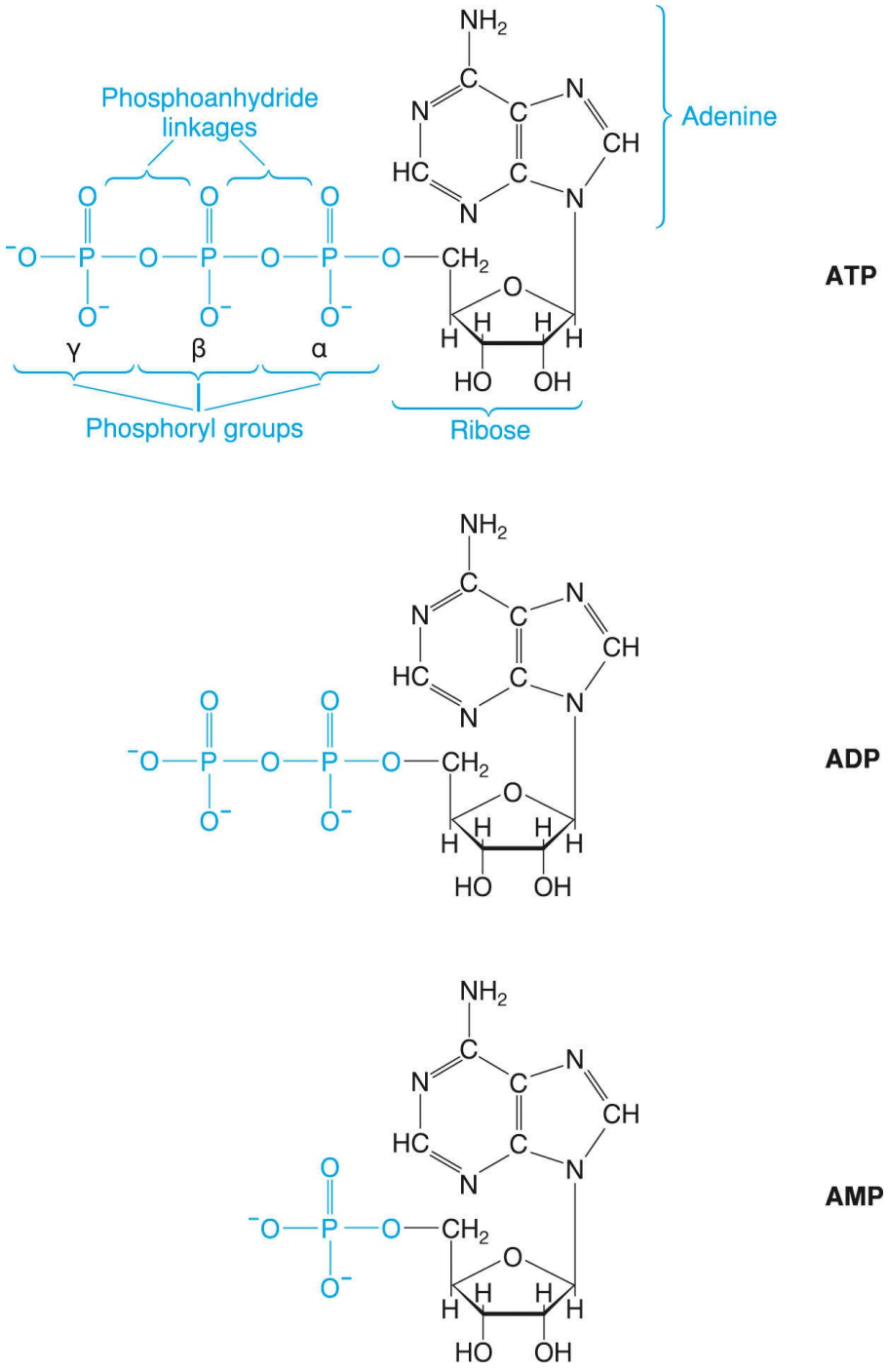


Figure 2.4 **ATP, ADP, and AMP.** ATP stars in the energy transactions taking place in

biological systems. It is converted into ADP or AMP while releasing energy. At physiological pH, the phosphoryl groups are ionized, and the three compounds have charges of –4, –3, and –2, respectively. The phosphorus atoms and phosphoryl groups are marked α (alpha), β (beta), and γ (gamma), beginning on the side of ribose. The ribose ring is perpendicular to the plane of the paper. To create an illusion of three-dimensionality, biochemists take the liberty of drawing more thickly the single bond that is closest to the viewer. Four of the five atoms in the ribose ring are carbons (located at the intersections of bonds), but, for the sake of simplicity, they are not shown. Another liberty taken by biochemists is that of drawing some bonds longer than other, identical ones in order to accommodate bulky groups on paper. This is the case of the bond connecting adenine to ribose.

ATP (figure 2.4) is a complex molecule consisting of three discrete units. The first one is **adenine**, a nitrogenous base (i.e., one that contains N), which we will examine in a systematic fashion in chapter 4. The second unit is **β-D-ribose** (or simply *ribose* for brevity), which is a carbohydrate with five carbon atoms that we will examine in a systematic fashion in chapter 5. Ribose and adenine combine to form adenosine. Finally, ATP contains three phosphoryl groups connected by two **phosphoanhydride linkages**.

A phosphoryl group consists of a P surrounded by four O. After an O has left, a phosphoanhydride linkage connects two phosphoryl groups, forming a —P—O—P— bridge.

The high energy content of ATP resides in its phosphoanhydride linkages, whose hydrolysis releases high amounts of energy. In biological systems, ATP can be hydrolyzed at either phosphoanhydride linkage but not at both simultaneously. Hydrolysis

ΔG°' values throughout the book are drawn from the eQuilibrator web interface (http://equilibrator.weizmann.ac.il).

at the outermost linkage—the one between the β and γ phosphoryl groups—is more common and takes place according to the equation

$$ATP + H_2O \rightleftharpoons ADP + P_i + H^+ \qquad \Delta G^{\circ\prime} = -6.3\ \text{kcal}\cdot\text{mol}^{-1} \quad \text{(equation 2.5)}$$

in which **ADP** is **adenosine diphosphate** (figure 2.4) and **P_i** is **inorganic phosphate** (figure 2.5). Note that the energy data next to reaction 2.5 indicate that the standard free-energy change at pH 7 accompanying the hydrolysis of

one mole of ATP is –6.3 kcal, one of the lowest among single metabolic reactions.

Hydrolysis at the other phosphoanhydride linkage—the one between the α and β phosphoryl groups—follows the equation

$$ATP + H_2O \rightleftharpoons AMP + PP_i + H^+ \qquad \Delta G^{\circ\prime} = -8.2 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 2.6)}$$

AMP is also called **adenylate** (the anion of adenylic acid).

in which **AMP** is **adenosine monophosphate** (figure 2.4) and **PP_i** is **inorganic pyrophosphate** (figure 2.5). PP_i can be further hydrolyzed to two P_i, thus liberating additional energy.

$$PP_i + H_2O \rightleftharpoons 2P_i + H^+ \qquad \Delta G^{\circ\prime} = -3.8 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 2.7)}$$

We can look at the total energy liberated by the sequential hydrolysis of the two phosphoanhydride linkages of ATP by adding equations 2.6 and 2.7 in the same way that we add mathematical equations. That is, we can write a "daughter" equation, in which the reactants consist of the sum of the reactants of the "mother" equations, the products consist of the sum of the products of the "mother" equations, and $\Delta G^{\circ\prime}$ is the algebraic sum of the two "mother" $\Delta G^{\circ\prime}$. In this process, PP_i cancels out, as it is both a product of reaction 2.6 and a reactant in reaction 2.7.

$$ATP + 2H_2O \rightleftharpoons AMP + 2P_i + H^+ \qquad \Delta G^{\circ\prime} = -12 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 2.8)}$$

The very negative $\Delta G^{\circ\prime}$ of the combined reaction (equation 2.8) shows that it is extremely favored thermodynamically.

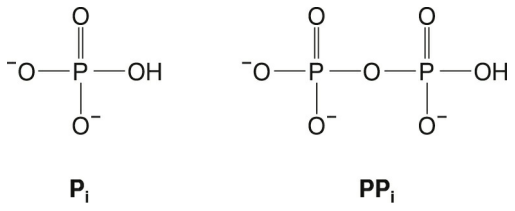


Figure 2.5 P_i and PP_i. Inorganic phosphate (P_i, introduced in figure 1.8 as hydrogen phosphate) is produced when ATP is hydrolyzed to ADP. Inorganic pyrophosphate (PP_i) is produced when ATP is hydrolyzed to AMP. The ionic forms shown here prevail at physiological pH. The term *inorganic* is used to stress that these phosphates are not parts of organic molecules, as opposed to the phosphoryl groups in ATP, ADP, AMP, and many other

biomolecules.

ATP is often referred to as a **high-energy compound**. By analogy, the phosphoanhydride linkages are called **high-energy bonds**, and they are symbolized as ~P (squiggle P). It is the hydrolysis of the phosphoanhydride linkages that yields high amounts of energy, since the products have lower *G* than do the reactants. An equally accurate way to phrase this is to say that ATP possesses a **high phosphoryl-transfer potential** (this term will become clearer in chapter 9).

2.4 Phases of Metabolism

We divide metabolism into two phases, **catabolism** and **anabolism**.

Catabolism

Catabolism includes degradation processes—that is, series of reactions by which biomolecules are broken down into smaller molecules. These processes have a double utility:

- They produce raw materials for the synthesis of larger molecules that are needed for bodily functions (a process that we will define as *anabolism*).
- They release energy, part of which is used in the synthesis of ATP.

Catabolism is often mistaken for the entire metabolism, even by knowledgeable sources. For example, some reputable and otherwise trustworthy websites define metabolism as the complex process in which the body turns food into energy. That process, however, accounts for only half of metabolism, the other half of which is anabolism.

To better comprehend the second utility of catabolism, let's assume that the breakdown of a large molecule, M, into *n* smaller molecules, m, has a $\Delta G^{\circ\prime}$ of –10 kcal per mole.

$$\mathrm{M} \rightleftharpoons n\,\mathrm{m} \qquad \Delta G^{\circ\prime} = -10\ \mathrm{kcal \cdot mol^{-1}} \qquad \text{(equation 2.9)}$$

Now let's write the reaction of ATP synthesis from ADP and P_i by reversing

reaction 2.5.

$$\mathrm{ADP + P_i + H^+ \rightleftharpoons ATP + H_2O} \qquad \Delta G^{\circ\prime} = 6.3\ \mathrm{kcal \cdot mol^{-1}} \quad \text{(equation 2.10)}$$

This synthesis is highly endergonic (since it requires the input of 6.3 kcal · $\mathrm{mol^{-1}}$) and therefore not favored. However, it can proceed if it is chemically coupled to exergonic reaction 2.9. Addition of the two reactions yields

$$\mathrm{M + ADP + P_i + H^+ \rightleftharpoons} \textit{n}\ \mathrm{m + ATP + H_2O} \qquad \Delta G^{\circ\prime} = -3.7\ \mathrm{kcal \cdot mol^{-1}} \quad \text{(equation 2.11)}$$

The negative $\Delta G^{\circ\prime}$ value of the combined reaction shows that it is favored. This, in principle, is how *catabolic processes fuel the synthesis of ATP*.

Anabolism

In contrast to catabolism, anabolism includes biosynthetic processes, in which cells form molecules from smaller units. Cells need anabolism in order to grow, divide, replace molecules that wear out, and create energy depots.

Biosynthetic reactions use intermediate products of catabolism as starting materials and are endergonic. Therefore, they are not favored; they can proceed, however, if they are coupled to an exergonic reaction such as ATP hydrolysis. Suppose we wish to synthesize the large molecule M.

$$\textit{n}\ \mathrm{m} \rightleftharpoons \mathrm{M} \qquad \Delta G^{\circ\prime} = 10\ \mathrm{kcal \cdot (mol\ M)^{-1}} \quad \text{(equation 2.12)}$$

If this synthesis is coupled to reaction 2.5, the following reaction will ensue.

$$\textit{n}\ \mathrm{m + ATP + H_2O} \rightleftharpoons \mathrm{M + ADP + P_i + H^+} \qquad \Delta G^{\circ\prime} = 3.7\ \mathrm{kcal \cdot (mol\ M)^{-1}} \quad \text{(equation 2.13)}$$

The $\Delta G^{\circ\prime}$ of this reaction is positive; therefore, the synthesis of M is still not favored, which means that the hydrolysis of one ATP is not sufficient to make the synthesis of one M feasible. However, the hydrolysis of two ATP will do, since it liberates twice as much energy, thus forcing the total $\Delta G^{\circ\prime}$ to plummet below zero [10 + 2 · (–6.3) = –2.6].

$$\textit{n}\ \mathrm{m + 2\ ATP + 2\ H_2O} \rightleftharpoons \mathrm{M + 2\ ADP + 2\ P_i + 2\ H^+} \qquad \Delta G^{\circ\prime} = -2.6\ \mathrm{kcal \cdot (mol\ M)^{-1}} \quad \text{(equation 2.14)}$$

Relationship of Phases

The example of M degradation and synthesis, as presented with the aid of

reactions 2.9 through 2.14, illustrates a central concept of metabolism: *Catabolism yields energy for the synthesis of our energy currency, ATP, which in turn is spent in biological functions requiring an input of energy.* One of these functions is anabolism (others, including muscle activity, will be presented in parts II and III). Thus, anabolism depends on catabolism in terms of both raw materials and energy (figure 2.6). Moreover, the example of M synthesis shows that *a biosynthetic process is more expensive than the reverse degradation process is lucrative.* This "extravagance" of ATP ensures that not only catabolism, but also anabolism is thermodynamically favored.

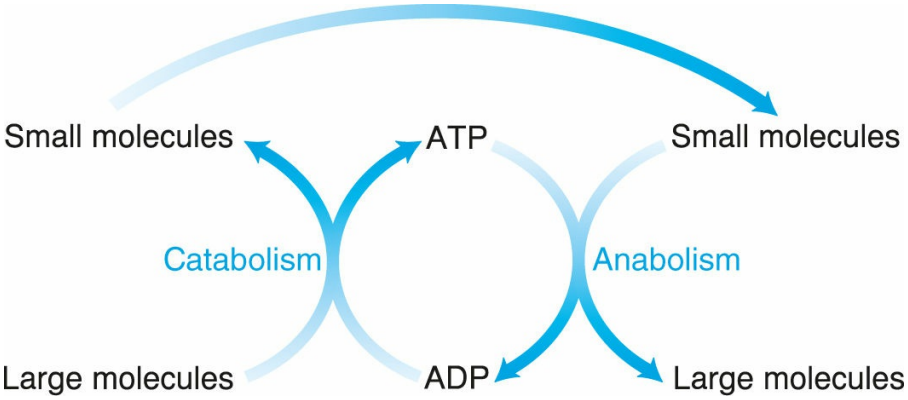


Figure 2.6 Interconnection of catabolism and anabolism. Starting at the bottom left, large molecules are broken down through catabolism to provide small molecules that provide the starting material for anabolism. At the same time, catabolism fuels the synthesis of ATP, which is degraded into ADP while fueling anabolism. Note that although an arrow connects the small molecules on the side of catabolism with the small molecules on the side of anabolism, no such arrow connects the large molecules in this fashion. The reason for this difference is that the large molecules on the two sides are not necessarily the same. For example, the proteins in food have to be broken down into their constituent amino acids in order to be used in building our own proteins, which differ from the ones in our food sources. In contrast, the small molecules produced by catabolism are the very same as those used in anabolism.

2.5 Redox Reactions

Metabolic reactions often involve **oxidations** and **reductions** of biological substances. *We say that a substance is oxidized when it loses one or more electrons and reduced when it accepts one or more electrons.* However, the transfer of electrons is not always evident in a reaction. Instead, it may be

more helpful to look at the transfer of hydrogen or oxygen atoms in order to decide whether a substance is oxidized or reduced. The following rules apply.

- When a compound loses H (not H^+), it is oxidized.
- When a compound accepts H, it is reduced.
- When a compound loses O, it is reduced.
- When a compound accepts O, it is oxidized.

The fact that no atoms are created or lost in a reaction (according to the principle of mass conservation, introduced in section 1.8) means that when a substance is oxidized, another is necessarily reduced and vice versa. That is why we speak of oxidation–reduction reactions or, in short, **redox reactions**.

Catabolic processes usually include oxidations of metabolites by the removal of H, and such oxidations are specifically called **dehydrogenations**. Conversely, anabolic processes usually include reductions of metabolites by the addition of H (**hydrogenations**). Where do the H that are removed go, and where do the H that are added come from? These hydrogen transactions are performed by specialized compounds, the most common of which is **nicotinamide adenine dinucleotide**, or **NAD**.

> The full name of NAD contains the term *nucleotide,* which characterizes nucleic acids and will be introduced in section 4.1. I will explain the connection in section 4.19.

NAD

NAD (figure 2.7) consists of an AMP unit and a **nicotinamide mononucleotide** (abbreviated **NMN**) unit differing from AMP in that it contains **nicotinamide** in place of adenine. Nicotinamide features a six-membered (hexagonal) ring of atoms and is a form of the vitamin niacin, which we will examine in chapter 6. The two units of NAD are connected by a phosphoanhydride linkage identical to the ones in ATP and ADP.

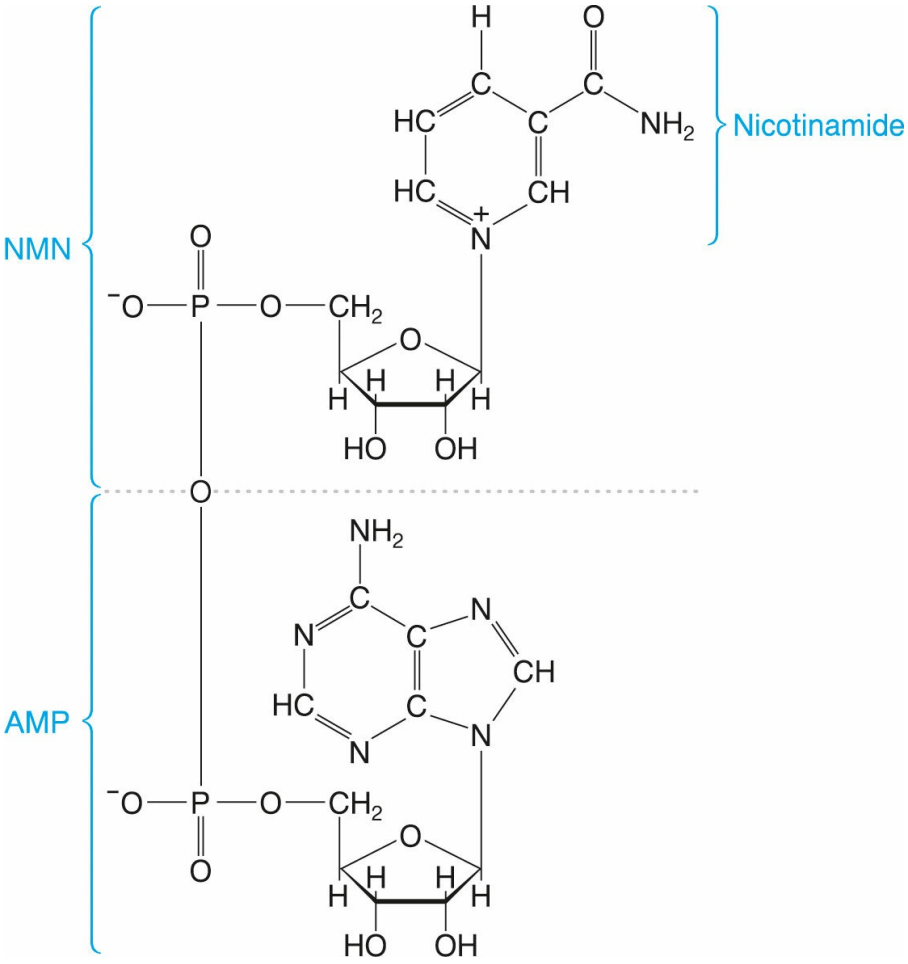
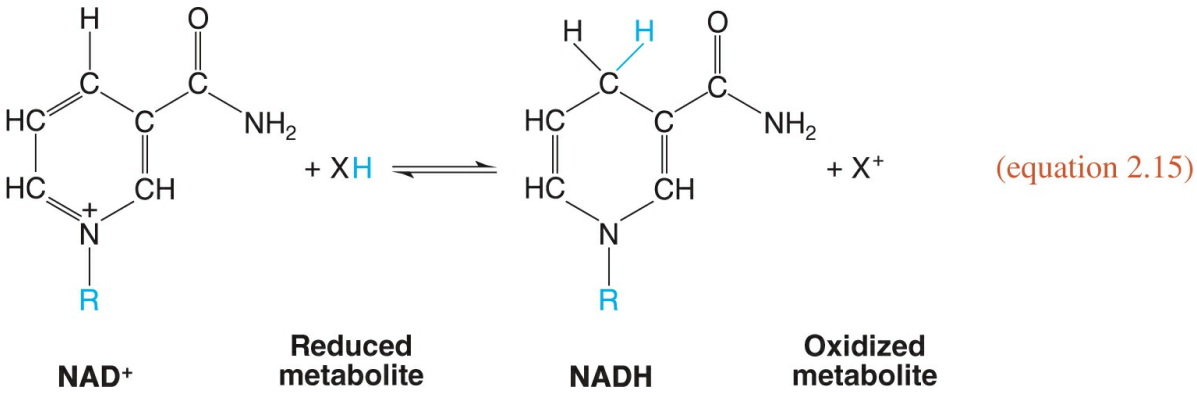


Figure 2.7 **NAD⁺.** Nicotinamide adenine dinucleotide (NAD) is a major mediator of hydrogen transactions in metabolic redox reactions. Presented here is one of its two forms, the oxidized one, which is symbolized as NAD^+ and serves as an H acceptor.

NAD exists in two forms, one oxidized and one reduced, thus forming a **redox pair**. In the oxidized form, the nicotinamide ring—which happens to be the reactive part of the whole molecule—bears one positive charge on its sole nitrogen. For this reason, we symbolize the oxidized form as **NAD^+** (although the molecule bears a net negative charge overall thanks to its two phosphoryl groups).

When a metabolite, say XH, participating in a redox reaction along with NAD^+ is oxidized, the metabolite loses a hydrogen, which is detached with both electrons of the bond connecting it to the rest of the molecule. Thus, H^- (called a **hydride ion**) is removed. H^- is then transferred to the nicotinamide ring, which neutralizes it and converts NAD^+ into **NADH**; this is the reduced

form of NAD.



(equation 2.15)

R is the rest of the NAD molecule as shown in figure 2.7.

Reaction 2.15 is reversible. Hence, the oxidized form of the metabolite, X^+, may regain its H^- from NADH and revert to the reduced form, while NADH turns into NAD^+. Be prepared to encounter a lot of NAD^+ and NADH when we discuss exercise metabolism in part III.

NADP

Some redox reactions employ a compound very similar to NAD: **nicotinamide adenine dinucleotide phosphate**, or **NADP**. NADP differs from NAD in having an additional phosphoryl group attached to the ribose of the AMP unit (figure 2.8). Like NAD, NADP comes in two interconvertible forms—**$NADP^+$** and **NADPH**. The latter is the main hydride donor in reactions involving reductions of metabolites in anabolic processes such as fatty acid synthesis (section 11.13).

FAD

Another hydrogen acceptor in metabolite oxidations is **flavin adenine dinucleotide**, or **FAD**. This H acceptor (figure 2.9) consists of an AMP unit and a **flavin mononucleotide** (**FMN**) unit, the two of which are linked by a phosphoanhydride linkage. FMN derives from vitamin B_2, also called riboflavin, which will be examined in chapter 6.

By analogy to nicotinamide in NAD, the **isoalloxazine** three-ring unit of FMN is the reactive part in FAD. Isoalloxazine accepts two H (to be precise, one H^+ and one H^-) from the compound being oxidized, say YH_2, thus

converting FAD into its reduced form, **$FADH_2$**.

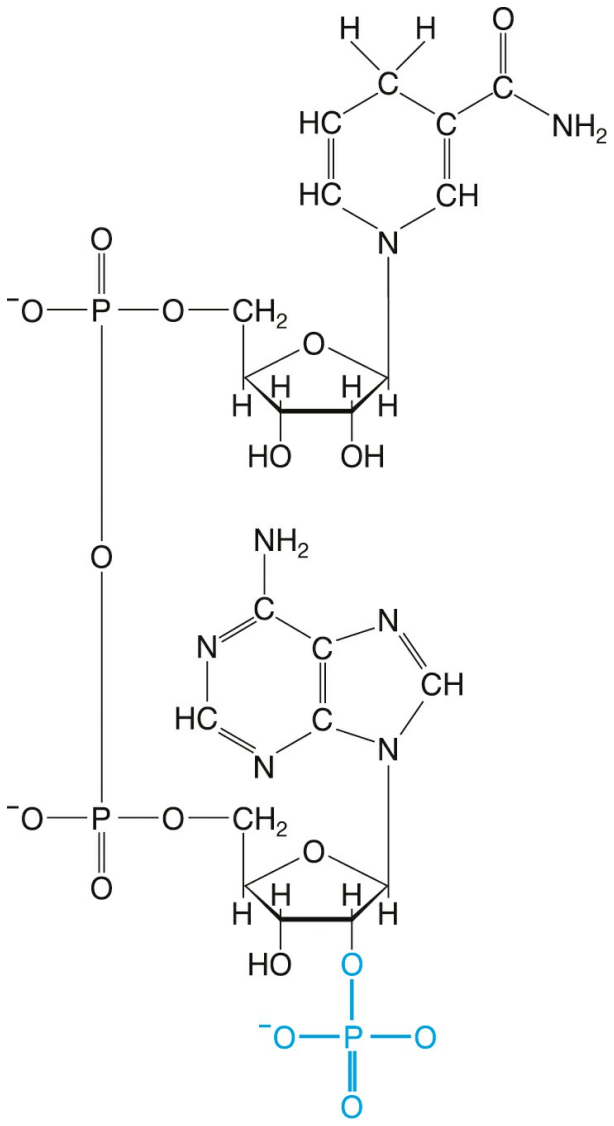


Figure 2.8 **NADPH.** Nicotinamide adenine dinucleotide phosphate (NADP) differs from NAD in that it has one phosphoryl group more (shown here in color). This addition earmarks the reduced form (NADPH) for participation in biosynthetic pathways.

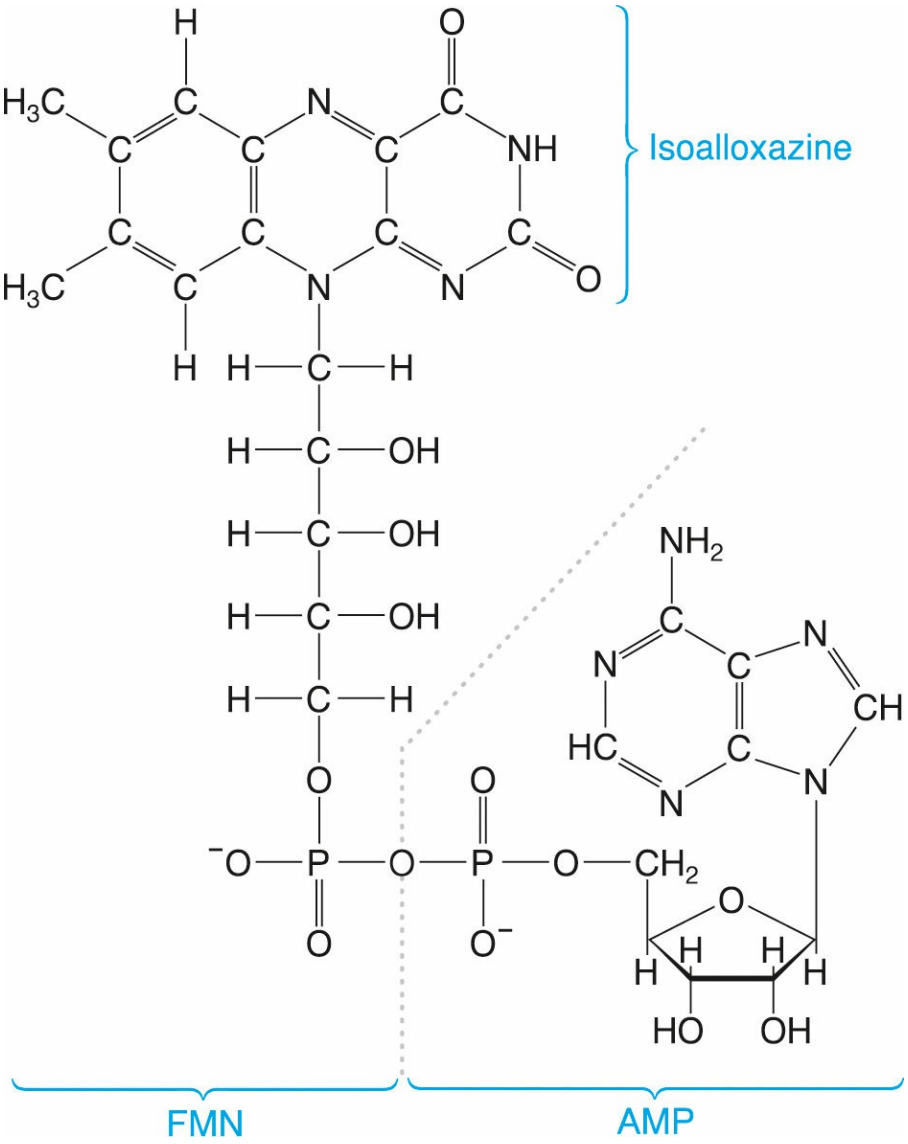
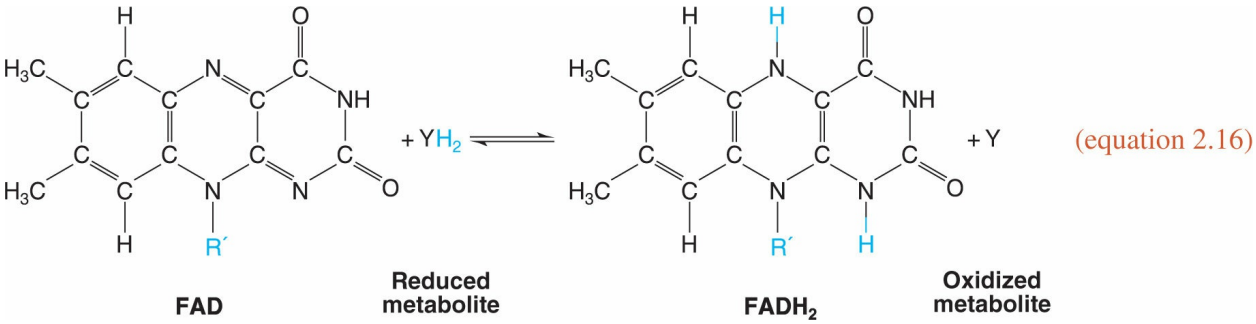


Figure 2.9 **FAD.** Flavin adenine dinucleotide (FAD, oxidized form) participates in metabolite oxidations as a hydrogen acceptor.



(equation 2.16)

R′ is the rest of the FAD molecule, as shown in figure 2.9.

Again, reaction 2.16 is reversible. Hence, the oxidized form of the

metabolite, Y, may regain its two H from $FADH_2$ and revert to the reduced form, while $FADH_2$ turns into FAD. We will meet FAD and $FADH_2$ again in chapters 10 and 11.

Relationship of Oxidants and Reductants

NAD^+, $NADP^+$, and FAD serve as **oxidizing agents**, or **oxidants**, by accepting H from metabolites. Conversely, NADH, NADPH, and $FADH_2$ serve as **reducing agents**, or **reductants**, by donating H to metabolites. The six compounds form three redox pairs. In addition, NADH and $FADH_2$ donate H (to be precise, electrons) to molecular oxygen. In fact, the transfer of electrons from NADH and $FADH_2$ to O_2 marks the end of the oxidation of metabolites within cells, as we will see in the next section briefly and in chapters 10 and 11 in detail. This transfer of electrons renders oxygen the ultimate oxidant in the body, whereas NAD^+ and FAD act as intermediaries in a redox relay (figure 2.10), which results in the regeneration of NAD^+ and FAD for use as oxidants anew. If this makes you wonder how O_2 is regenerated, you will receive an answer when we get to section 3.9.

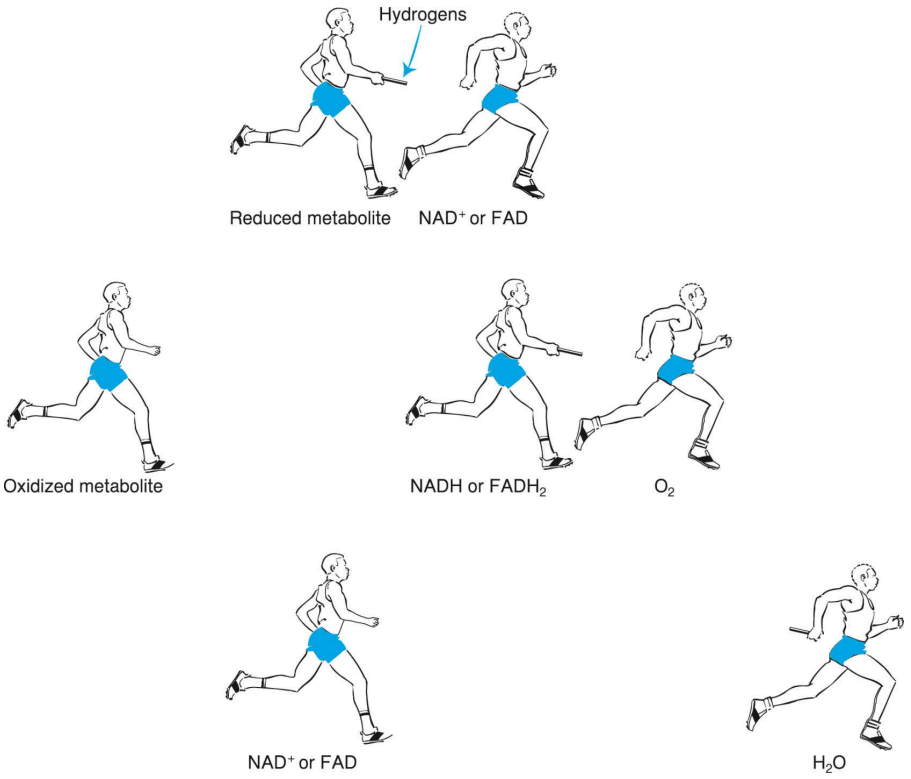


Figure 2.10 Redox relay. Metabolites are oxidized by donating their hydrogens to intermediate oxidants, such as NAD^+ and FAD. The resulting NADH and $FADH_2$ pass the hydrogens to oxygen—the ultimate oxidant—turning it into water, while they revert to NAD^+ and FAD.

2.6 Overview of Catabolism

Of the two phases of metabolism, this book emphasizes catabolism because muscle activity requires increased amounts of ATP, which is synthesized along catabolic pathways. For this reason, I will close the present chapter with a bird's-eye view of the processes that produce the vast majority of ATP in animal cells—processes that will be the focus of part III.

As mentioned in section 2.3, animals obtain their energy by burning foodstuffs. The components of food that yield energy belong to three of the five classes of nutrients presented in section 1.14, which coincide with three of the four classes of biomolecules presented in section 1.13. These classes are—in order of abundance in the usual human diet—**carbohydrates**, **fats** (or **lipids**), and **proteins**. The free energy released by burning one gram of each is about 4, 9, and 4 kcal, respectively. Thus, the ΔG (section 2.1) will be –4, –9, and –4 kcal $\cdot$ g^{-1}, respectively. Another energy-yielding nutrient is **ethanol** (contained in alcoholic beverages), one gram of which releases 7 kcal when burned.

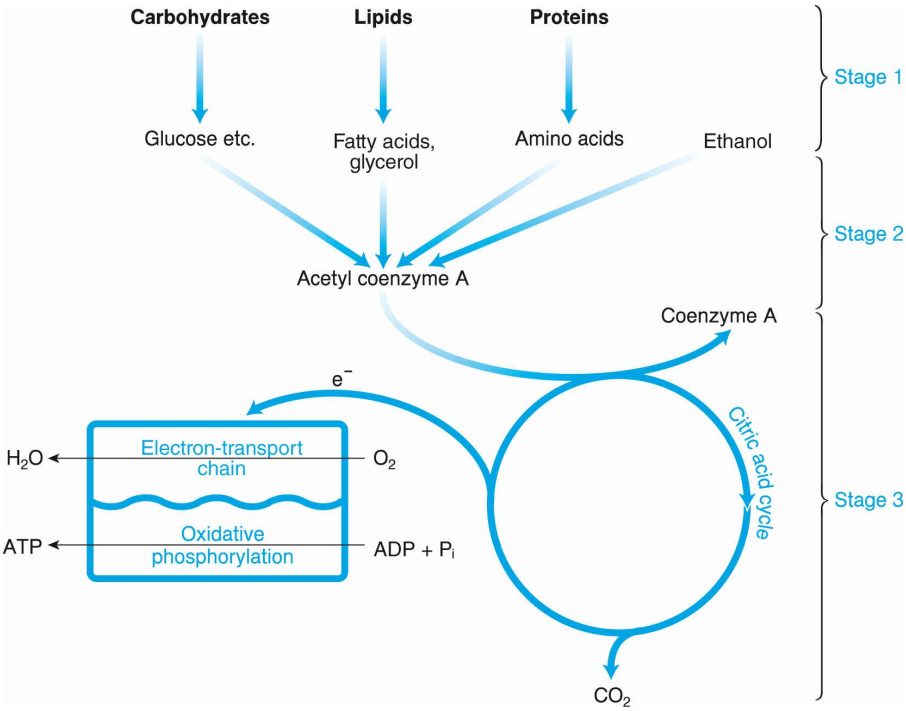


Figure 2.11 Stages of catabolism. Animals, including humans, extract energy from foodstuffs in three stages. In stage 1, large molecules are broken down into smaller molecules. In stage 2, the smaller molecules are converted into acetyl coenzyme A (except for some amino acids, which follow different courses). In stage 3, the acetyl group of acetyl coenzyme A is funneled into the citric acid cycle—leaving coenzyme A behind—and is converted into carbon dioxide. The electrons produced in the cycle are channeled to the electron-transport chain, and the energy released feeds the synthesis of ATP from ADP and P_i through oxidative phosphorylation.

We can divide the course of extracting energy from carbohydrates, lipids, and proteins into three stages (figure 2.11). In stage 1, the macromolecules in food, or similar macromolecules in our cells, are broken down into their monomers. Carbohydrates are converted into **glucose** and related compounds; lipids are converted into **fatty acids** and **glycerol**; and proteins are converted into **amino acids**. The reactions of stage 1 take place in the digestive fluids as part of the process of digestion or in the cytosol of most cells. No ATP is synthesized in this stage 1; rather, this stage is a preparatory step for ATP synthesis in subsequent stages.

Stages 2 and 3 take place solely inside cells. In stage 2, the products of stage 1 are degraded into a few simpler metabolites lying at the core of metabolism. Glucose is catabolized through the pathway of **glycolysis**, which

accommodates glycerol from the breakdown of lipids as well. Fatty acids are subjected to **β oxidation**, while amino acids follow individual routes. Most of the compounds entering stage 2 end up in a chemical group of two carbons—the **acetyl group**, which is part of **acetyl coenzyme A**. Ethanol is also converted into acetyl coenzyme A. Stage 2 produces a small amount of ATP.

In stage 3, the acetyl group is oxidized to carbon dioxide through the **citric acid cycle**. The electrons released in this oxidation are taken up initially by NAD^+ and FAD. Next, the electrons are transferred to oxygen, which reaches the cells from the lungs through the bloodstream. Oxygen takes up the electrons and is reduced to water in a series of exergonic reactions forming the **electron-transport chain**. This process is coupled to **oxidative phosphorylation**, in which part of the energy of the electron-transport chain is harnessed to synthesize most of a cell's ATP. All of stage 3 and part of stage 2 take place in the mitochondria.

Phosphorylation is the addition of a phosphoryl group to a compound. Thus, ATP is produced by phosphorylation of ADP.

Because carbohydrates, lipids, and proteins are finally burned by O_2 to CO_2 in order to produce energy, they are referred to as **fuels** for cells—and therefore for the functions of the human body, including exercise.

Summary

Metabolism is the sum of the chemical reactions that take place in a living organism or in part of it. Metabolism is characterized by high organization and great complexity, which is mitigated by the existence of common patterns among reactions and metabolites (the participants in metabolic reactions). In addition, groups of reactions are connected like caterpillars walking in a row, thus forming metabolic pathways. The direction in which a metabolic reaction proceeds is determined by its free-energy change, or ΔG, which needs to be negative in order for a reaction to be favored. Such a reaction is called *exergonic*, and

its Δ*G* can be used to produce useful work. Reactions that have positive Δ*G* are not favored and are termed *endergonic*. Finally, a reaction with zero Δ*G* is a reaction at equilibrium. Two variations of Δ*G*—specifically, Δ*G*° and Δ*G*°'—simplify the study of bioenergetics, which is the branch of chemistry and physics dealing with energy changes in living systems.

Cells handle most of the energy needed for their functions by synthesizing and degrading adenosine triphosphate, or ATP, which enjoys high energy content thanks to its two phosphoanhydride linkages. When these linkages are hydrolyzed through highly exergonic reactions, ATP is converted into ADP or AMP.

Metabolism can be divided into catabolism (which includes degradation processes) and anabolism (which includes biosynthetic processes). Catabolism produces two commodities: (1) raw materials for anabolism and (2) energy (mainly in the form of ATP) for biological processes including anabolism. In turn, anabolism is necessary for the development and maintenance of an organism, as well as for the formation of energy depots. Catabolic processes are usually accompanied by oxidation of metabolites, whereas anabolic processes are usually accompanied by reduction of metabolites. Oxidizing agents include NAD^+, $NADP^+$, and FAD, whereas reducing agents include NADH, NADPH, and $FADH_2$. The oxidizing agents can acquire hydrogens, whereas the reducing agents can donate hydrogens. The six compounds form three redox pairs. Oxygen is the ultimate hydrogen acceptor (hence, oxidizing agent) in redox reactions in animals; it is converted into water in the process.

Catabolism, the phase of metabolism that predominates during exercise, can be divided into three stages. In stage 1, our macromolecular fuels—that is, carbohydrates, lipids, and proteins—are broken down into their building blocks: glucose and similar compounds; fatty acids and glycerol; and amino

acids, respectively. In stage 2, the products of phase 1 are converted into a few smaller metabolites, among which the acetyl group, embedded in acetyl coenzyme A, stands out. Finally, in stage 3, the acetyl group is oxidized to CO_2, O_2 is reduced to H_2O, and most of our ATP is synthesized from ADP and P_i.

Problems and Critical Thinking Questions

1. The following six reactions take place in our cells. (Never mind about not knowing many compounds; they will all appear in a later chapter.) Your task is to order them in such a way as to form a metabolic pathway. Take into account that one of the reactions does not fit into the pathway and that all of the reactions are reversible (meaning that you may have to reverse some).
   a. 1,3-bisphosphoglycerate + NADH + H^+ ⇌ glyceraldehyde 3-phosphate + NAD^+ + P_i $\Delta G°'$ = –1.9; kcal · mol^{-1}
   b. dihydroxyacetone phosphate + NADH + H^+ ⇌ glycerol 3-phosphate + NAD^+ $\Delta G°'$ = –6.3 kcal · mol^{-1}
   c. fructose 1,6-bisphosphate ⇌ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate $\Delta G°'$ = 4.7 kcal · mol^{-1}
   d. phosphoenolpyruvate + H_2O ⇌ 2-phosphoglycerate $\Delta G°'$ = 1 kcal · mol^{-1}
   e. 1,3-bisphosphoglycerate + ADP ⇌ 3-phosphoglycerate + ATP $\Delta G°'$ = –4.4 kcal · mol^{-1}
   f. 3-phosphoglycerate ⇌ 2-phosphoglycerate $\Delta G°'$ = 1 kcal · mol^{-1}
2. Based on the $\Delta G°'$ data provided in problem 1, classify the reactions in the pathway that you formed as exergonic or endergonic. Then calculate the $\Delta G°'$ of the entire pathway

and declare whether it is exergonic or endergonic.

3. Calculate the K_{eq} of reaction *c* in problem 1 at 37 °C with pH = 7. If the concentrations of the participating compounds in the cytosol of a muscle cell are, in series, 0.8, 0.6, and 0.6 mmol · L^{-1}, what is the Δ*G*?
4. Which one of the following is true? The higher the equilibrium constant of a reaction,
    a. the more positive the free-energy change
    b. the more positive the standard free-energy change
    c. the more negative the free-energy change
    d. the more negative the standard free-energy change
5. Draw a table showing the number of phosphoryl groups and phosphoanhydride linkages in ATP, ADP, and AMP. Explain why one of the three is not a high-energy compound, whereas the other two are.
6. Draw another table distinguishing the two phases of metabolism in terms of how metabolite size changes in each, whether ATP is converted into ADP or the opposite, and whether metabolites are usually oxidized or reduced.
7. Gluconeogenesis is an anabolic pathway consisting of the synthesis of glucose from pyruvate (to be discussed in chapter 10). The Δ*G*°' of this conversion is 32 kcal · mol^{-1}. How many moles of ATP must be hydrolyzed to make the synthesis of one mole of glucose favorable?
8. When a substance burns, is it oxidized or reduced? Explain.
9. Draw a table containing the dual forms of NAD, NADP, and FAD in two columns. Then distinguish
    a. the oxidized forms from the reduced forms,
    b. the forms that get oxidized from the forms that get reduced in redox reactions,
    c. the H acceptors from the H donors, and
    d. the oxidants from the reductants.

10. Draw a table showing where each stage of catabolism takes place and roughly how much energy each stage produces.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

metabolism
metabolic reaction
metabolite
metabolic pathway
chemical thermodynamics
bioenergetics
enthalpy change, ΔH
entropy change, ΔS
free-energy change, ΔG
exergonic
endergonic
standard free-energy change, $\Delta G°$
$\Delta G°'$
kilocalorie, kcal
joule, J
adenosine triphosphate, ATP
adenine
β-D-ribose
phosphoanhydride linkage
adenosine diphosphate, ADP
inorganic phosphate, P_i
adenosine monophosphate, AMP
adenylate
inorganic pyrophosphate, PP_i

high-energy compound
high-energy bond
high phosphoryl-transfer potential
catabolism
anabolism
oxidation
reduction
redox reaction
dehydrogenation
hydrogenation
nicotinamide adenine dinucleotide, NAD
nicotinamide mononucleotide, NMN
nicotinamide
redox pair
NAD^+
hydride ion
NADH
nicotinamide adenine dinucleotide phosphate, NADP
$NADP^+$
NADPH
flavin adenine dinucleotide, FAD
flavin mononucleotide, FMN
isoalloxazine
$FADH_2$
oxidizing agent, oxidant
reducing agent, reductant
carbohydrate
fat
lipid
protein

ethanol
glucose
fatty acid
glycerol
amino acid
glycolysis
β oxidation
acetyl group
acetyl coenzyme A
citric acid cycle
electron-transport chain
oxidative phosphorylation
phosphorylation
fuel

References and Suggested Readings

Berg JM, Tymoczko JL, Gatto GJ, Stryer L (2015). *Biochemistry* (Freeman, New York), pp. 423-448.

Garrett RH, Grisham CM (2012). *Biochemistry* (Cengage, Boston), pp. 51-76.

Mathews CK, van Holde KE, Appling DR, Anthony-Cahill SJ (2013). *Biochemistry* (Pearson, New York), pp. 57-81, 414-444.

Nelson DL, Cox MM (2017). *Lehninger Principles of Biochemistry* (Freeman, New York), pp. 505-542.

CHAPTER 3

Proteins

Learning Objectives

After reading this chapter, you should be able to do the following:

- List the common and unique features in the structure of the amino acids that make up proteins.
- Describe the properties that distinguish amino acid side chains and classify the side chains according to those properties.
- Draw a peptide unit and identify the atoms that participate in its formation.
- Define the four levels of protein structure and explain why not all proteins display the uppermost level.
- Discuss the characteristics of the four levels of protein structure, what stabilizes them, and what destabilizes them.
- Define the proteome and proteomics.
- Discuss the various functions of proteins and classify specific proteins into functional categories.
- Compare the two oxygen carriers in the human body in terms of structure and function.
- Describe the cooperative binding of oxygen to hemoglobin and discuss the role of hemoglobin in aerobic capacity.

- Define enzymes and describe their properties; explain what the substrates, active site, and enzyme activity are.
- Contrast the energetic aspect of a metabolic reaction that an enzyme influences with the energetic aspect that an enzyme does not influence.
- List factors that affect enzyme activity and discuss how each one does so.

Proteins are the first class of biological macromolecules that we are going to explore. Their name derives from the Greek *proteíos*, which means "first in rank" and denotes the protagonistic role of these compounds in biological processes. As for the -in ending, it characterizes a category of organic compounds named *amines* and reflects the fact that protein molecules contain amino groups. In fact, the nitrogen of amino groups constitutes a substantial part of the protein mass (16% on average).

We will examine proteins (as with all classes of biological macromolecules) from two sides—structure and function. We will start with structure, ranging from building blocks to the full, three-dimensional shape of proteins; then we will consider some of their diverse functions.

3.1 Amino Acids, the Building Blocks of Proteins

Amino acids are the monomers, or building blocks, of proteins. Biological fluids contain a multitude of amino acids, but those composing proteins are limited to 20. One may find additional amino acids in a protein molecule, but they arise by modification of some of the 20 fundamental amino acids after a protein has been synthesized. There are two astounding exceptions, one of which is found in us humans (as well as other organisms). We will encounter several cases of modified amino acids throughout the book—for example, in sections 3.5, 6.1, 6.2, 6.4, and 10.6.

All but one of the 20 amino acids of proteins can be depicted by the same general formula (figure 3.1). Each is made up of a carbon atom to which four groups are attached through single covalent bonds: a carboxyl group, an amino group, a hydrogen, and a variable group. Thus, the term *amino acid* stems from the presence of an amino group and a carboxyl group, the latter of which is the trademark of organic acids.

The variable group determines the identity of an amino acid; the variable group is also called a **side chain** for a reason that will become clear when we examine how a protein is assembled. Amino acids of the kind that make up proteins are called **α-amino acids** when one wants to emphasize or clarify that the amino group is connected to the carbon next to the carboxyl group; this carbon was denoted α carbon in older nomenclature.

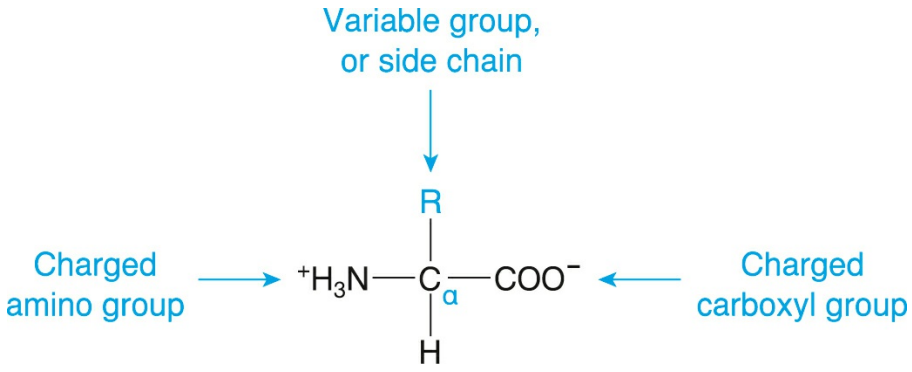


Figure 3.1 **General formula of an α-amino acid.** An amino acid is shown with indication of its α carbon and with both the amino and carboxyl groups ionized. This is the predominant form in most biological fluids.

Amino acids are ionized in aqueous solutions. The predominant form at physiological pH is negatively charged at the carboxyl group, which has a p*K* (see section 1.11) of around 2, and positively charged at the amino group, which has a p*K* of 9 to 11. Because the two charges cancel each other out, an amino acid is electrically neutral (that is, zwitterionic) unless the side chain is charged.

Another detail regarding the structure of amino acids is that they can exist in two isomeric forms, one of which is the mirror image of the other. We distinguish these forms by using the prefixes D- and L-. Amino acids in proteins are of the L form, and all formulas in this book depict L-amino acids

according to a convention known as the Fischer projection, after the German chemist Emil Fischer, who devised it in 1891. The convention dictates that horizontal bonds project toward the viewer, whereas vertical bonds project away from the viewer.

Isomers that are mirror images of each other are called **enantiomers**.

Table 3.1 presents the names of the 20 protein amino acids. Each has a three-letter abbreviation, which in most cases coincides with the three first letters of its name. For greater brevity, biochemists refer to amino acids by means of one-letter symbols, which are also included in the table.

As already mentioned, amino acids differ with respect to the side chain only. Side chains vary in size, charge, polarity, and chemical reactivity. You can review their versatility in figure 3.2, which presents the structural formulas of all 20 protein amino acids. Amino acids can be as small as glycine (number 1) or as large as tryptophan (number 9). They can have no net charge (numbers 1 to 13, 16, and 17), negative charge (numbers 14 and 15), or positive charge (numbers 18 and 19) at physiological pH. In particular, aspartate and glutamate (numbers 14 and 15) owe their negative charges to the fact that their conjugate acids—aspartic acid and glutamic acid—have pK values of around 4 at their side chains. Likewise, lysine and arginine (numbers 18 and 19) owe their positive charges to the fact that their pK values at the side chains are 10.5 and 12.5, respectively.

Table 3.1 Amino Acid Names and Abbreviations

Name	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I

Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Histidine (number 20) is present in considerable amounts in two forms (with either positive or no charge on the side chain), because the p*K* of the side chain is 6 (that is, close to the physiological pH) and can increase considerably depending on the surrounding side chains in a protein molecule. Proline (number 6) is the one amino acid that does not obey the general formula presented in figure 3.1, because its side chain is connected to the amino nitrogen. Finally, there are amino acids (such as number 2, alanine) with hydrophobic side chains and some (such as number 10, serine) with hydrophilic, though uncharged, side chains.

3.2 The Peptide Bond

Amino acids form proteins when they are joined covalently in a row by the tens or hundreds or even thousands like links in a chain. Short stretches of amino acids are called **peptides**, a term deriving from the Greek verb *pépto* ("to digest") because, as we will see in section 12.1, protein chains are digested to produce shorter chains. Depending on the number of amino acids constituting them, peptides may be specified as dipeptides, tripeptides, and so on, and proteins are often referred to as **polypeptide chains**. The part of an amino acid participating in a peptide is termed a **residue**.

The term *residue*—meaning what remains of a molecule after it gives up a few of its atoms to be linked with other molecules and become part of a polymer—is used to describe monomers not only in proteins but also in nucleic acids and carbohydrates.

The bonds linking amino acids are formed between the amino group of

one and the carboxyl group of another. To see how this linking is done, let's consider two amino acids: phenylalanine and aspartate. The nitrogen of aspartate can be linked to the carboxyl carbon of phenylalanine by a bond called **amide bond** in organic chemistry and **peptide bond** in biochemistry (figure 3.3).

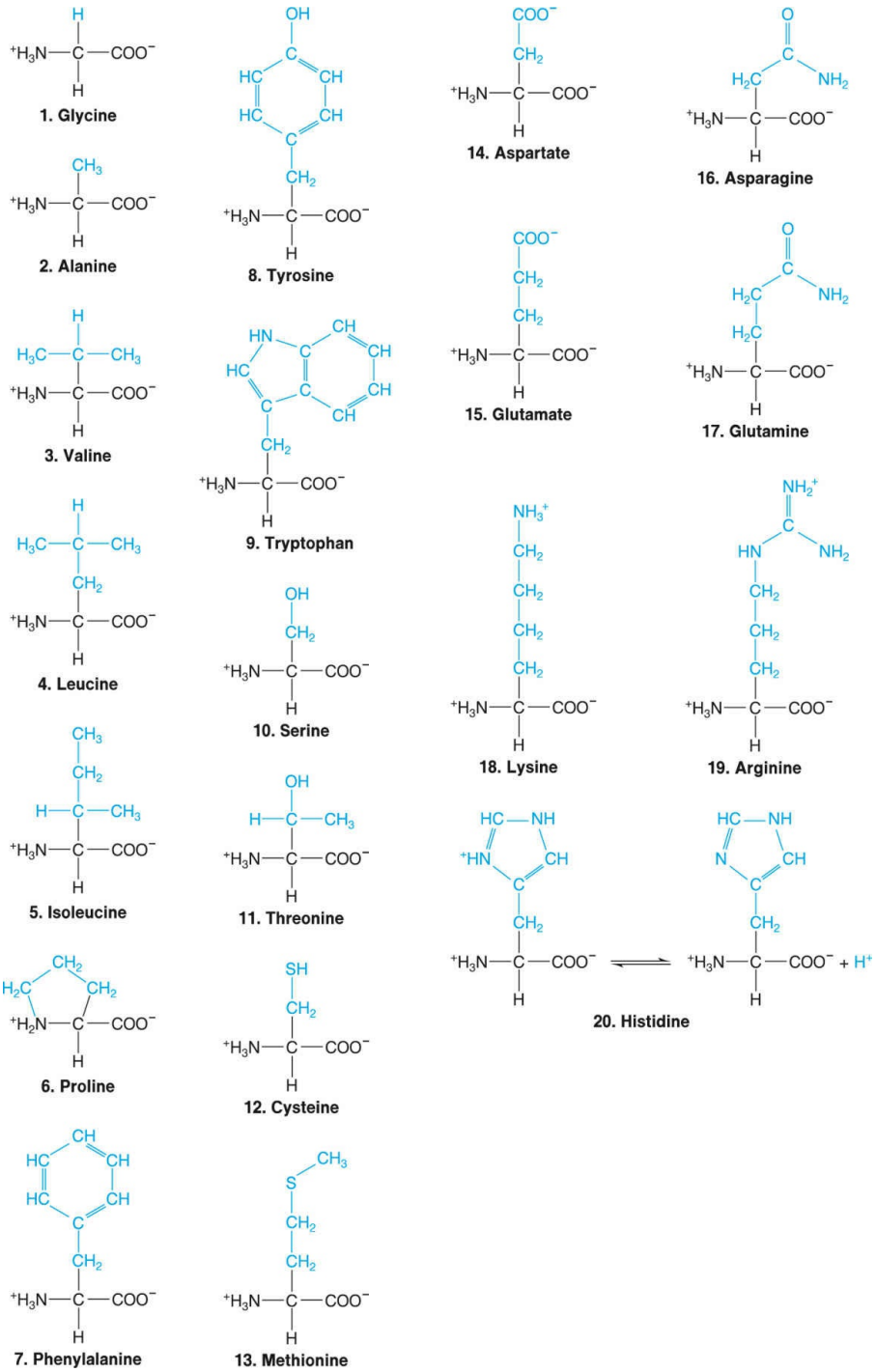


Figure 3.2 **Structural formulas of the amino acids.** The 20 amino acids of proteins have been arranged in columns and grouped according to the similarity of their side chains.

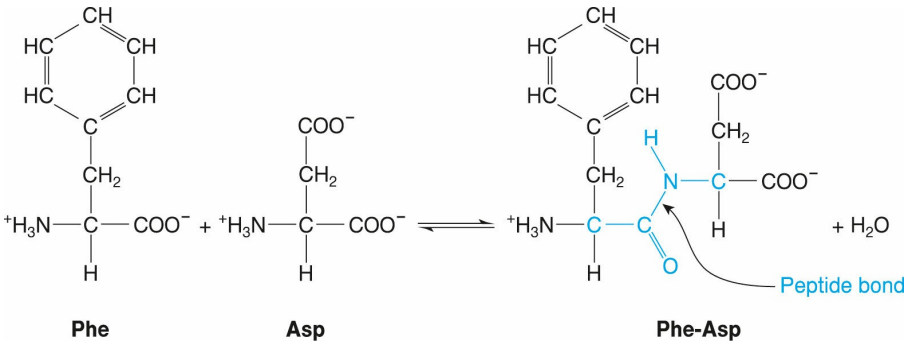


Figure 3.3 **Peptide bond.** Two amino acids can be joined by a peptide bond, thus forming a dipeptide and water. The six atoms in color are coplanar (that is, they lie on the same plane). Of these, H, N, C, and O form a **peptide unit**.

Before we proceed, I need to clarify that the reaction shown in figure 3.3 is endergonic and thus not favored. (In contrast, the reverse reaction—that is, the hydrolysis of the dipeptide—is favored.) I have presented it only to describe the peptide bond. In reality, peptide bond formation for protein synthesis takes place in a more complex way, which I will describe in the next chapter.

The dipeptide shown in figure 3.3 has a free (unbound) amino group deriving from phenylalanine and a free carboxyl group belonging to aspartate. It is called phenylalanyl aspartate and is abbreviated as Phe-Asp. If the two amino acids were linked in the reverse way, the resulting dipeptide, Asp-Phe, would have a free amino group deriving from aspartate and a free carboxyl group belonging to phenylalanine. It would thus be a different compound (an isomer of Phe-Asp).

The dipeptide Asp-Phe, with the addition of a methyl group to the carboxyl group of Phe, is the artificial sweetener **aspartame**, which is added to diet beverages and other low-calorie products. Although not devoid of calories, aspartame is about 200 times as sweet as sugar and can replace it in much smaller quantities.

To avoid confusion, biochemists have agreed to write and name peptides —and proteins—starting from the free amino group and going to the free

carboxyl group. The residue bearing the free amino group is termed the **amino-terminal residue**, or ***N*-terminal residue**, whereas the residue bearing the free carboxyl group is termed the **carboxyl-terminal residue**, or ***C*-terminal residue**. Thus, the conventional direction of a polypeptide chain is $N \rightarrow C$.

3.3 Primary Structure of Proteins

Proteins are intricate three-dimensional entities in which we can discern four levels of structure of increasing complexity: **primary**, **secondary**, **tertiary**, and **quaternary**. The simplest of these, primary structure, is defined as *the amino acid sequence in a protein.*

The general formula of the primary structure of a protein is shown in figure 3.4*a*. The "backbone" of the polypeptide chain is formed by the alternation of three atoms: nitrogen, the α carbon, and what was formerly the carboxyl carbon and is presently the amide carbon. The variable groups of the residues protrude from the backbone, and that is why they have been named side chains.

The primary structure of a protein can be likened to a human chain (figure 3.4*b*) in which each human represents an amino acid and the hands represent the amino groups and carboxyl groups. The person on one end of the chain has a free right hand (representing, say, the free amino group), while the person on the other end has a free left hand (the free carboxyl group). Everybody else in the chain has a right hand linked to somebody else's left hand and a left hand linked to somebody else's right hand (the hand linkages representing the peptide bonds). Finally, everyone's head and legs, just like the side chain and hydrogen of every amino acid, take no part in the linking.

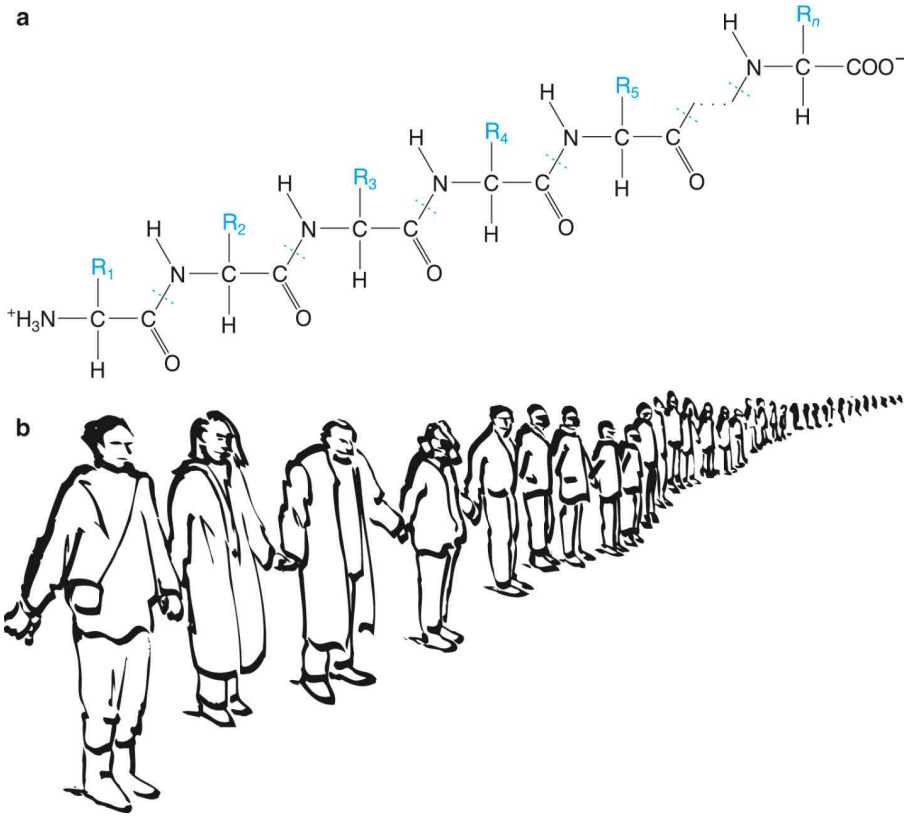


Figure 3.4 **Primary structure of a protein.** *(a)* A protein is a chain of amino acid residues linked by peptide bonds. The broken lines mark the boundaries of each residue. The symbols R_1-R_n represent the side chains. Note the distinct ends of the polypeptide chain. *(b)* A protein is like a human chain. See the text for parallels.

Protein molecules are usually assembled from hundreds or thousands of amino acids. Thus, the repertoire of the 20 amino acids—which can be joined in any possible sequence—permits the existence of an inconceivably large number of proteins. Their real number, of course, is finite, though impressive. For example, we humans are thought to have 100,000 to 200,000 different proteins. The entire set of proteins in an organism, tissue, cell type, or biological fluid constitutes its **proteome**, and the large-scale, comprehensive study of proteomes in terms of protein identity, quantity, and function makes up the field of **proteomics**. This field, like other -omics that we will encounter in subsequent chapters, relies heavily on **bioinformatics**, an interdisciplinary field of science that applies computer technology to manage biological information.

What determines the primary structure of a protein? We will explore this

question in detail in the next chapter. For now, we may say that the amino acid sequence in a protein is dictated by the genetic information contained, in encoded form, in what we call the gene.

Let me now reverse the question: What does the primary structure of a protein determine? The answer is simple: It helps determine the next, more complex levels of structure (figure 3.5). Until three decades ago, biochemists stressed that the shape (and, hence, function) of a protein was determined *exclusively* by its primary structure—in particular, by the kind of the side chains of its residues. However, later findings showed that many proteins are aided by other proteins (aptly named **molecular chaperones**) in assuming their definite shapes. Thus, it is safer to say that the primary structure is an important (probably the most important) determinant of the shape of a protein.

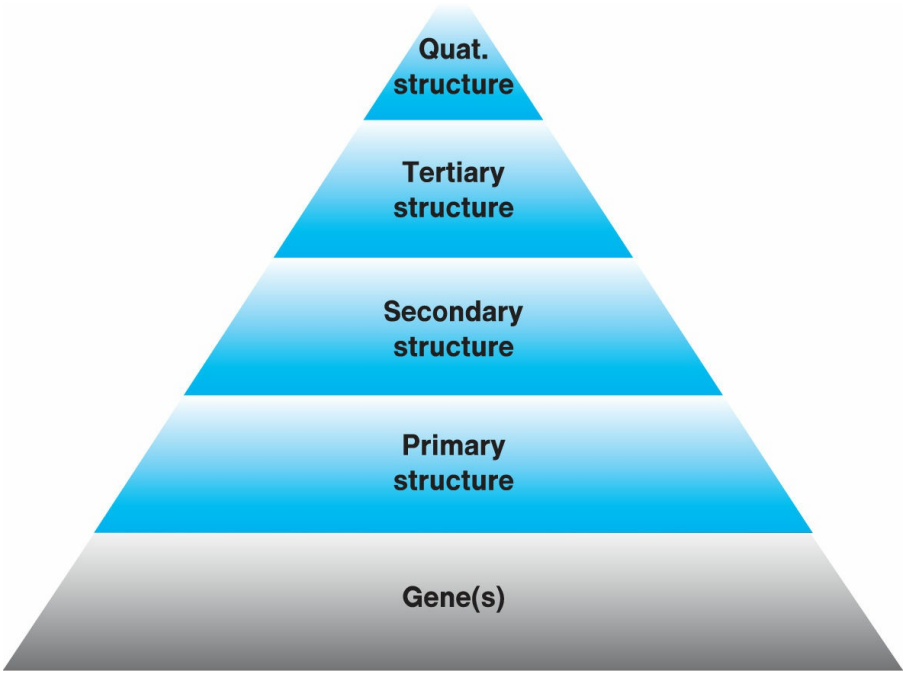


Figure 3.5 **Buildup of information.** The primary structure of a protein forms the basis for its higher levels of structure (secondary, tertiary, and quaternary structures). The primary structure itself is encoded by the gene(s).

3.4 Secondary Structure

A polypeptide chain does not extend in a straight line, as figure 3.4 may

suggest. Instead, the chain folds in various ways, giving rise to a multitude of three-dimensional structures. *The folding of a short stretch of the polypeptide chain in a usually characteristic and regular pattern is the secondary structure.*

The main force responsible for the secondary structure in a protein is the **hydrogen bond**. This is a primarily noncovalent bond—though with some covalent character—appearing between a hydrogen atom with partial positive charge and an atom with partial negative charge, usually oxygen or nitrogen (figure 3.6). Remember that section 1.6 introduced you to the concept of partial charge, its symbol, and the knowledge that the nuclei of N and O are stronger than those of H and C in attracting bonding electrons. Thus, when a strong electron attractor bonds with a weak electron attractor, the former acquires a δ^-, the latter acquires a δ^+, and the two form a dipole. If a dipole with δ^+ on H approaches another dipole (which can be several or many residues away in terms of primary structure in a protein), the two are attracted and a hydrogen bond develops.

A hydrogen bond is 10 to 100 times weaker than a covalent bond. However, there are many H atoms with δ^+ and many O and N atoms with δ^- in a protein, thus giving rise to many hydrogen bonds. The sum of the resulting forces is sufficient to stabilize the secondary structure.

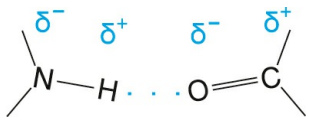


Figure 3.6 Hydrogen bond in a protein. The hydrogen in a peptide unit of a protein, bearing a δ^+ because of its covalent bonding with N, can be attracted by the oxygen in another peptide unit, bearing a δ^-, and form a hydrogen bond (colored dots). This is but one of several kinds of hydrogen bond that stabilize protein structure.

The secondary structure can be either irregular (though strictly defined) or, more frequently, regular, with repeating motifs. In the latter case, we speak of periodic secondary conformations. The two that are most widely known and most frequently encountered are the **α helix** and the **β pleated sheet**, or simply **β sheet**.

In the α helix, the backbone of the polypeptide chain forms the core of a

spiral having a pitch of 5.4 Å and encompassing 3.6 residues per turn (figure 3.7*a*), while the side chains of the residues point outward. The helix is right-handed (meaning that if you look through its axis, it twists clockwise moving away) and is stabilized by hydrogen bonds between H and O atoms of peptide units in adjacent turns.

> We are not going in circles, we are going upwards. The path is a spiral; we have already climbed many steps.—Herman Hesse, *Siddhartha*

In the β sheet, two or more segments of a polypeptide chain (called **β strands**) are almost fully stretched, and they undulate in such a way that the planes of every other peptide unit are parallel (figure 3.7*b*). The side chains of adjacent residues in a β strand point in opposite directions. β Strands are held parallel by hydrogen bonds between H and O atoms of peptide units in neighboring strands, thus forming the β sheet.

> An amino acid occupies 3.5 Å along a β strand, as opposed to only 1.5 Å along an α helix.

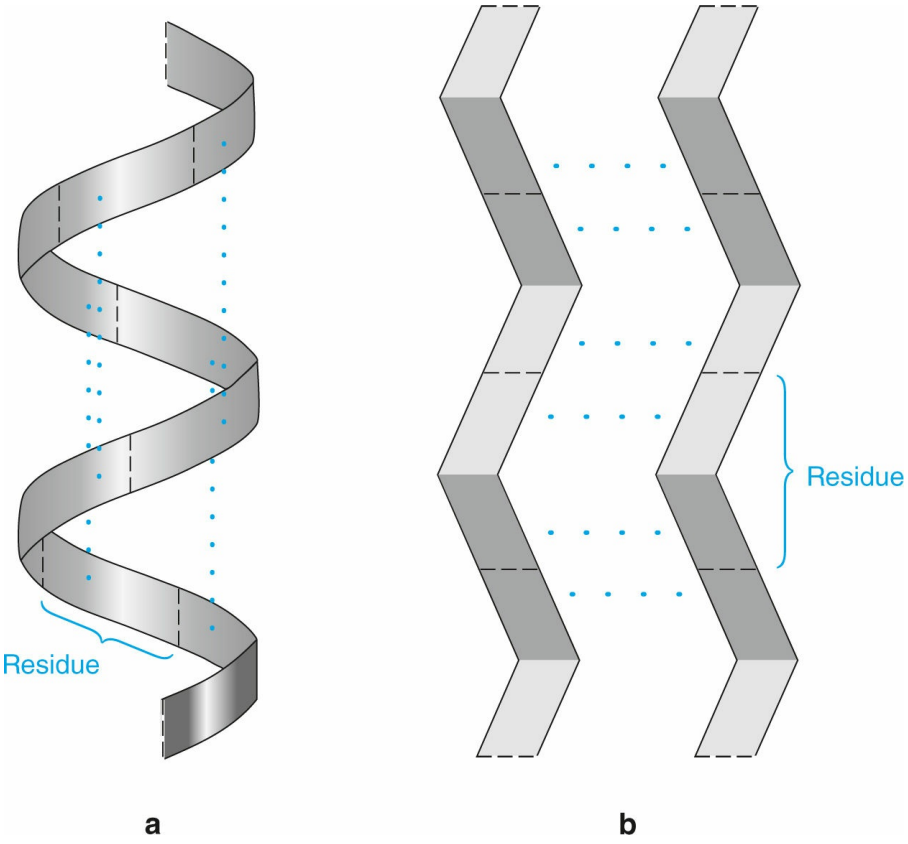


Figure 3.7 **Secondary structure of proteins.** Two of the most common conformations of short stretches of polypeptide chains are the α helix *(a)* and β (pleated) sheet *(b)*. The polypeptide chains are presented as ribbons. Broken lines intersecting the ribbons mark the boundaries of the amino acid residues and the positions of peptide bonds. Colored dots represent hydrogen bonds. The α helix is a right-handed spiral held in place by hydrogen bonds almost parallel to its axis. The β sheet is an undulating structure. Two or more β strands may approach in parallel and form a sheet stabilized by hydrogen bonds perpendicular to the axes of the strands.

3.5 Tertiary Structure

The conformation of an entire polypeptide chain is its tertiary structure. The chain is flexible and can fold to bring together residues that lie far apart in terms of primary structure. Like the secondary structure, the tertiary one is stabilized by hydrogen bonding; it also employs four other kinds of interactions: the **electrostatic bond**, the **van der Waals interaction**, the **disulfide bond**, and the **hydrophobic interaction**.

Electrostatic Bond

The electrostatic, or ionic, bond is formed between positively and negatively charged groups and is due to the attraction of opposite charges (figure 3.8). Positively charged groups in a protein are the terminal amino group and the side chains of lysine, arginine, and (possibly) histidine (refer to figure 3.2). On the other hand, negatively charged groups are the terminal carboxyl group and the side chains of aspartate and glutamate. Electrostatic bonds within or between molecules in biological fluids are weaker than hydrogen bonds.

All of the positively charged groups in a protein can donate H^+, whereas all of the negatively charged groups in a protein can accept H^+. This contrast endows proteins with considerable buffer capacity (section 1.12) and plays an important role in maintaining the pH of biological fluids.

Figure 3.8 **Electrostatic bond in a protein.** Two oppositely charged groups, like an amino group and a carboxyl group in a protein, may be attracted to form an electrostatic, or ionic, bond.

van der Waals Interaction

A van der Waals interaction is a noncovalent attraction that can develop between any two chemical entities because of the movement of electrons in atomic or molecular orbitals. Because of this movement, momentary asymmetries appear in the distribution of charges inside atoms or molecules. These asymmetries induce similar ones (by repulsion of like charges and attraction of opposite charges) in neighboring atoms, molecules, or parts of the same molecule. Thus, attractive electrostatic forces develop. If such forces appear between parts of a protein molecule, they contribute to the stabilization of its tertiary structure. Van der Waals interactions are weaker than electrostatic bonds.

Disulfide Bond

The disulfide bond (figure 3.9) is a covalent bond formed between residues of cysteine, an amino acid with a **sulfhydryl group** (—SH) at the end of its side chain (number 12 in figure 3.2). The Cys residues can be anywhere along the

polypeptide chain. In forming a disulfide bond, two sulfhydryl groups shed their H, and their S atoms are linked. (Would you call this an oxidation or a reduction?)

This is one case of amino acid modification after protein synthesis (mentioned at the beginning of section 3.1). The disulfide bond is stronger than the peptide bond. As a result, when a protein is broken down into its constituent amino acids, the two cysteine residues remain united, forming **cystine**. The location of disulfide bonds in a protein is part of what characterizes its primary structure.

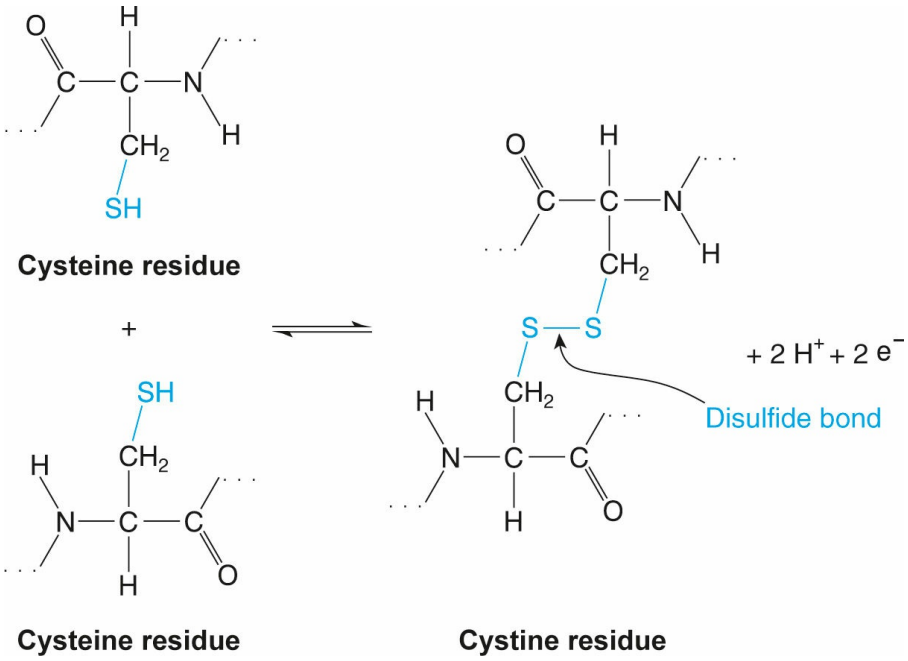


Figure 3.9 **Disulfide bond**. Two cysteine residues in a polypeptide chain can come together in such a way that their side chains are joined through a disulfide bond after each one loses a full hydrogen atom (that is, a proton and an electron).

Hydrophobic Interaction

Finally, hydrophobic side chains—such as those of alanine, valine, and leucine (numbers 2 to 4 in figure 3.2)—may come together and recede inside a protein molecule. Such hydrophobic interactions result not so much from mutual attraction as from repulsion by the surrounding water. We first encountered this tendency when we discussed hydrophobic substances in

section 1.7. The clustering of hydrophobic groups limits their surface of contact with water. This compact arrangement is more stable than an arrangement in which every hydrophobic group would be exposed to water. Hydrophobic interactions are the weakest of the interactions that we have considered.

Universality of Interactions

Except for the disulfide bond, the interactions presented so far in the chapter are not confined to protein molecules. Thus, hydrogen bonds, electrostatic bonds, van der Waals interactions, and hydrophobic interactions develop both within and across all classes of biomolecules. In fact, *these types of rather weak interactions among molecules in biological fluids enable biochemical processes to occur.*

Figure 3.10 presents an example of the tertiary structure of a protein. The protein, ribonuclease, features three α helices and six β strands, four of which form two β sheets (one on the right and one on the left). These periodic secondary structures are joined by short irregular stretches of residues. Ribonuclease contains eight cysteine residues linked in pairs by four disulfide bonds.

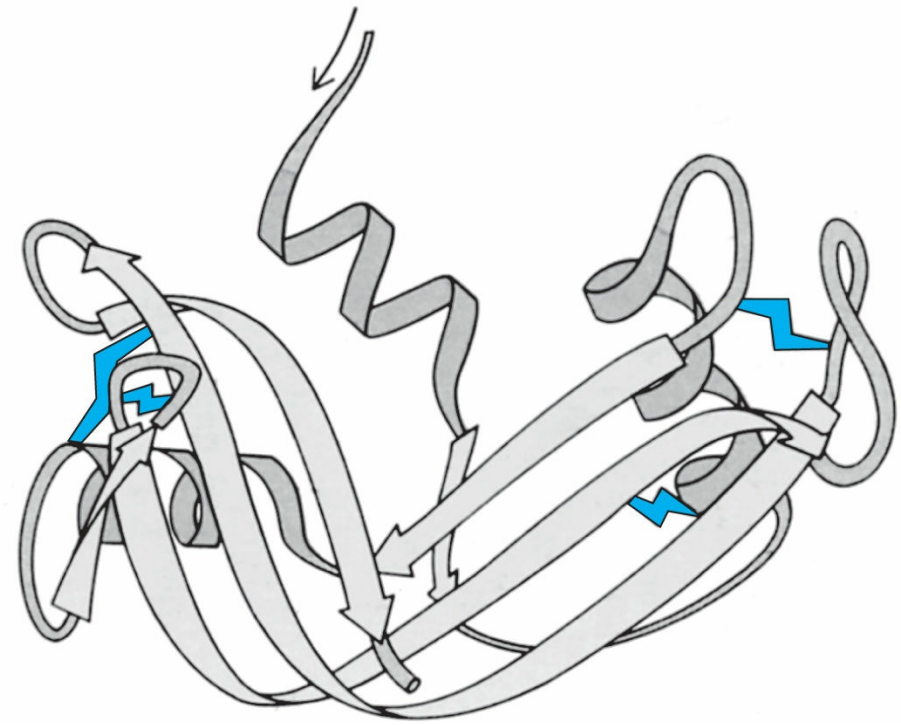


Figure 3.10 Tertiary structure of a protein. Ribonuclease is a relatively small protein combining α helices and β pleated sheets in its structure. α Helices are depicted as spiraling ribbons, and β sheets are depicted as flat ribbons with arrowheads showing the conventional direction of the polypeptide chain (*N* → *C*). The thin arrow at the top indicates the amino end. Four disulfide bonds (two on the left and two on the right, all shown as colored lightning bolts) stabilize the tertiary structure. Try to follow the polypeptide chain from one end to the other. In case you are wondering what ribonuclease does, it is an enzyme—that is, a protein that speeds up chemical reactions, as we will see in section 3.13. Specifically, ribonuclease speeds up the breakdown of a nucleic acid (RNA).

Courtesy of Jane S. Richardson.

3.6 Denaturation

Being stabilized by the bonds mentioned in the preceding section, each protein acquires a definite tertiary structure. Structure determines the interaction of the protein with other molecules and, therefore, protein function. The tertiary structure is sensitive, because it results mostly from weak interactions.

The delicate structure of a protein can easily be disrupted by factors such as high temperature and a highly acidic or alkaline environment. High temperature increases the thermal movement of the segments of a polypeptide chain and of the surrounding molecules, which results in alteration of the

chain's structure. Extreme pH values, on the other hand, cause the addition or removal of protons to or from chemical groups in the protein that have the tendency to exchange H^+ (listed in the preceding section). This change, in turn, elicits changes in hydrogen and electrostatic bonding, which affect protein structure. In all such cases, we speak of protein **denaturation**, which is usually permanent (irreversible).

We encounter many cases of protein denaturation in relation to our nutrition. For example, when we cook an egg, its contents solidify because of two factors: the egg's high protein content (13% of its mass, or more than half of its solids) and the collapse of protein structure when heated to 62 °C or higher. The combination of these factors makes the proteins lose their solubility in water, which results in solidification of the egg contents.

Yogurt, on the other hand, offers an example of denaturation due to a combination of heating and a drastic drop in pH: Milk proteins lose their solubility and settle when milk is first heated to 85 to 95 °C and then, after cooling down to about 42 °C, microorganisms are added to it. The microorganisms degrade the carbohydrate of milk (lactose, which is presented in section 5.3) into lactate and H^+, resulting in a decrease of pH from about 6.5 to 4.5.

3.7 Quaternary Structure

One would expect the tertiary structure (which shows how the entire polypeptide chain folds) to be the highest level of organization in a protein. However, many proteins consist of not just one but two or more polypeptide chains, which are called **subunits** or simply chains. Proteins of this kind present an additional level of organization—quaternary structure—which is defined as *the conformation of the entire complex of subunits.*

The quaternary structure is as precise as the tertiary one: The position of each subunit relative to the others is strictly defined, and the subunits are held in place by the same bonds as those that stabilize the tertiary structure. A classic example of a protein having subunits (and, therefore, quaternary structure) is hemoglobin, which we will examine in terms of both structure and function in sections 3.11 and 3.12. The quaternary structure of another

protein is shown in figure 3.11.

The quaternary structure, like the tertiary structure, is stabilized by hydrogen bonds, electrostatic bonds, van der Waals interactions, disulfide bonds, and hydrophobic interactions.

3.8 Protein Function

Proteins have a vast repertoire of functions, which endows them with the ability to participate in any biochemical process. Examples of biological processes that we have considered or will consider in this book include metabolism; transport of substances; movement of components, cells, and whole organisms; reproduction; cell division; cell growth; cell differentiation; cell organization and biogenesis; homeostasis; defense response; response to stimulus and signal transduction; and cell communication. Finally, strange as it might seem, the regulation of a biological process constitutes a biological process in itself.

To facilitate the study of proteins participating in such diverse processes, we assign them to functional categories such as the following:

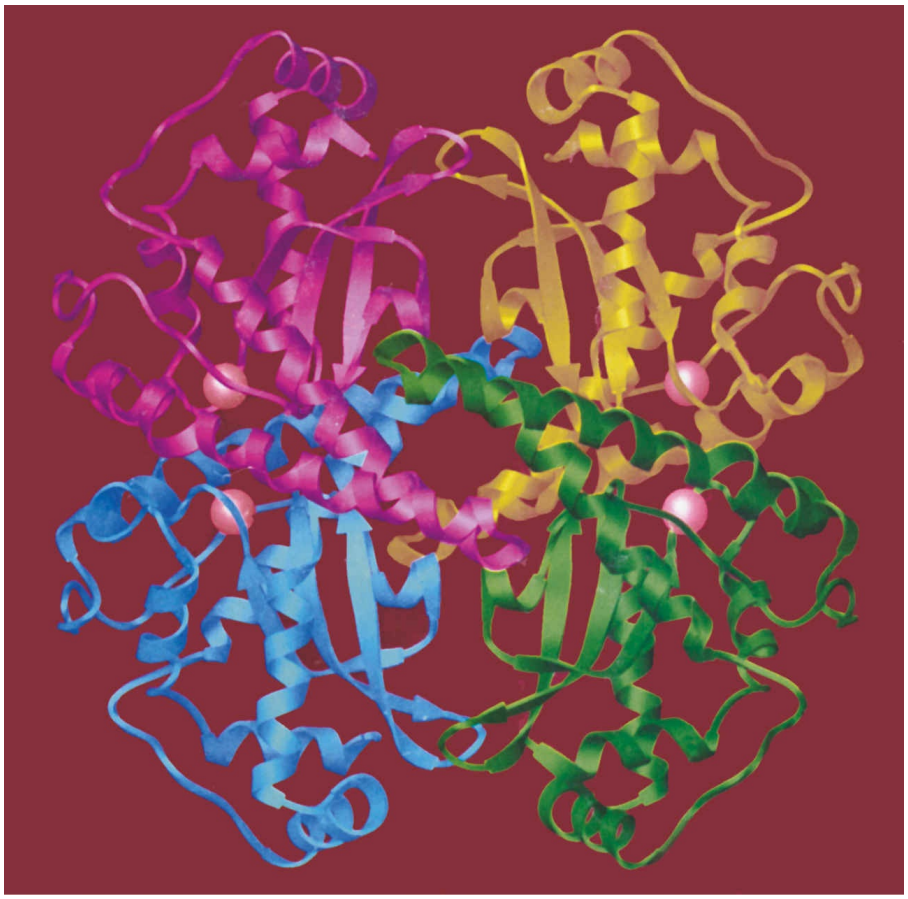


Figure 3.11 **Quaternary structure of a protein.** Mitochondrial superoxide dismutase is an enzyme with antioxidant activity. Specifically, as discussed in section 14.24, it prevents the superoxide radical (introduced in section 1.5) from acting as an oxidant. The protein consists of four identical polypeptide chains shown in different colors. Can you discern α helices and β sheets in each subunit? The four pink orbs—one in each subunit—are manganese cations (Mn^{2+}) attached to the polypeptide chains through a kind of bond (called *coordinate*) that I will explain in section 3.10. Mn^{2+} is required for the enzyme to function. You may mark manganese as our 12th acquaintance with an element found in living organisms.

Image by M. Pique, G. Borgstahl, and J. Tainer.

- **Catalytic proteins.** A plentiful group of proteins catalyze (that is, accelerate) metabolic reactions. These proteins are called **enzymes** and will be the topic of subsequent sections.
- **Transport proteins.** Many substances are transported in and out of cells, in and out of intracellular organelles, and from one site in the body to another with the help of specific proteins. For example, oxygen from the air is carried to our cells by hemoglobin, the major blood protein.

- **Storage proteins.** Other proteins store cellular components. This category includes ferritin, which stores iron in the body (see section 16.6).
- **Motile proteins.** Movement in living organisms is made possible by the interaction of organized protein assemblies. Muscle contraction, for example, is feasible thanks to the movement of myosin relative to actin, as we will see in part II.
- **Structural proteins.** Proteins offer mechanical support to cells and tissues. The cytoskeleton, which gives cells a shape (otherwise they would be amorphous sacs of cytoplasm), consists of proteins. The elasticity of connective tissue is due to the remarkable properties of proteins like collagen and elastin, and another family of structural proteins—the keratins—are key components of the outer layer of skin, hair, wool, nails, claws, horns, hooves, scales, feathers, and beaks.
- **Defensive proteins.** This category comprises the **antibodies**, components of the immune system, which recognize and neutralize foreign substances and cells very selectively, thus protecting us from invaders.
- **Messenger proteins.** Multicellular organisms require communication among their cells to ensure concerted action. Many of the compounds transmitting signals from one cell to another are proteins. This category includes hormones such as insulin, as well as neurotransmitters such as endorphins. In addition, proteins participate in signal transduction within cells; one example is protein kinase A (discussed in section 10.6).
- **Receptors.** Molecular messengers—whether protein in nature or not—carry out their missions indirectly by binding to proteins that recognize them. These proteins are called **receptors**, and one example is the insulin receptor. The binding of a messenger to its receptor changes the structure of the latter, thus altering its biological activity. There is at least one receptor for every messenger.
- **Other regulatory proteins.** The proteins of most of the preceding categories control biological processes and could thus be characterized

as regulatory in one sense or another. There are additional regulatory proteins that do not fit into any of these categories. They include, for example, the proteins that control the flow of genetic information by binding to nucleic acids. Biochemical research is revealing a rapidly growing number of regulatory roles for proteins.

I must stress that the classification just presented is not meant to confine proteins within mutually exclusive categories but, rather, to demonstrate the inexhaustible variety of functions that proteins perform. Many proteins do not limit themselves to one function. For example, myosin is a motile protein and an enzyme at the same time, as we will see in section 8.3.

Most proteins carry out their specialized functions by binding other compounds reversibly and with high specificity, or selectivity. Such compounds are called **ligands** and may be any kind of compound. For example, insulin is a ligand for the insulin receptor.

In the ensuing sections of this chapter, we will turn our attention to some proteins of special interest. First, we will examine myoglobin and hemoglobin as representatives of storage and transport proteins. Our interest in them lies in the fact that they bind the oxygen we need in order to live and exercise. Then we will examine the enzymes, since almost none of the reactions described in the book can take place to any appreciable extent unless an enzyme is present.

3.9 Oxygen Carriers

Animals and many microorganisms cannot live without oxygen; thus, they are **aerobic** organisms. Oxygen is vital to them because it serves as the ultimate oxidant in the electron-transport chain, which provides the energy needed to synthesize the vast majority of their ATP (section 2.6). The first aerobic bacteria appeared on Earth approximately in the middle of its existence, some two billion years ago. Their evolution became possible thanks to the liberation of oxygen in the primeval atmosphere as a product of **photosynthesis**. Photosynthesis had enabled more primitive bacteria to harness solar energy and use it to power the extremely endergonic synthesis of glucose from carbon dioxide and water.

$$6\,CO_2 + 6\,H_2O \rightleftharpoons C_6H_{12}O_6 + 6\,O_2 \qquad \Delta G^{\circ\prime} = 707\ \text{kcal}\cdot\text{mol}^{-1} \quad \text{(equation 3.1)}$$

Photosynthesis is currently carried out mainly by plants. Aerobic organisms flourished on Earth thanks to the large amounts of energy released when O_2 oxidizes (burns) fuel molecules and thanks to the vigorous regeneration of O_2 by plants (figure 3.12). The entry of O_2 into microorganisms posed no problem, since O_2 could diffuse easily from the surrounding air or water—where it was dissolved—into the cells by crossing their membranes. Things were not so simple, however, for multicellular organisms, since only the cells at their surface contacted air or water. Thus, the imperative arose to carry O_2 to every internal cell—a prerequisite for the evolution of large and strong animals.

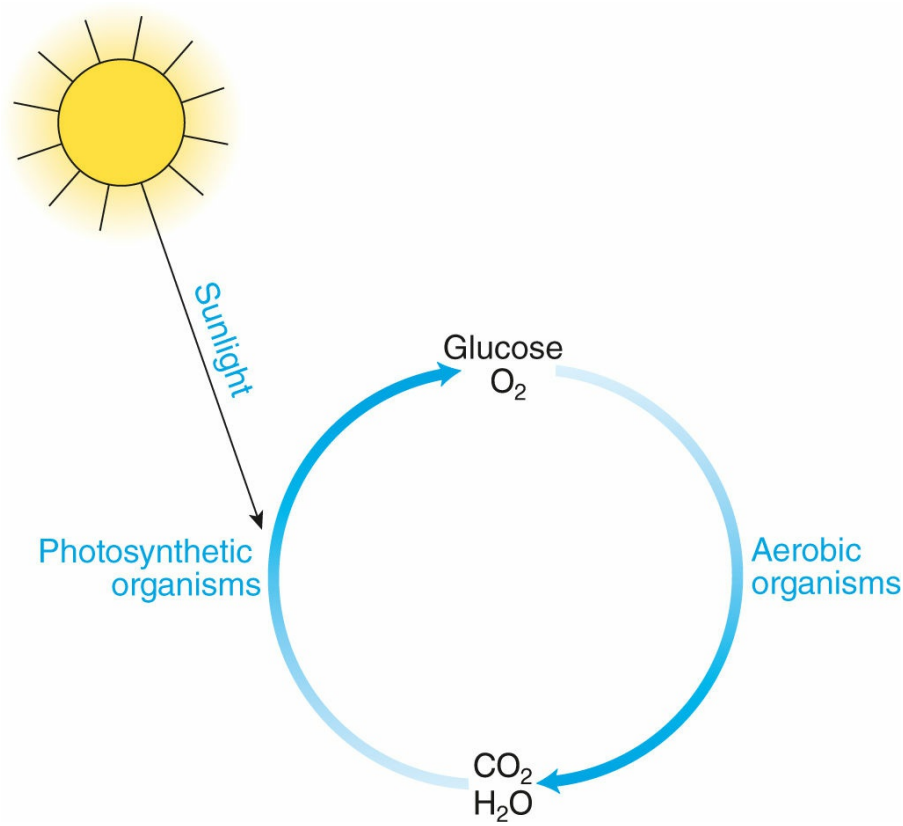


Figure 3.12 **Indispensable photosynthesis.** Aerobic organisms such as humans could not have existed on Earth had photosynthetic organisms (such as cyanobacteria, algae, and plants) not evolved first. Photosynthetic organisms managed to put sunlight at the service of producing an organic compound (glucose) from inorganic raw materials (CO_2 and H_2O). The other product of photosynthesis, O_2, proved to be an excellent oxidant of not only glucose but also other fuels (such as lipids and proteins) produced from glucose by photosynthetic organisms. This set of circumstances enabled aerobic organisms to evolve and thrive on

Earth.

Unfortunately, being nonpolar, O_2 is so poorly soluble in water that its mere transport through the body fluids of the first animals could not meet the needs of their active cells. Thus, natural selection favored the evolution of proteins with high capacity to carry and store oxygen, which appeared some 500 million years ago. We will explore two such proteins in the next three sections, starting with the simpler one.

3.10 Myoglobin

Myoglobin is a relatively small protein. The human form contains 153 amino acid residues and has a molecular mass of 17 kDa. Myoglobin is located primarily in skeletal and cardiac muscle. Its function is to transport oxygen from the plasma membrane (which oxygen has reached through the bloodstream) to the mitochondria, where it is used in stage 3 of catabolism for ATP production (see section 2.6). In addition, myoglobin serves to store oxygen in the cytosol of muscle cells, which helps muscle cope with sudden increases in oxygen demand during exercise.

As summarized in a review of the cognate scientific literature, published by Gerolf Gros and coworkers, human skeletal muscle contains about 6 grams of myoglobin per kilogram, or 0.6% of its mass, whereas the myoglobin content of the muscles of sea mammals, such as seals and whales, is about 4.5%. This big difference is understandable, since sea mammals benefit from large reservoirs of O_2 for their long dives.

Myoglobin owes its high oxygen-carrying and oxygen-storing capacity to the fact that it binds O_2 with high **affinity**, that is, binding strength. In fact, the ability to bind O_2 resides not in the polypeptide chain of myoglobin per se but in a nonprotein compound attached to the polypeptide chain. This is not uncommon: Many proteins owe their function to relatively small organic molecules that are tightly bound to the polypeptide chain. These molecules are collectively known as **prosthetic groups**.

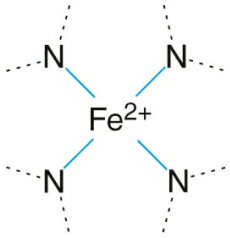


Figure 3.13 **Heme iron.** Iron sits in the middle of the heme molecule, forming four coordinate bonds (in color) with N atoms that belong to a complex, almost flat organic ring (not shown in its entirety). This bonding is possible because N harbors a lone electron pair that is available for coordinate bonding.

The prosthetic group of myoglobin, called **heme**, is a complex red compound with one cation of **iron** (Fe^{2+}) in the middle. (Count iron as the 13th element we meet in living organisms.) Iron is held in place by forming **coordinate bonds** with four surrounding nitrogen atoms (figure 3.13). A coordinate bond is formed by the unilateral contribution of an electron pair from one of the two bound atoms. In the case of heme, the electron pairs come from the nitrogen atoms.

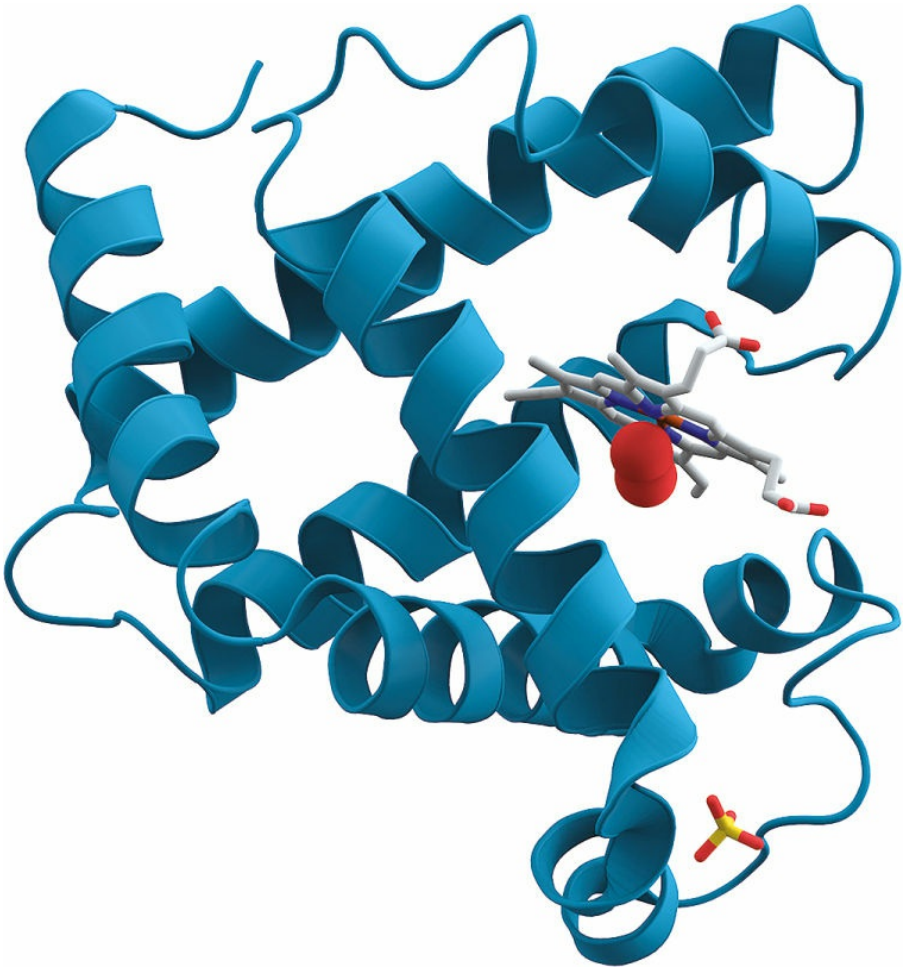


Figure 3.14 **Myoglobin.** Most of the polypeptide chain in myoglobin is folded into α helices. Heme is depicted on the right side as a disc in gray (representing carbon atoms), red (O), blue (N), and orange (Fe, at the center). An O_2 molecule (the pair of red orbs) is bound to iron. The seemingly empty space between α helices is in fact occupied by the side chains of the amino acid residues.

Reprinted from Wikimedia Commons, "File:Myoglobin.png," Available: https://commons.wikimedia.org/w/index.php?title=File:Myoglobin.png&oldid=260048661.

The tertiary structure of myoglobin can be seen in figure 3.14. Its molecule is compact, measuring 45 by 35 by 25 Å, and most of the polypeptide chain takes the form of α helices. Heme lies in a cavity of the molecule and is held in place through bonds with the side chains of residues from adjacent α helices. One of these residues, histidine, binds the heme iron through a fifth coordinate bond that is almost perpendicular to the heme plane and is formed by the donation of a lone electron pair from a nitrogen in the histidine side chain (see figure 3.2). Iron binds O_2 on the other side of the

heme plane through a sixth (and final) coordinate bond formed by the donation of a lone electron pair from one of the two atoms in the O_2 molecule.

Myoglobin is the first protein to have its three-dimensional structure revealed by X-ray crystallography, an experimental technique in which crystals (including crystals of biological substances) are bombarded with X-rays. The rays are diffracted by the crystal structure, and the patterns produced are imprinted on film (as in a radiograph). By studying these imprints, researchers draw conclusions about the structure of the substances under study. The English biochemist and crystallographer, John Kendrew, who led the scientific group that reported this feat in 1958, shared the 1962 Nobel Prize in Chemistry with Max Perutz, an English molecular biologist, for their studies on the structures of globular proteins.

The oxygenated form of myoglobin is called **oxymyoglobin**, whereas the one lacking oxygen is **deoxymyoglobin**. Thus, one can write the equation

$$\text{Deoxymyoglobin} + O_2 \rightleftharpoons \text{oxymyoglobin} \qquad \text{(equation 3.2)}$$

According to the discussion in section 2.2, the reaction is shifted to the right when there is ample supply of oxygen to the muscles, resulting in oxygen storage within oxymyoglobin. However, when the oxygen concentration drops because of, for example, a long dive performed by a sea mammal, or exercise performed by any animal, the reaction is shifted to the left, and oxymyoglobin releases its oxygen to support energy production in the mitochondria.

If only iron binds oxygen, what is the rest of myoglobin doing? The answer is that neither iron nor heme alone can hold O_2 because O_2 is so reactive that it oxidizes Fe^{2+} to Fe^{3+}, thus rendering it incapable of attracting O_2. What is more, the process produces radicals, which can harm cellular constituents, as we will see in section 14.24. However, the polypeptide chain offers a protective environment that prevents iron oxidation by keeping oxygen at a safe distance. Thus, it takes the entire myoglobin molecule to carry out the task of oxygen transport and storage.

3.11 Hemoglobin Structure

Hemoglobin is the most abundant protein of the **erythrocytes**, or red blood

cells. Erythrocytes are small, donut-shaped cells that usually take up 35% to 50% of the blood volume. The rest is occupied by **plasma**, which is the fluid of blood, as well as other cells that we will discuss in chapter 16. Hemoglobin is also the most abundant protein of blood as a whole; a typical concentration in humans is 14 $g \cdot dL^{-1}$. (For more on blood hemoglobin concentration, see section 16.1).

Hemoglobin binds atmospheric oxygen in the capillaries surrounding the alveoli of the lungs, thus turning from **deoxyhemoglobin** into **oxyhemoglobin**. The erythrocytes then transport oxyhemoglobin to all tissues. Oxygen dissociates from hemoglobin in the capillaries of the tissues, mainly because the oxygen concentration there is lower than in the alveoli, and diffuses into the cells. The dissociation of O_2 from hemoglobin is facilitated by the presence of CO_2 and H^+ in the capillaries, as we will see in the next section.

Hemoglobin binds O_2 through heme, exactly as myoglobin does. However, hemoglobin enjoys a higher level of organization: quaternary structure. Indeed, hemoglobin consists of not just one but four polypeptide chains, called **globins**. In adult humans, the hemoglobin complex contains two globins of the so-called α type and two of the β type (figure 3.15). The tertiary structure of each globin is very similar to that of myoglobin, although the primary structures differ markedly. Globins contain α helices of almost the same arrangement as in myoglobin. In addition, each globin contains one heme. As a result, there are four oxygen-binding sites in hemoglobin. The presence of heme in hemoglobin gives blood its red color.

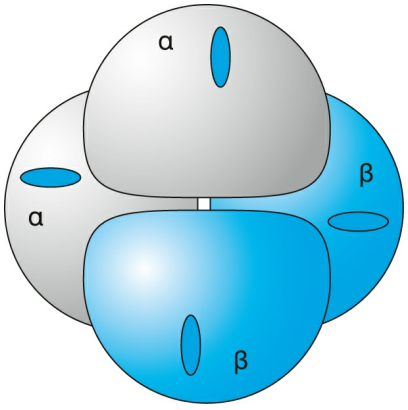


Figure 3.15 **Hemoglobin.** The oxygen carrier in our blood is a tetramer (a complex of four

subunits) having the subunit composition $\alpha_2\beta_2$. Each hemoglobin subunit (globin) resembles myoglobin and is in touch with all three other subunits. The colored discs, one in each globin, represent heme.

The four globins are held together by an array of hydrogen bonds, electrostatic bonds, van der Waals interactions, and hydrophobic interactions, all of which give rise to a tetrahedral structure of impressive spatial economy. The resulting complex is nearly globular and has a diameter of 55 Å. This globular shape is reflected in the name of hemoglobin—and, by extension, of myoglobin. (As for *hemo* and *myo*, they derive from the Greek words *héma*, meaning "blood," and *mys*, meaning "muscle.") The heme molecules are located on the surface of the complex.

3.12 The Wondrous Properties of Hemoglobin

The complex structure of hemoglobin is reflected in its function. The protein is not a mere oxygen carrier but an accurate sensor and regulator of the blood environment. In this section, we will explore those properties of hemoglobin that justify this claim and fall within the scope of the book. These properties would be absent had hemoglobin the simpler structure of myoglobin. The examination of the two proteins exemplifies the fact that complexity in biological systems is not a purposeless luxury; instead, it appears in the course of evolution under the pressure to serve new, more intricate needs.

Cooperativity

Let's begin with the primary role of hemoglobin: oxygen binding and transport. The binding of oxygen by hemoglobin is **cooperative**, which means that the affinity of the protein for O_2 is not fixed but depends on the number of O_2 molecules already bound. Thus, if we started adding O_2 molecules to deoxyhemoglobin by increasing the $[O_2]$ in the surrounding fluid, each O_2 would bind to a globin more tightly than the previous one (as if each molecule entering the hemoglobin complex paved the way in for the next one). In fact, the fourth O_2 binds more than 20 times as tightly as the first one. Conversely, if we started unloading oxyhemoglobin by decreasing

the [O_2], then each O_2 would be removed more easily than the previous one (as if each molecule leaving the hemoglobin complex paved the way out for the next one).

The importance of cooperative binding becomes evident if we consider that the [O_2] is high in the lungs but low in the rest of the body. Thus, thanks to cooperativity, we achieve a higher loading of hemoglobin with O_2 in the lungs and a more efficient unloading in the other organs. In the end, *cooperativity increases oxygen delivery to the cells.*

How can the binding of oxygen to one globin affect the binding to an adjacent one? The answer lies in their interaction. The binding of O_2 causes a translocation of iron by a mere 0.4 Å outside the heme plane, which triggers a series of translocations of amino acid residues inside the globin molecule. This conformational change modifies the interface with the other globins and is transmitted to them, thus facilitating O_2 binding. The result is an increasingly tighter binding of subsequent O_2 molecules.

Losing to Myoglobin

Oxygen delivery from blood to skeletal muscles and the heart is facilitated not only by cooperativity but also by another factor. Specifically, as compared with hemoglobin, myoglobin has a higher affinity for O_2 under physiological conditions. To explain this important effect, I need to introduce two terms: *oxygen saturation* and *partial pressure*.

Oxygen saturation, or **SO_2**, is the percentage of the total oxygen-binding sites in the molecules of an oxygen carrier that are occupied by O_2. SO_2 serves as a handy measure of the balance between the oxy and deoxy forms of hemoglobin and myoglobin. The SO_2 of hemoglobin ranges between 96% and 99% in healthy humans when they are at sea level and in the resting state. It goes down at high altitude, in certain disease states, and during exercise.

Partial pressure of a gas in a mixture (such as O_2 in the air) is the pressure the gas would exert if it alone occupied the volume of the mixture. It can also be thought of as the share of a gas in the total pressure of a mixture. A usual unit of partial pressure is the millimeter of a mercury column (mm Hg), which equals 1/760 of the standard atmospheric pressure.

The partial pressure of oxygen, or pO_2, in blood (resulting from the

dissolution of atmospheric air in blood) affects the SO_2 of hemoglobin. That is, the higher the blood pO_2, the higher the hemoglobin SO_2. Likewise, the higher the muscle pO_2, the higher the myoglobin SO_2. However, the curves depicting these relationships differ for the two proteins (figure 3.16). Thus, whereas hemoglobin is almost fully saturated in the lungs, where the pO_2 is about 100 mm Hg, it gets largely desaturated when it reaches the other tissues (including the muscles at rest), which have a pO_2 of about 40 mm Hg. In contrast, myoglobin remains highly saturated at that pO_2. This difference means that myoglobin has a higher affinity for oxygen than does hemoglobin in the muscles and results in hemoglobin relinquishing much of its O_2 to myoglobin.

Thus, myoglobin binds and sequesters the O_2 molecules that diffuse into the muscle cells after being detached from oxyhemoglobin in the capillaries. Myoglobin's greater "power" than hemoglobin contributes to the superior oxygenation of the muscles as compared with tissues that lack myoglobin. The myoglobin SO_2 decreases during exercise, as the active muscles use more O_2 in the electron-transport chain to feed the synthesis of ATP through oxidative phosphorylation. I will return to this effect of exercise in section 10.21.

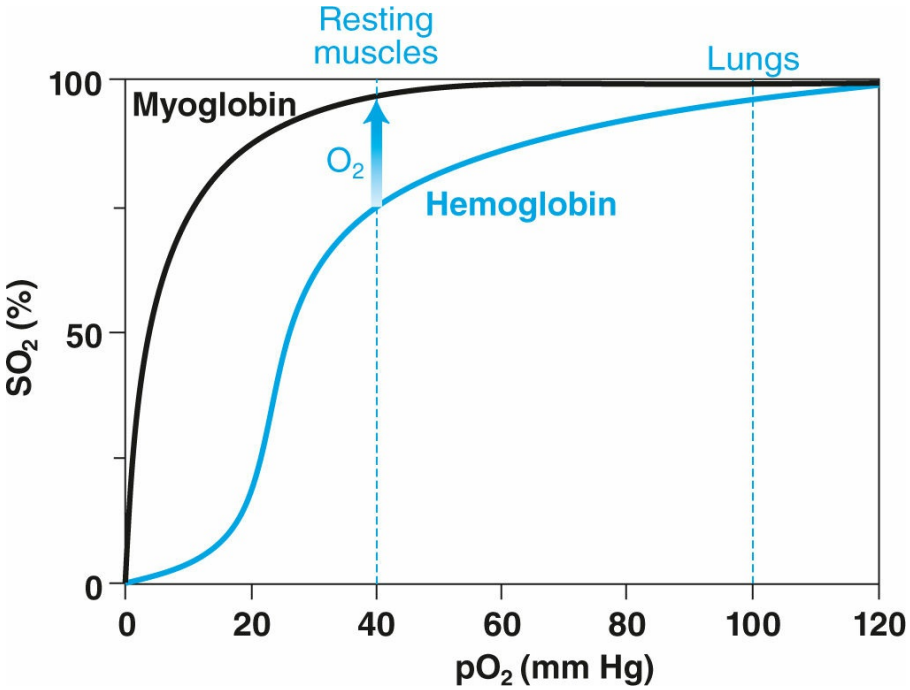


Figure 3.16 SO_2 as a function of pO_2. Note the difference in the curves describing how hemoglobin and myoglobin oxygen saturation levels (SO_2) change in relation to the partial pressure of oxygen (pO_2) in our tissues. As a result of this difference, hemoglobin loses much of its O_2 when it goes from the lungs to the muscles. In turn, myoglobin, which has a higher affinity for O_2, picks it up (arrow) and gets nearly fully saturated.

Carrying CO_2 and H^+

Apart from oxygen, hemoglobin carries carbon dioxide produced by the burning of fuel molecules in the cells. Nevertheless, most (about three quarters) of CO_2 is carried in the bloodstream as soluble bicarbonate (HCO_3^-), formed inside the erythrocytes according to the sequential reactions

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \qquad \text{(equation 3.3)}$$

The compound in the middle is carbonic acid. The reactions are reversed in the alveolar capillaries because of the low CO_2 content of the atmospheric air. Thus, CO_2 passes to the exhaled air. The conversion of CO_2 into HCO_3^- in the erythrocytes and back into CO_2 in the lungs works as an important buffer system, known as the **bicarbonate system**, which cooperates with proteins and other solutes to maintain the pH of blood.

Hemoglobin plays an additional role in binding and transporting part of the H^+ produced in reaction 3.3 and in the anaerobic breakdown of carbohydrates during hard exercise. Removal of H^+ is essential for protecting blood from a dangerous drop in pH, known as **metabolic acidosis**. The binding sites for CO_2 and H^+ on hemoglobin are different from the oxygen-binding sites.

The Bohr and Haldane Effects

The binding of CO_2, H^+, or both to hemoglobin reduces hemoglobin's affinity for O_2. This reduction is known as the **Bohr effect**, after the Danish physician Christian Bohr, who discovered it in 1904. Conversely, the binding of O_2 to hemoglobin reduces hemoglobin's affinity for CO_2 and H^+. This reduction is known as the **Haldane effect**, after the Scottish physiologist John Scott Haldane, whose team described it in 1914.

Both effects occur due to minute intramolecular rearrangements like the

ones described earlier when explaining the mechanism of cooperativity. Both are also of great physiological importance: CO_2 and H^+ abound in the capillaries of active organs, such as exercising muscles. This abundance facilitates the detachment of O_2 from oxyhemoglobin and the entry of O_2 into the cells that need it. Hemoglobin takes up CO_2 and H^+ on the way back to the alveoli of the lungs. There, the abundance of O_2 forces hemoglobin to release CO_2 and H^+.

Allostery

When the binding of a ligand to a protein affects the binding of another ligand to a different site in the protein, we speak of **allostery** (meaning "other site" in Greek). We encountered two cases of allostery in hemoglobin:

- Any bound O_2 molecule(s) favor(s) the binding of additional O_2 molecules until hemoglobin is fully saturated.
- The binding of CO_2, H^+, or both favors the dissociation of O_2 and vice versa.

Hemoglobin and Aerobic Capacity

As the transporter of oxygen to our cells, hemoglobin is intimately connected with aerobic capacity, that is, the maximal amount of oxygen delivered to the tissues during maximal exertion. This capacity is expressed as **maximal oxygen uptake** and symbolized as **$\dot{V}O_2max$**, in which $\dot{V}$ denotes volume per time. To determine $\dot{V}O_2max$, exercise scientists measure the maximal difference between the amount of O_2 in the inspired air and that in the expired air during carefully programmed and executed exercise tests to exhaustion. We measure $\dot{V}O_2max$ in either liters of oxygen per minute ($L \cdot min^{-1}$) or milliliters per kilogram of body mass per minute ($mL \cdot kg^{-1} \cdot min^{-1}$). The latter measure is used to control for body mass, which affects oxygen uptake in a positive manner.

Studies have shown that $\dot{V}O_2max$ depends on the total amount of

hemoglobin in the body. We can calculate total hemoglobin if we multiply the blood hemoglobin concentration by the total blood volume (since concentration is amount per volume). Although it is easy to measure the hemoglobin concentration in a drop of blood, or even bloodlessly, determining total blood volume is more sophisticated; nonetheless, it is feasible. Athletes with high aerobic capacity usually possess a combination of hemoglobin concentration and blood volume that, when multiplied, produce a high amount of hemoglobin. This advantage is supplemented by optimal state of other parameters that affect $\dot{V}O_2max$, such as cardiac function and body composition.

3.13 Enzymes

Enzymes are biological catalysts, substances that accelerate metabolic reactions without being altered in the end. A reaction catalyzed by an enzyme is an **enzyme reaction**, and the reactants of an enzyme reaction are called the **substrates**. Enzymes are proteins, with one intriguing exception that we will discuss in section 4.19.

Enzymes catalyze nearly all reactions that take place in living organisms. Thus, there is almost no biological substance that is not a substrate for an enzyme. Even enzymes themselves are formed and degraded by other enzymes (or, in some cases, by their very selves). Now, if this makes you wonder how the first enzyme came into existence, you will have to be patient until you get to section 4.19.

Enzymes are usually named after their substrate(s), or the kind of reaction they catalyze, or both. Their most frequent ending is *-ase*. For example, the enzyme in figure 3.10 was named *ribonuclease* after its substrate, ribonucleic acid (RNA). The enzyme in figure 3.11, on the other hand, was named *superoxide dismutase* after both its substrate (superoxide) and the kind of reaction it catalyzes (a dismutation, to be explained in section 14.24).

To provide complete information about an enzyme reaction, biochemists like to write the name of the enzyme near the arrow(s) in the chemical equation. Thus, the conversion of substrate S into product P with the aid of

enzyme E would be symbolized as

$$S \underset{}{\overset{E}{\rightleftharpoons}} P \qquad \text{(equation 3.4)}$$

Enzymes share three main features:

- *They speed up reactions* by 10^5 to 10^{17} times. Most of the reactions that occur in living organisms would be so slow without enzymes that they would be practically incapable of supporting life. Some enzymes make a reaction take place several hundred thousand times per second.
- *They display high specificity*. Each enzyme catalyzes one reaction or a limited number of reactions involving substrates that have something in common, such as a chemical group or bond. As an example of high specificity, an enzyme usually distinguishes the isomers of a compound, recognizing only one as substrate.
- *Their catalytic power is regulated.* The speed at which an enzyme reaction proceeds is affected by a variety of factors. This susceptibility renders the enzymes extremely versatile as catalysts and lets living organisms control how fast they synthesize or break down their components depending on their needs.

We encounter an example of isomer discrimination by enzymes in protein synthesis (see sections 4.14 to 4.17): Enzymes involved in this process use only L-amino acids (not D-amino acids) as substrates. This discrimination is how proteins come to contain amino acids of this form only.

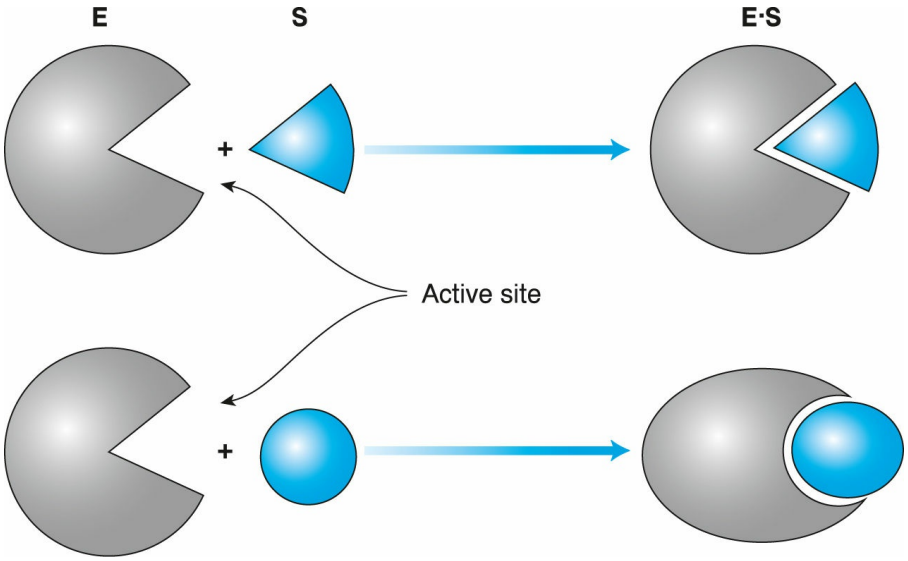


Figure 3.17 **Mutual recognition of enzyme and substrate.** Two means of recognition between enzyme (E) and substrate (S) have been proposed. According to the lock-and-key model (top), S fits the active site of E as a key fits a lock. According to the induced-fit model (bottom), E interacts with S and undergoes a conformational change that results in S fitting the active site.

3.14 The Active Site

The specificity of an enzyme (the second of the features mentioned in the preceding section) is due to the complementary structure of part of its molecule in relation to the molecule(s) of the substrate(s). Specifically, mutual attraction exists between a relatively small area on the surface of the enzyme, called the **active site**, and the substrate(s). This attraction facilitates product formation.

Two hypotheses explain how an enzyme and its substrate recognize each other (figure 3.17). The older one—proposed in 1894 by Emil Fischer (the German chemist introduced in section 3.1) and expressed through the so-called **lock-and-key model**—maintains that the substrate precisely fits the active site just as a key fits the proper lock. The newer hypothesis, put forward in 1958 by the American biochemist Daniel Koshland and described by the **induced-fit model**, posits that the active site is not complementary to the substrate a priori. However, when the substrate touches the active site, their interaction modifies the shape of the enzyme (and, in some cases, the substrate) in such a way that they fit each other precisely. This model seems

to hold true for most enzymes.

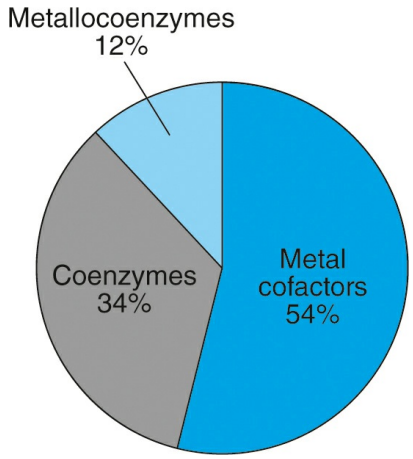


Figure 3.18 **Cofactor distribution.** About 1,800 enzymes contain cofactors in their active sites. The cofactors in most of these enzymes are metal ions, whereas one third contain organic compounds called coenzymes. A small number contain coenzymes with metal ions in the middle (mainly heme).

To catalyze reactions, many enzymes require the presence of nonprotein chemical entities, termed **cofactors**, in their active sites. Cofactors are of two kinds: metal ions and organic compounds. The latter are specifically called **coenzymes**. Most coenzymes derive from vitamins, as we will see in chapter 6, and several of the minerals that we will discuss in the same chapter serve as cofactors. According to the ExPASy bioinformatics resource portal (www.expasy.org), of the approximately 5,500 characterized enzymes in all biological species, close to one third (1,800) require a cofactor. Of those, the majority (54%) contains metal cofactors, and 34% contain coenzymes (figure 3.18). The remaining 12% could be credited to both categories, because they contain **metallocoenzymes**, that is, metal ions embedded in organic compounds. The major metallocoenzyme is heme, which is present not only in myoglobin and hemoglobin but also in many enzymes.

3.15 How Enzymes Speed Up Metabolic Reactions

Every chemical reaction has two distinct sides. One is *which way and how far*

it goes, and the other is *how fast it gets there*. The first aspect is examined by chemical thermodynamics, as presented in sections 2.1 and 2.2. The latter is examined by **chemical kinetics** and will be presented here.

A key term in chemical kinetics is **reaction velocity**, or, more commonly, **reaction rate**. The rate (to be exact, the average rate) of a reaction in a solution is *the change in the concentration of a reactant or product divided by the time in which the change occurs.* Thus, for reaction 3.4, rate, ***V***, is

$$V = \frac{\Delta[\mathrm{P}]}{\Delta t} = -\frac{\Delta[\mathrm{S}]}{\Delta t} \qquad \text{(equation 3.5)}$$

The last fraction has a negative sign in front of it because Δ[S] is negative (since the substrate concentration decreases), whereas Δ[P] is positive (since the product concentration increases). Rate is exactly what an enzyme increases in a reaction. The increase in *V* attributable to an enzyme indicates its catalytic power and is referred to as **enzyme activity**.

The slow hydrolysis of ATP in the absence of specific enzymes is another reason (apart from its high energy content) that it was selected as the energy currency of the cells in the course of evolution. Were it prone to uncatalyzed hydrolysis, cells would have a hard time amassing adequate amounts of it and controlling its allotment for their functions.

The rate of a reaction is irrelevant to how favored the reaction is. For example, ATP hydrolysis is highly favored (exergonic), but if the appropriate enzyme is missing, it is slow. The reason is that *V* is not connected to the free-energy change from reactants to products (Δ*G*). Rather, *V* relates to the free energy of an intermediate and temporary state of the reaction, termed the **transition state**. This is an obligatory passage of high energy for the substrates—and the reactants of chemical reactions in general—on their way to the products. We may think of it as a mountain pass that one must cross in order to get from one valley to another. The transition state is denoted by a double dagger (‡).

$$\mathrm{S} \rightleftharpoons \mathrm{S}^{\ddagger} \rightleftharpoons \mathrm{P} \qquad \text{(equation 3.6)}$$

The free-energy change of the transition of S to S‡ is called the **free energy of activation** and symbolized as Δ***G***‡; it is always positive. The higher its value, the harder the activation of the substrates and the slower the

reaction. (Think of the mountain pass analogy again: The higher the pass, the harder to cross it.) This is exactly where enzymes—and catalysts in general—intervene: By binding the substrates at their active sites, enzymes expedite the formation of the transition state, in effect lowering $\Delta G^{\ddagger}$ (figure 3.19).

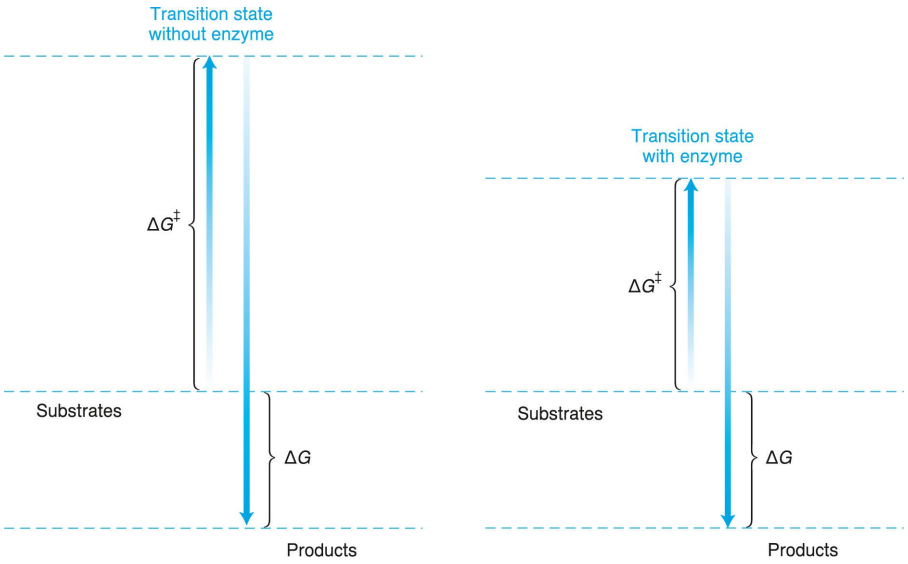


Figure 3.19 **Decreasing the free energy of activation.** Enzymes speed up reactions by decreasing $\Delta G^{\ddagger}$. However, they do not determine the direction of reactions, because they do not affect ΔG.

By lowering $\Delta G^{\ddagger}$, enzymes increase reaction rates. Nevertheless, they do not alter the outcome of the reactions they catalyze. Rather, the outcome is determined by the value of ΔG (without the double dagger), which, as discussed in section 2.2, depends on the nature and concentrations of substrates and products, regardless of the route—catalyzed or uncatalyzed—the reaction takes. From this, it follows that *if an enzyme catalyzes a certain metabolic reaction, it will also catalyze the reverse one*, provided the concentrations of the participating substances are modified so as to change the sign of ΔG.

3.16 Factors Affecting the Rate of Enzyme Reactions

The rate of an enzyme reaction hinges on several factors, including

- substrate concentration,
- enzyme concentration,
- temperature,
- pH, and
- ionic strength.

The following subsections examine these factors one by one. You can find further discussion of the factors that affect the rate of enzyme reactions (including enzyme activation and inhibition), with a focus on the control of exercise metabolism, in the introduction to part III (section III.4).

Substrate Concentration

The concentration of substrate(s) in a solution or biological fluid affects the reaction rate in a positive manner: Increasing the substrate concentration (while keeping the enzyme concentration fixed) results in more substrate molecules binding to the active site on the enzyme molecules and reacting within a given time; therefore, the reaction rate increases. However, there is a limit to this effect: When the active sites of all available enzyme molecules become occupied by substrate molecules, any further increase in the substrate concentration will not affect the reaction rate. At this point, the enzyme is said to be saturated, and **maximal rate**, symbolized as $\boldsymbol{V_{max}}$, has been achieved. The dependence of reaction rate on substrate concentration is shown in figure 3.20.

Two indices of the catalytic properties of an enzyme are the Michaelis constant and the turnover number. The **Michaelis constant**, symbolized as $\boldsymbol{K_M}$, equals the substrate concentration corresponding to half-maximal rate (figure 3.20). It is desirable for an enzyme to have a low K_M because this means that the enzyme requires a low substrate concentration to reach half of its maximal activity and, by extension, any fraction of its maximal activity. To recruit a term that I used extensively when discussing the binding of O_2, CO_2, and H^+ to the oxygen carriers in previous sections, *a low* K_M *means a high affinity of the enzyme for the substrate*. For most enzymes, K_M ranges from 10^{-7} to 10^{-1} mol · L^{-1}.

The Michaelis constant is named after the German biochemist Leonor Michaelis, who, along with the Canadian physician Maud Menten, made an important contribution to the study of enzyme kinetics in 1913 by proposing a mathematical model of enzyme reactions.

The **turnover number** is the number of substrate molecules converted into product by an enzyme molecule in a specified time (usually 1 s) when the enzyme is saturated with substrate. The turnover number shows how fast an enzyme dispatches a reaction and ranges from 1 to 10^6 s^{-1} for most enzymes.

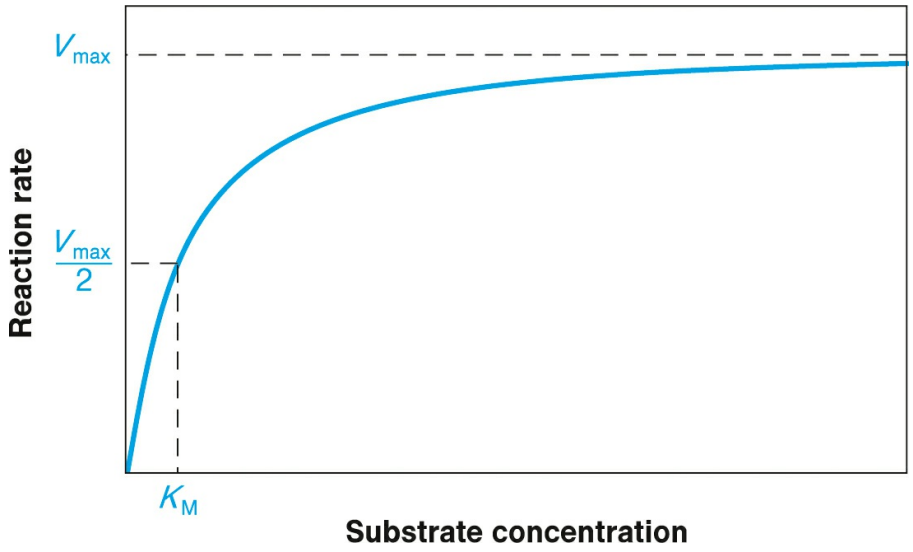


Figure 3.20 **Reaction rate–substrate concentration plot.** The rate of an enzyme reaction increases with the substrate concentration, up to a maximal value corresponding to the saturation of the enzyme. The substrate concentration at which half-maximal rate is reached is indicated by K_M, the Michaelis constant.

Enzyme Concentration

The rate of an enzyme reaction is obviously influenced by the enzyme concentration. Increasing the latter results in a proportional increase in the former, since it makes available more active sites for substrate binding. The dependence of reaction rate on enzyme concentration is depicted in figure 3.21.

Temperature

In general, raising the temperature increases the rate of a chemical reaction, because it increases the kinetic energy of molecules, which results in a higher number of effective collisions (that is, collisions leading to reaction). The same holds true for enzyme reactions, except that raising the temperature above a certain limit—about 50 °C for many enzymes—leads to denaturation (section 3.6). Then enzyme activity drops or even disappears (figure 3.22).

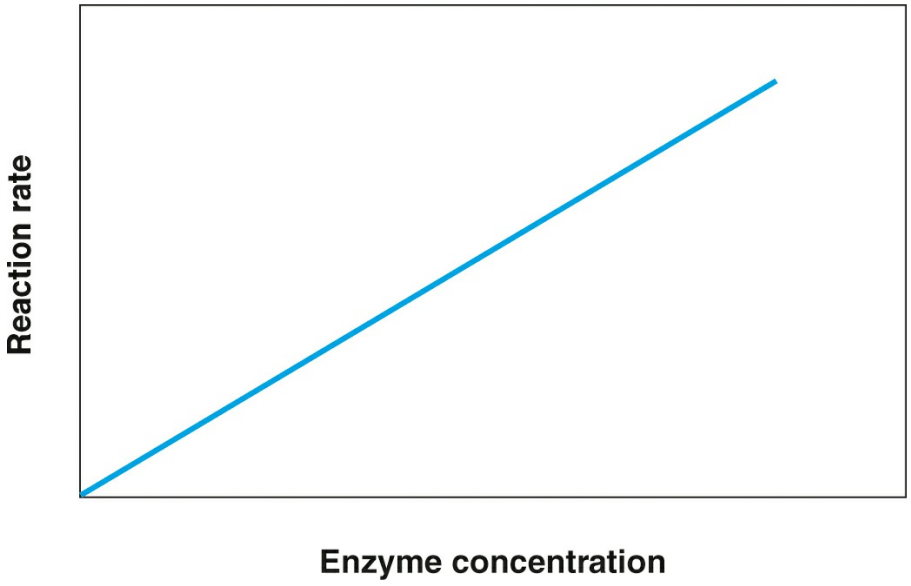


Figure 3.21 **Reaction rate–enzyme concentration plot.** The rate of an enzyme reaction is proportional to the concentration of the enzyme.

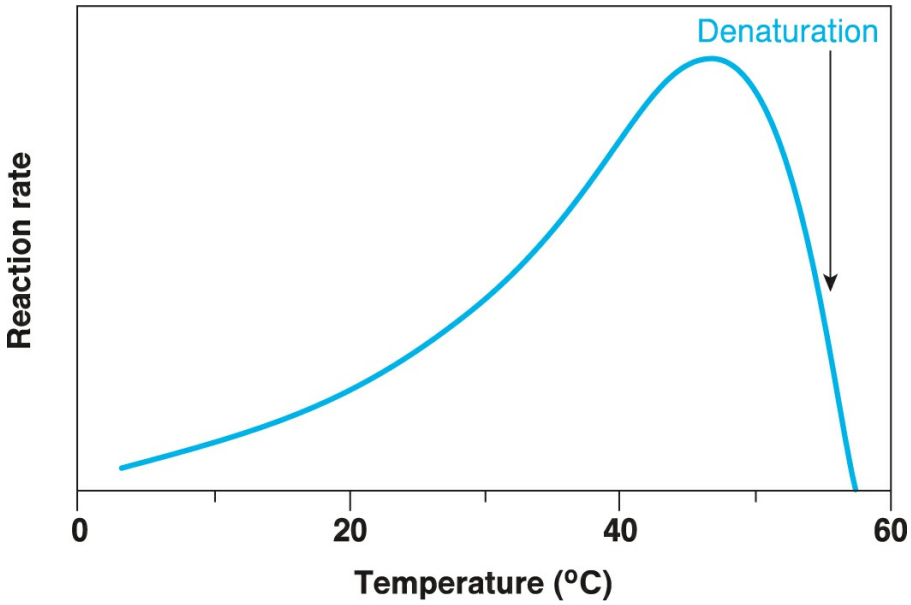


Figure 3.22 Enzyme activity–temperature plot. Enzyme activity increases with temperature up to the point of denaturation of the enzyme (and substrate, if it too is susceptible to denaturation).

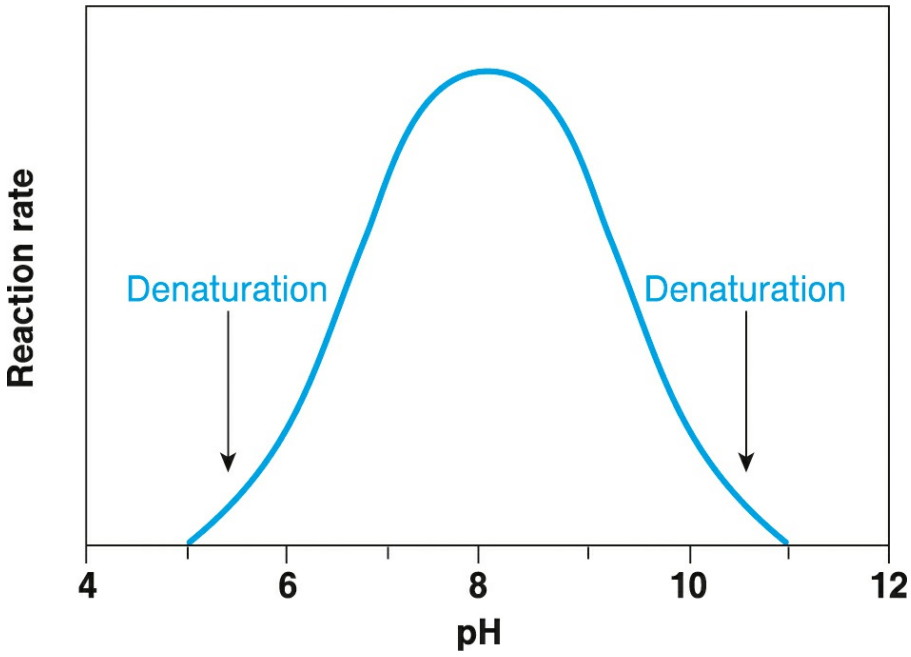


Figure 3.23 Enzyme activity–pH plot. The activity of an enzyme depends on the pH of the surrounding medium. The graph refers to glucose 6-phosphatase, an enzyme involved in glucose metabolism (see section 10.24).

pH

The catalytic power of enzymes depends on the pH of the solution they are in. Changes in the $[H^+]$ cause the addition or removal of protons from an enzyme molecule and may thus alter the conformation of the active site. Enzymes are active in a more or less narrow pH range and usually exhibit maximal activity at a value close to the physiological pH. An example of the dependence of enzyme activity on pH is presented in figure 3.23.

Ionic Strength

Enzyme activity is affected by **ionic strength**, which is the sum of the concentrations of all ions in a solution. Ions interact with protein molecules through electrostatic bonds, thus modifying the tertiary and quaternary

structure of proteins. When the ionic strength is shifted above or below the physiological value (which is about 0.3 mol · L^{-1} for most of our body fluids), many enzymes are denatured.

Summary

Proteins are macromolecules that play pivotal roles in all biological processes. They are made up of amino acids, which are joined, by the hundreds or thousands, through peptide bonds to form polypeptide chains. Cells use 20 amino acids to synthesize their proteins. These amino acids differ in their side chains, which can be hydrophobic, hydrophilic with no charge, negatively charged, or positively charged at physiological pH. The amino acid sequence of a protein is its primary structure, which determines, wholly or largely, the protein's three-dimensional structure and, through that, its function. Short stretches of a polypeptide chain can form regular shapes, such as the α helix and β pleated sheet, which are parts of the protein's secondary structure.

Contributors to the final conformation of a protein include the hydrogen bond, the electrostatic bond, the van der Waals interaction, the disulfide bond, and the hydrophobic interaction. All of these attractive forces develop among atoms of both the side chains and the backbone in a polypeptide chain, resulting in a defined three-dimensional figure: the tertiary structure. Certain proteins consist of more than one polypeptide chain; these chains also have a defined arrangement in space, which is the protein's quaternary structure.

The tens of thousands of existing proteins serve a multitude of functions, such as acceleration of chemical reactions, transport and storage of substances, movement, mechanical support of cells, defense against intruders, signal transduction, and regulation of biological processes. Of this entire medley, we have examined two oxygen carriers and the enzymes.

Hemoglobin and myoglobin fulfill the crucial tasks of carrying atmospheric oxygen in blood and muscles, respectively. Hemoglobin is more complex than myoglobin, consisting of four interacting polypeptide chains rather than one, which is the case for myoglobin. Each chain carries heme, a prosthetic group with iron at its center. Iron is where O_2 binds. The presence of four chains in hemoglobin accounts for the cooperative binding of oxygen, that is, the attachment of each consecutive O_2 to hemoglobin more easily than the previous one in the lungs and the detachment of each consecutive O_2 from hemoglobin more easily than the previous one in the other tissues. Hemoglobin also binds CO_2 and H^+, both of which favor the detachment of O_2 (the Bohr effect). Conversely, the binding of O_2 favors the detachment of CO_2 and H^+ from hemoglobin (the Haldane effect). In addition, hemoglobin has a lower affinity for O_2 than does myoglobin. All of these interactions facilitate the saturation of hemoglobin with O_2 in the lungs; the unloading of O_2 and the uptake of CO_2 and H^+ by hemoglobin in the other tissues (muscles, in particular); and the unloading of CO_2 in the lungs.

Enzymes catalyze metabolic reactions by lowering their free energy of activation and, hence, increasing their rate spectacularly. Each enzyme catalyzes one or just a few reactions thanks to the specialized structure of its active site. Enzymes do not affect the outcomes of reactions; they only help them to go faster in the direction dictated by their ΔG. The rate of an enzyme reaction depends on substrate (reactant) concentration (up to the point of saturation of the enzyme with substrate), enzyme concentration, temperature, pH, and ionic strength.

Problems and Critical Thinking Questions

1. Based on the information provided in section 1.6 about which groups are nonpolar and which are polar, classify the side

chains of the 20 protein amino acids into these two categories.

Nonpolar	Polar

Note: This is the first in a number of "integrative problems," which you will encounter throughout the rest of the book and which require you to combine knowledge acquired in the present chapter with knowledge acquired in previous ones. To prepare you, I will point out this fact at the beginning of such problems.

2. Consider a protein of 1,000 amino acid residues.
   a. How many peptide bonds are in it?
   b. How many *N*-terminal residues does it have?
   c. How many *C*-terminal residues does it have?
   d. What are the chances of any amino acid being at any of the protein's ends?
   e. How many residues have bound (not free) amino groups?
   f. How many residues have bound carboxyl groups?
   g. If all amino acids were equally represented in the protein, how many residues of each would be there?
   h. In the previous case, what would be the maximal possible number of disulfide bonds in the protein?
3. Which of the following proteins have a quaternary structure?

- Myoglobin
- Hemoglobin
- Ribonuclease
- Mitochondrial superoxide dismutase

4. In a process known as *sterilization*, surgical instruments are heated to or above 100 °C for some time to kill microbes that might cause infections in a patient. Explain how sterilization works.
5. When people suffering from oral allergy syndrome eat fresh fruits or vegetables, they get an itchy, burning, or swelling sensation in the lips and mouth. In most cases, this unpleasant effect may be avoided by cooking the fruits or vegetables before eating them. Explain how cooking solves the problem.
6. Classify the proteins listed in problem 3 into the functional categories listed in section 3.8. Do the same for the following proteins:

    - Tropomyosin and troponin, two proteins bound to actin, which control when a muscle contracts (discussed in section 8.10)
    - Transferrin, an iron carrier in blood (discussed in section 16.2)
    - Transferrin receptor (a protein in the plasma membrane of cells that binds transferrin, as discussed in section 16.5)
    - Immunoglobulin A
    - Growth hormone, a protein signaling a variety of anabolic responses in the body (discussed in section 13.5)
    - Nuclear respiratory factors, proteins that control the flow of genetic information by binding to nucleic acids (discussed in section 13.6)

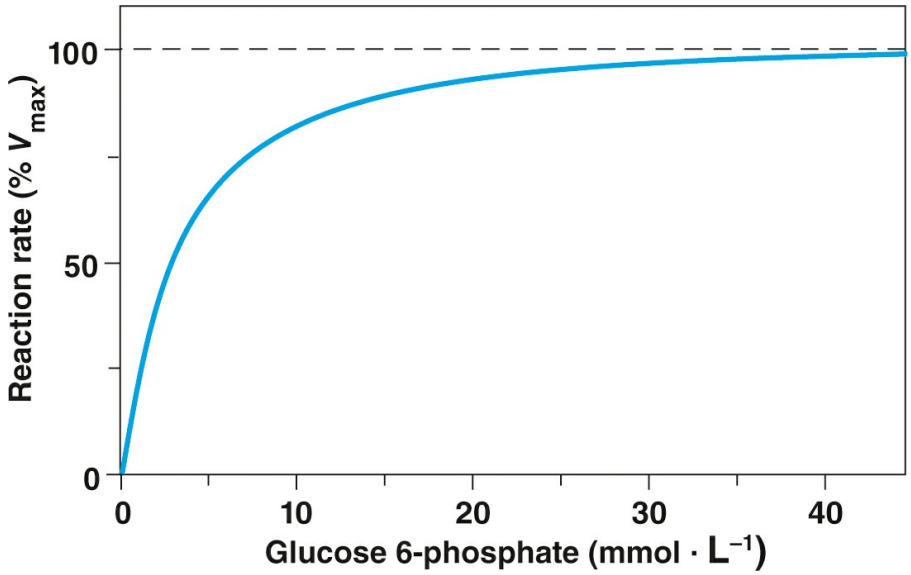
7. Construct a table showing the similarities and differences between myoglobin and hemoglobin in terms of structure and function.
8. List all the ways by which muscles extract O_2 from blood.
9. Glucose 6-phosphatase (the enzyme of figure 3.23) catalyzes the hydrolysis of glucose 6-phosphate according to the following reaction:

$$\text{glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

How will the reaction rate be affected by the following factors?

   a. An increase in the concentration of glucose 6-phosphate
   b. An increase in pH from 7 to 8
   c. An increase in pH from 8 to 9
   d. An increase in temperature from 37 °C to 55 °C
   e. An increase in the concentration of glucose 6-phosphatase

10. Refer to the enzyme in problem 9.
   a. Which compounds would you expect to encounter in the active site?
   b. From the following graph, calculate the K_M.



c. Consider a solution in which the enzyme has a concentration of $1 \cdot 10^{-10}$ mol · L^{-1} and is saturated with glucose 6-phosphate. If the reaction rate is $4 \cdot 10^{-6}$ mol · min^{-1} · L^{-1}, what is the turnover number?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

protein
amino acid
side chain
α-amino acid
enantiomer
peptide
polypeptide chain
residue
amide bond
peptide bond
peptide unit

aspartame
amino-terminal residue, *N*-terminal residue
carboxyl-terminal residue, *C*-terminal residue
primary structure
secondary structure
tertiary structure
quaternary structure
proteome
proteomics
bioinformatics
molecular chaperone
hydrogen bond
α helix
β pleated sheet, β sheet
β strand
electrostatic bond
van der Waals interaction
disulfide bond
hydrophobic interaction
sulfhydryl group
cystine
denaturation
subunit
catalytic protein
enzyme
transport protein
storage protein
motile protein
structural protein
defensive protein

antibody
messenger protein
receptor
ligand
aerobic
photosynthesis
myoglobin
affinity
prosthetic group
heme
iron
coordinate bond
oxymyoglobin
deoxymyoglobin
hemoglobin
erythrocyte
plasma
deoxyhemoglobin
oxyhemoglobin
globin
cooperative
oxygen saturation, SO_2
partial pressure
bicarbonate system
metabolic acidosis
Bohr effect
Haldane effect
allostery
maximal oxygen uptake, $\dot{V}O_2max$
enzyme reaction

substrate
active site
lock-and-key model
induced-fit model
cofactor
coenzyme
metallocoenzyme
chemical kinetics
reaction velocity, reaction rate, V
enzyme activity
transition state
free energy of activation, $\Delta G^{\ddagger}$
maximal rate, V_{max}
Michaelis constant, K_M
turnover number
ionic strength

References and Suggested Readings

Berg JM, Tymoczko JL, Gatto GJ, Stryer L (2015). *Biochemistry* (Freeman, New York), pp. 27-64, 191-250.

Garrett RH, Grisham CM (2012). *Biochemistry* (Cengage, Boston), pp. 77-192, 407-446.

Gros G, Wittenberg BA, Jue T (2010). Myoglobin's old and new clothes: from molecular structure to function in living cells. *Journal of Experimental Biology* 213: 2713-2725.

Mathews CK, van Holde KE, Appling DR, Anthony-Cahill SJ (2013). *Biochemistry* (Pearson, New York), pp. 126-255, 360-413.

Nelson DL, Cox MM (2017). *Lehninger Principles of Biochemistry* (Freeman, New York), pp. 75-242.

CHAPTER 4

Nucleic Acids and Gene Expression

Learning Objectives

After reading this chapter, you should be able to do the following:

- Describe the basic scheme of the flow of genetic information in living organisms.
- Describe the structure of the building blocks of DNA and of RNA and list their differences.
- Discuss the characteristics of the DNA double helix and name the force that stabilizes it.
- Apply the rule of base pairing to deduce the sequence of a DNA strand from the sequence of its complementary strand in a DNA duplex and calculate the percentages of three bases from the percentage of one.
- Describe the process of DNA replication—what it means to be semiconservative, what it requires, which proteins are involved, and how it proceeds.
- Explain the causes of mutations and describe their potential effects.
- State the differences between DNA replication and transcription,

including the differences in action between DNA polymerase and RNA polymerase.
- Define *gene*, *genome*, and *genomics*.
- Describe the size of the human genome in terms of base pairs and genes and explain how the genome is organized.
- Define gene expression and explain the roles of the different kinds of RNA (both classical and novel) in gene expression.
- Discuss the role of our genes in determining performance and fitness.
- Describe the process of translation—what it requires, which cell components are involved, how it begins, how it proceeds, and how it ends.
- Describe the genetic code—what it means that it is degenerate and universal, how many codons it contains, and what they encode.
- Apply the genetic code to deduce the amino acid sequence of a protein from the base sequence of its mRNA.
- Explain which problem is solved by the hypothesis of the existence of RNA before DNA and proteins—and how. Also list the pieces of evidence for this hypothesis.

Nucleic acids and gene expression are the “hottest” areas in modern biochemistry, that is, the ones enjoying the most fervent research activity. They are newer than other scientific areas in the biological sciences. For example, just a little over six decades have passed since the discovery of the basic structure of DNA (the double helix), and the genetic code—a fundamental concept in the flow of genetic information—was deciphered less than half a century ago. Recognition of the roles played by the different kinds of RNA is even more recent, and a new kind is discovered every few years. In addition, the revolutionary laboratory techniques that signaled the birth of genetic engineering appeared only about four decades ago, and the

sequencing of the human genome was completed only in our current century. As for our knowledge of the control of gene expression, it resembles the knowledge of a child exploring the surrounding world. You will be initiated into this magnificent world in the present chapter.

4.1 Introducing Nucleic Acids

Nucleic acids are the biomolecules of heredity. They are of two kinds:

- **Deoxyribonucleic acid**, or **DNA**
- **Ribonucleic acid**, or **RNA**

Nucleic acids are macromolecules made of monomers named **nucleotides**. In particular, the monomers of DNA are called **deoxyribonucleotides**, and those of RNA are called **ribonucleotides**. The terms *nucleic* and *nucleotide* derive from the word *nucleus*. Indeed, DNA is the "trademark" of the (eukaryotic) cell nucleus, and this is where it was discovered. However, the nucleus is not the only home for nucleic acids. RNA—although synthesized in the nucleus—is also found in the cytosol and endoplasmic reticulum. In addition, nucleic acids are present inside the mitochondria. Thus, we are dealing with terms of historical rather than literal meaning.

As for the term *acids*, it reflects the acidic character of DNA and RNA owing to the presence of many phosphoryl groups in their molecules. Finally, the prefixes *deoxyribo-* and *ribo-* refer to the most striking difference in structure between DNA and RNA, one that we will discuss in due time.

4.2 Flow of Genetic Information

We characterize nucleic acids as the biomolecules of heredity because they store and transmit genetic information (that is, information about how an organism is constructed and how it functions) from one generation to another. In fact, a "division of labor" exists between DNA and RNA: The former stores genetic information, whereas the latter transmits it and participates in controlling its flow. Much of the genetic information ends up in protein synthesis. Thus, the fundamental scheme of flow of genetic information is

We call the sum of the genetic material of an organism its **genome**. The genome is like a library (figure 4.1): It consists of large DNA molecules, organized together with proteins in the **chromosomes**, just as a library consists of books. (Picture this book as a chromosome!) Each chromosome contains many **genes**, which correspond to the sections of a book. *A gene is a piece of DNA containing a unit of genetic information*, such as the information on the amino acid sequence of a protein. (Imagine this section of the current chapter you are reading as a gene!) The information about an amino acid is like a word in a section, and deoxyribonucleotides are the letters in that word.

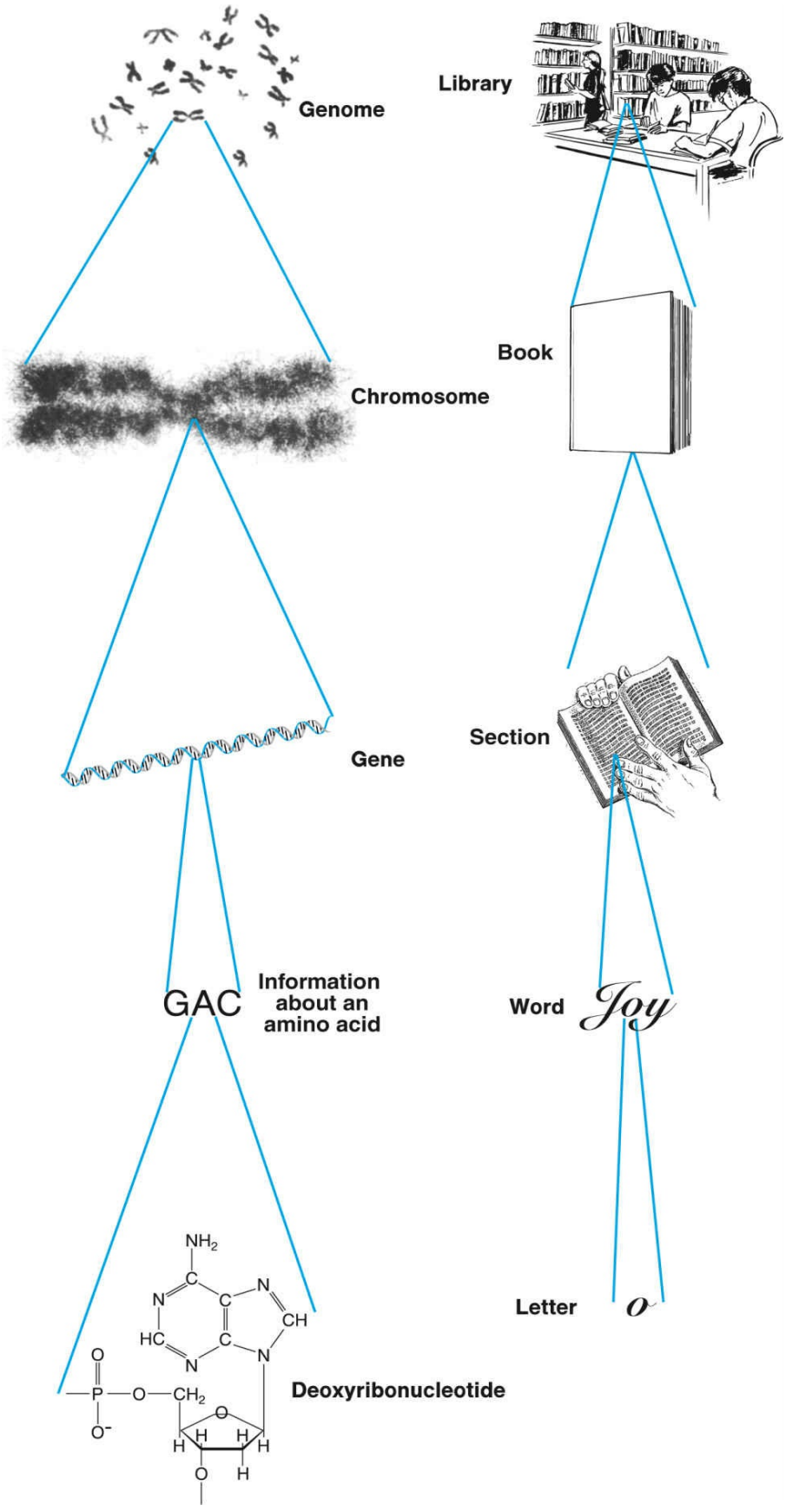

Genome
Library
Chromosome
Book
Gene
Section
GAC
Information about an amino acid
Word
Joy
Letter
o
Deoxyribonucleotide

Figure 4.1 **Parallel between the genome and a library.**

4.3 Deoxyribonucleotides, the Building Blocks of DNA

Deoxyribonucleotides consist of three parts: a nitrogenous base, a deoxyribose unit, and one to three phosphoryl groups (figure 4.2).

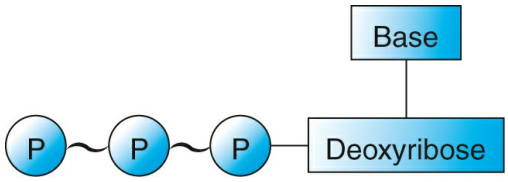


Figure 4.2 **Simplified structure of a deoxyribonucleotide.** A deoxyribonucleotide is made up of a nitrogenous base, a deoxyribose unit, and one to three phosphoryl groups. Each of the latter is depicted as an encircled P.

DNA contains four nitrogenous bases, which are presented in figure 4.3. Two of the bases consist of two rings each, with one ring being six membered and the other being five membered. In all, the two rings contain five carbon atoms and four nitrogen atoms. These two-ring bases are **adenine** and **guanine**, and they belong to the category of **purines**. The other two bases belong to **pyrimidines**. Their names are **thymine** and **cytosine**, and each consists of a six-membered ring containing four C and two N. The rings of both purines and pyrimidines are planar. All four compounds shown in figure 4.3 are alkaline, and that is why they are called bases. For the sake of brevity, we symbolize them by their initials (A, G, T, and C).

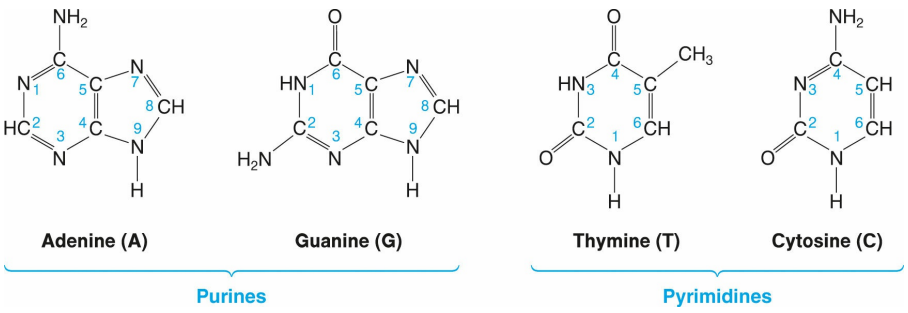


Figure 4.3 **The bases of DNA.** DNA contains four bases, two of which are purines and two

of which are pyrimidines. Note the numbering of the atoms in the rings. Which one of the bases have you met in a previous chapter?

The second component of deoxyribonucleotides, **β-D-2-deoxyribose** (figure 4.4), is the one that gave DNA the first part of its name and the initial *D*. β-D-2-Deoxyribose derives from ribose (see figure 2.4) after elimination of the oxygen connected to carbon 2 (thence the prefix *2-deoxy-*). As for the prefix β-*D*-, I will explain its meaning in section 5.2. For the sake of simplicity, I will omit β-D-2- when referring to β-D-2-deoxyribose in most cases from now on.

Each DNA base is connected to deoxyribose through a covalent bond formed either between nitrogen 9 of the purines and carbon 1' of deoxyribose or between N1 of the pyrimidines and, again, C1' of deoxyribose. Finally, the structure of a deoxyribonucleotide is completed by the addition of one, two, or three phosphoryl groups to C5'. The phosphoryl groups are linked to each other by phosphoanhydride linkages, the same ones that we encountered in ATP, ADP, NAD, NADP, and FAD in chapter 2.

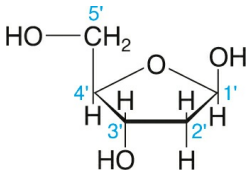


Figure 4.4 **Deoxyribose.** β-D-2-Deoxyribose is a distinctive component of DNA. Four of the five atoms in its ring are carbon, but for the sake of simplicity they are not depicted (see also figure 2.4). The numbers identifying the carbons are followed by a prime (') to distinguish them from the numbers identifying the atoms in the rings of the bases (figure 4.3) when bases are joined with deoxyribose to form deoxyribonucleotides.

The presence of either one of the four bases and one to three phosphoryl groups gives rise to 12 possible deoxyribonucleotides. The structural formula of one of them is shown in figure 4.5. This one is called **deoxyadenosine monophosphate** and is symbolized as **dAMP**. Had it two phosphoryl groups, it would be **deoxyadenosine diphosphate (dADP)**; had it three phosphoryl groups, it would be **deoxyadenosine triphosphate (dATP)**.

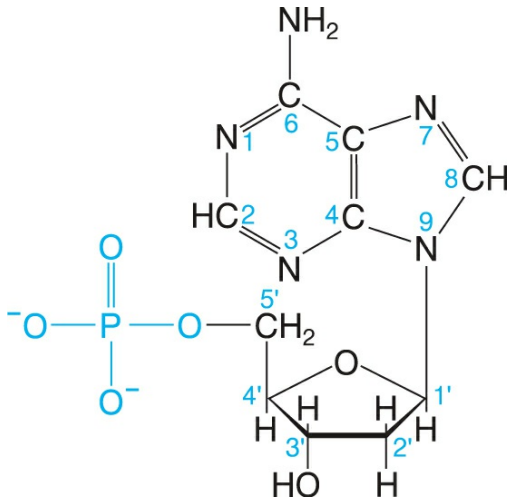


Figure 4.5 **dAMP.** Deoxyribonucleotides like the dAMP shown here are the building blocks of DNA. The unit in black is named deoxyadenosine (in general, a deoxyribonucleoside). What is the difference between this compound and AMP in figure 2.4?

In general, we distinguish deoxyribonucleotides according to their numbers of phosphoryl groups by calling them **deoxyribonucleoside monophosphates**, **deoxyribonucleoside diphosphates**, or **deoxyribonucleoside triphosphates**. We symbolize them as **dNMP**, **dNDP**, and **dNTP**, respectively. Note the new term, **deoxyribonucleoside**, which differs from *deoxyribonucleotide* by just one letter. A deoxyribonucleoside consists of a base tied to deoxyribose. In other words, *a deoxyribonucleoside is a deoxyribonucleotide without its phosphoryl group(s).* The specific deoxyribonucleosides of DNA are named **deoxyadenosine**, **deoxyguanosine**, **thymidine**, and **deoxycytidine**. The prefix *deoxy-* is omitted from thymidine for a reason that I will explain in section 4.9.

4.4 Primary Structure of DNA

Deoxyribonucleoside monophosphate residues, joined in a row by covalent bonds, form DNA, which is therefore referred to as a **polynucleotide chain**. The bond connecting two deoxyribonucleoside monophosphate residues is formed between the phosphorus of one and the oxygen at position 3' of the other (figure 4.6). In this way, every phosphoryl group in DNA (except for the terminal one) is linked to carbons 3' and 5' of two adjacent deoxyribose

units. This link is termed a **phosphodiester linkage**.

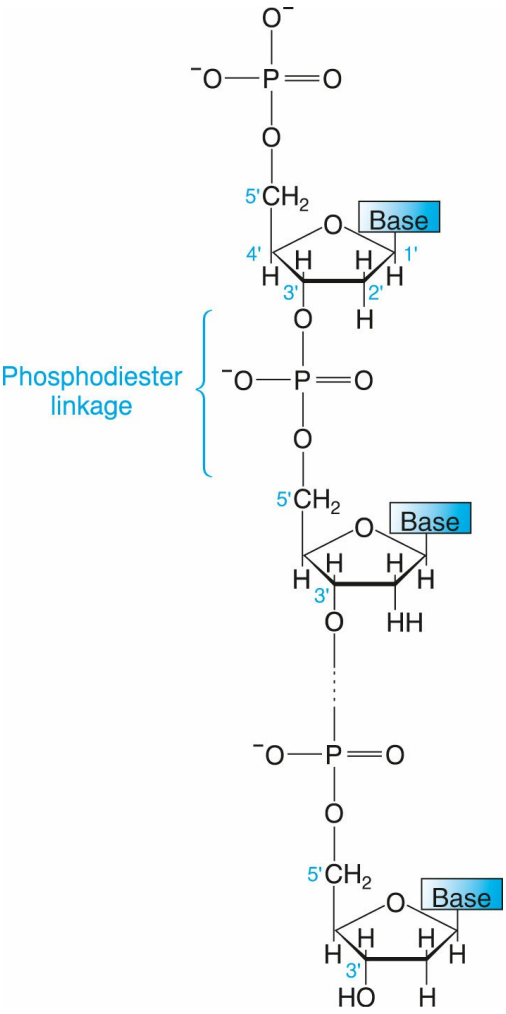


Figure 4.6 DNA chain. A DNA molecule is a chain of deoxyribonucleotide residues connected through phosphodiester linkages.

The sequence of the deoxyribonucleotides composing a DNA molecule is its **primary structure**. The only variable groups in this sequence are the bases. In contrast, the backbone, consisting of deoxyribose units alternating with phosphoryl groups, does not vary among DNA molecules. Therefore, if you want to describe the primary structure of a DNA, all you need to do is report its base sequence, say ACGTACCT.

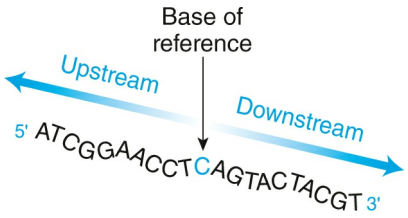


Figure 4.7 **Bases in a stream.** Because we read DNA in the 5' → 3' direction, we use the metaphorical terms *upstream* and *downstream* to describe the location of a base or a base sequence relative to another.

The bonding of each phosphoryl group of DNA with two different positions in deoxyribose (C5' and C3') results in DNA's having two distinct ends. On one end (top of figure 4.6), C5' of the terminal deoxyribose unit does not participate in a phosphodiester linkage, whereas C3' does. Conversely, on the other end, C3' of the terminal deoxyribose unit does not participate in a phosphodiester linkage, whereas C5' does. We therefore speak of the **5' end** and **3' end** of the polynucleotide chain, respectively. By convention, we write the primary structure in the 5' → 3' direction unless for some reason the opposite direction is preferable or necessary, in which case we need to indicate the two ends. Because of the convention, when we wish to indicate the position of a base or a base sequence relative to another in a polynucleotide chain, we use the terms **upstream** if it lies toward the 5' end and **downstream** if it is located toward the 3' end (figure 4.7).

Why not distinguish the ends of a DNA molecule according to which one bears a phosphoryl group? A phosphoryl group does not reside in one end only (as implied in figure 4.6). Both ends may have no phosphoryl groups, or one, or more. Thus, it is not safe to distinguish the ends by the presence or absence of phosphoryl groups.

4.5 The Double Helix of DNA

Neighboring deoxyribonucleotide residues in a DNA molecule assume unique conformations, which constitute its **secondary structure**. The most common secondary structure of DNA is the **double helix**. Its discovery in 1953—by the American molecular biologist James Watson and the British molecular biologist Francis Crick on the basis of experimental data obtained by Rosalind Franklin, Raymond Gosling, and Maurice Wilkins—constitutes a

landmark in the history of biological sciences.

For their discoveries concerning the structure of nucleic acids and its significance for information transfer in living organisms, Francis Crick, James Watson, and Maurice Wilkins were awarded the Nobel Prize in Physiology or Medicine in 1962.

Figure 4.8 **The double helix.** DNA usually has the shape of a right-handed helix containing two polynucleotide chains of opposite directions (5' → 3', 3' → 5'), attracted through their bases. In this simplified diagram, the backbones of the chains are represented by ribbons, and the base pairs are represented by two-colored bars. In reality, there is no empty space between base pairs.

We designate the double helix of Watson and Crick as **B-DNA** to distinguish it from other DNA conformations that were discovered afterward. The main features of B-DNA (figure 4.8) are as follows:

1. B-DNA is a **duplex** consisting of two polynucleotide chains wrapped around each other in a right-handed fashion. The chains are also called **strands**. The diameter of the double helix is 20 Å.
2. The backbones of the two strands lie on the surface of the double helix, whereas the bases are sequestered inside. The planes of the bases are parallel to each other and perpendicular to the longitudinal axis of the helix. Each base in one strand faces a base in the other strand and is attracted to it by hydrogen bonds (section 3.4).
3. The two strands have opposite directions, that is, one runs 5' → 3' and the other 3' → 5'. Thus, the 5' end of one strand and the 3' end of the other are present in each end of the double helix (if the DNA is not circular; see next section).
4. The four bases pair in a strictly defined manner: adenine pairs with thymine, and guanine pairs with cytosine. Thus, each **base pair** (abbreviated as **bp**), be it A–T or G–C, contains one purine and one

pyrimidine. The A–T pair is stabilized by two hydrogen bonds, whereas the G–C pair is stabilized by three (figure 4.9).

5. Each turn of the double helix encompasses 10.5 bp and occupies 36 Å along the helix.

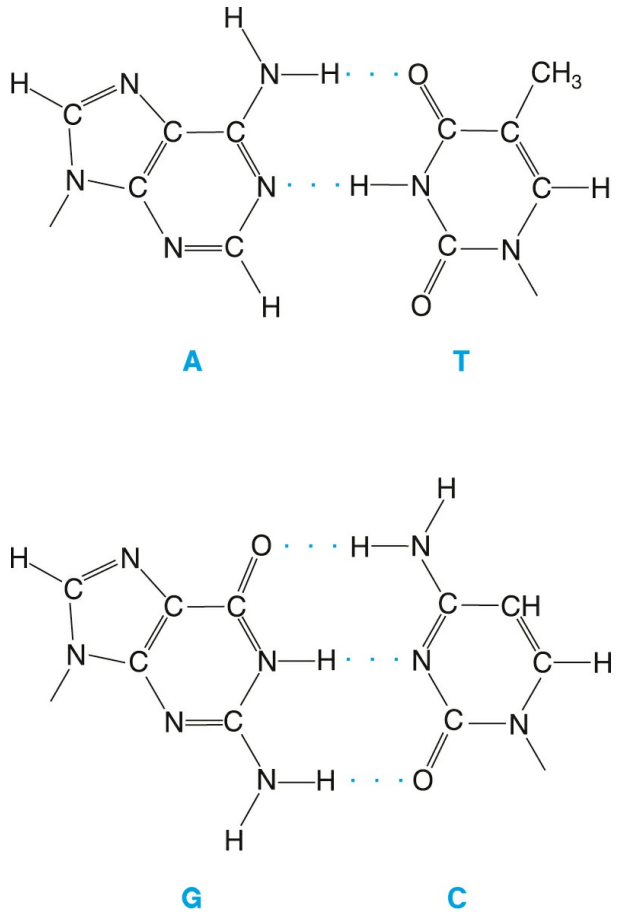


Figure 4.9 **Hydrogen bonds in DNA.** The DNA double helix is held in place by hydrogen bonds (colored dots) between its bases. Two bonds develop between A and T, whereas three bonds develop between G and C.

The issue of base pairing in the double helix is worth a comment. The obligatory presence of adenine opposite thymine and of guanine opposite cytosine has a very important corollary: *The base sequence in one strand determines the base sequence in the other*; as a result, we refer to the two strands as **complementary strands**.

By striking coincidence, a double helix is depicted in a beautiful painting of two ancestral Chinese deities (figure 4.10).

Figure 4.10 **Ancient double helix.** Nüwa (left) and Fuxi, considered the first ancestors of the Chinese and creators of the universe, are joined together by serpent bodies, which are entwined in a right-handed double helix like that in B-DNA. This painting on hemp dates to around 700 CE and was discovered in the Astana tombs, near present-day Turpan in northwest China. It is kept at the National Museum of Korea in Seoul.

4.6 The Genome of Living Organisms

DNA molecules are the largest of all biological molecules. In fact, their sizes correspond roughly to the complexity of organisms. The DNA of the virus **ϕX174** was the first to be sequenced, in 1977, by Frederick Sanger, a British biochemist and double Nobel laureate in chemistry, and his coworkers. It consists of 5,386 bp (though it exists in a single-stranded form as well). Its molecular mass is $3.3 \cdot 10^6$ Da, and its length is 1.8 μm. The DNA of ϕX174

is circular, as are the DNA of bacteria. One of them, ***Escherichia coli***, is an inhabitant of our colon and is the most fully characterized cell biochemically. Almost all of *E. coli*'s genome is gathered in a single circular DNA consisting of $4.6 \cdot 10^6$ bp.

Although double-stranded DNA consists of two molecules (its two polynucleotide chains), we often take the liberty of referring to it as a single molecule because of the tight connection of its two strands.

We use the term *circular* in regard to DNA to signify not its shape but the fact that it has no ends.

The genome of eukaryotes is much larger and is organized in multiple chromosomes, each of which contains an open-chain (not circular) duplex DNA. Human **diploid cells** contain 46 chromosomes in the nucleus, organized in 23 pairs. In 22 of these pairs, the two chromosomes are duplicates called **autosomes**. The 23rd pair consists of the **sex chromosomes**: an X and a Y chromosome in males and two X chromosomes in females. Our **haploid cells**—that is, sperm and ova (eggs)—contain one set of 23 chromosomes. The DNA of the 23 chromosomes consists of approximately $3.1 \cdot 10^9$ bp (figure 4.11), and most of its sequence was published in 2003 as the product of the **Human Genome Project** (see the paper by Francis Collins and collaborators). The large-scale, comprehensive study of genomes in terms of base sequence, gene identification, and gene function is the subject of **genomics**.

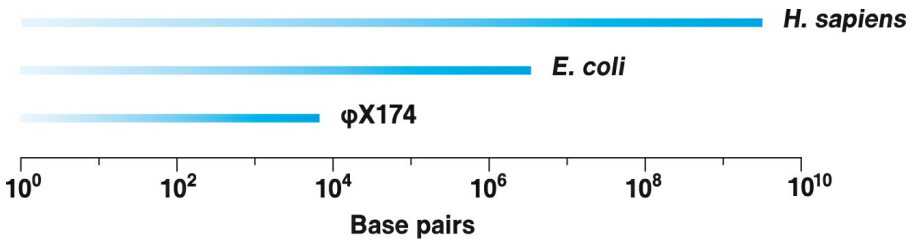


Figure 4.11 Genome sizes. The comparison of the size of the genetic material of a multicellular organism, such as the human *(H. sapiens)*, with that of primitive life forms, such as the *Escherichia coli* bacterium and the ϕX174 virus, is overwhelming. Note that the scale is logarithmic (that is, the number of base pairs increases by a factor of 10 from notch to notch).

Were it proportional, the band corresponding to the human would have to be about 570 m long in order for the band corresponding to the virus to be barely visible (1 mm).

If we could stretch the DNA of a human diploid cell in a straight line, it would be about 1.8 m long (!). Nevertheless, this length is contained in a nucleus that is just 5 to 10 μm in diameter. How is this tremendous condensation achieved? Remember that the double helix is extremely thin (only 20 Å in diameter). DNA is wrapped in an orderly manner with the aid of proteins to form the **chromatin** of the nucleus (see figure 1.9). The main proteins involved in DNA wrapping are called **histones** and play not only a structural but also a functional role by participating in the control of the flow of genetic information, as we will see in section 13.7.

Histones form "spools" around which the DNA double helix winds. Numerous positively charged side chains of lysine and arginine residues in the histone molecules form electrostatic bonds with the negatively charged phosphate groups in DNA. The resulting unit of eight polypeptide chains and about 146 bp is called a **nucleosome** (figure 4.12). This and higher levels of ordered coiling lead to an astonishing compression of the genome in the nucleus. Suffice it to say that the final length of a chromosome is about 10,000 times less than the length of its DNA.

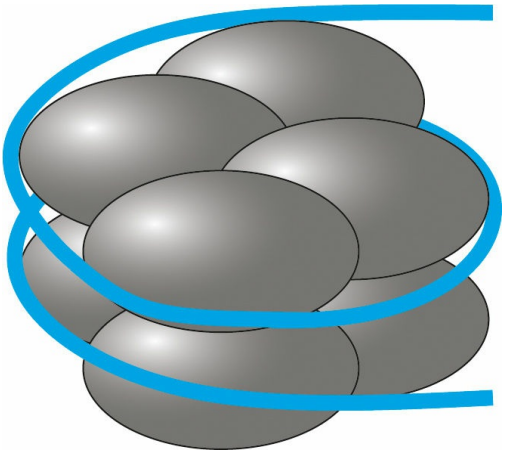


Figure 4.12 Nucleosome. A stretch of 146 bp of DNA (in color) winds around eight polypeptide chains of histones to form a nucleosome, the elementary unit of DNA packaging in eukaryotes.

In addition to nuclear DNA, eukaryotic cells contain multiple copies of a

small DNA molecule inside each of their mitochondria. The human mitochondrial DNA is a circular duplex of 16,569 bp and is packed with proteins that are different from the nuclear histones to form complexes known as **nucleoids**.

4.7 DNA Replication

To develop and stay alive, an organism needs to produce new cells. Most of our cells are produced by **mitosis**, that is, the division of a cell into two "daughter" cells, each equipped with an exact copy of the genome of the "parent" cell. Therefore, cells need to duplicate their DNA, and they do so through a process called **DNA replication**.

In DNA replication, a cell's DNA is doubled in preparation for cell division.

In eukaryotic cells, DNA replication takes place before mitosis, during a stage of the cell's life called the **S phase** (*S* stands for *synthesis*). DNA replication is a complex process requiring the combined action of a multitude of proteins. The leading part belongs to **DNA polymerase**, an enzyme catalyzing DNA polymerization, that is, the sequential addition of deoxyribonucleotide residues to a growing chain.

DNA is not duplicated in its double-stranded form; the two strands must first be separated. This separation is performed by **helicase**, a protein that unwinds the double helix at internal points of a chromosome. Helicase hydrolyzes ATP to produce the energy necessary to separate the two strands. Then DNA polymerase uses each strand as a **template** to synthesize a new, complementary strand. The substrates for this synthesis are deoxyribonucleoside triphosphates, which are synthesized in sufficient quantities to duplicate DNA.

Apart from deoxyribonucleoside triphosphates and a template strand, DNA polymerase requires a **primer**, because it is unable to start replicating DNA from scratch. The primer is usually a short RNA (not DNA) strand, complementary to the template strand and attached to it by base pairing. (As we will see shortly, RNA has bases that are almost the same as those in

DNA.) The primer is made by **primase**, an enzyme capable of synthesizing RNA by starting with just one ribonucleotide. When replication is on its way, the primer is excised from the newly formed DNA.

Figure 4.13 shows how DNA polymerase initiates replication. First, the primer strand is attached to part of the template strand by hydrogen bonding between complementary bases. Note that the two strands have opposite directions, just as in the DNA double helix. The stage is now set for DNA polymerization to begin. A dNTP having a base complementary to the first unbound base of the template after the 3' end of the primer is attached to the base by hydrogen bonding. For example, if the base in the template is T, dATP binds to it. Next, the oxygen at the 3' end of the primer forms a covalent bond with the α phosphorus of the dNTP, and the two phosphoryl groups at the end of the molecule are detached. As a result, a dNMP residue is added to the primer, and pyrophosphate is released.

Why are dNTP used as substrates for DNA polymerization if only the dNMP residue is incorporated in DNA? A reaction of the nascent strand with a dNMP would be endergonic, thus not favored. In contrast, the reaction with dNTP is exergonic because of the cleavage of a phosphoanhydride linkage. Moreover, as pointed out in section 2.3, the PP_i produced can be further hydrolyzed to two P_i, thus releasing additional energy.

All of this happens inside the active site of DNA polymerase. When the addition of a deoxyribonucleotide is completed, the enzyme moves slightly toward the 5' end of the template strand and adds the next deoxyribonucleotide to the nascent strand by the mechanism just described. Thus, the polymerase “walks” along DNA, replicating it on the way.

It is evident from the preceding description that the parental strand is copied in the 3' → 5' direction, whereas the new strand is synthesized in the 5' → 3' direction. The opposite directions and the complementarity of the two strands fit perfectly with the structure of the double helix. Thus, as the new strand grows, it wraps around the parental strand, forming a duplex DNA that is neither totally new nor totally old; instead, it is half new and half old. The same happens with the other parental strand that is being copied (figure 4.14).

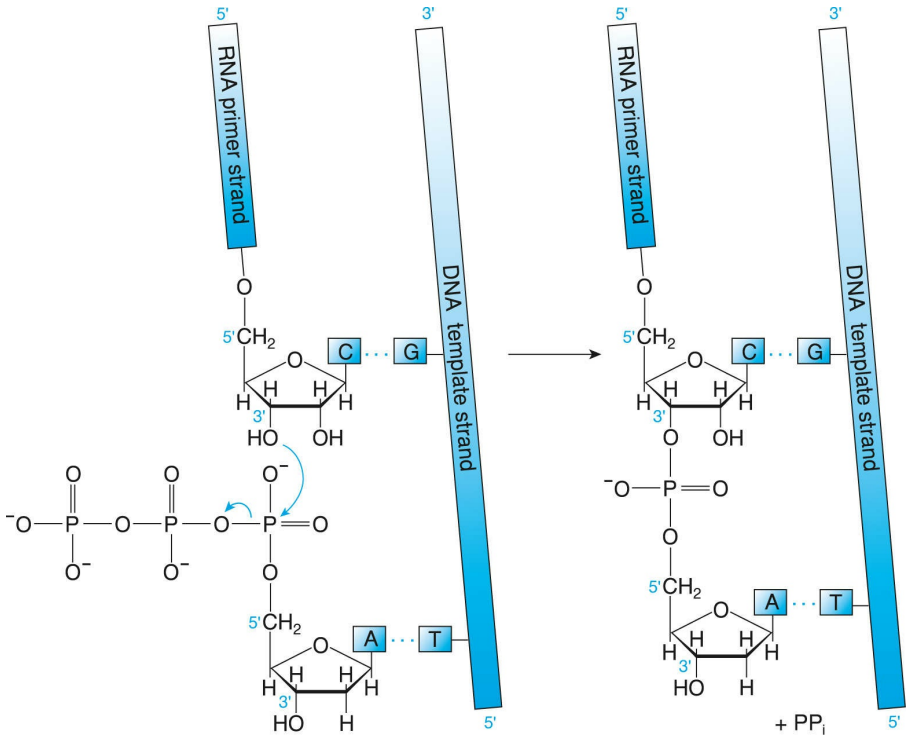


Figure 4.13 **DNA replication.** Each DNA strand in a duplex serves as a template to form a new strand during replication. DNA polymerase uses an RNA primer strand to add, one after another, deoxyribonucleotides that are complementary to the template strand. The colored arrows indicate the way in which a deoxyribonucleotide is added and PP_i is formed.

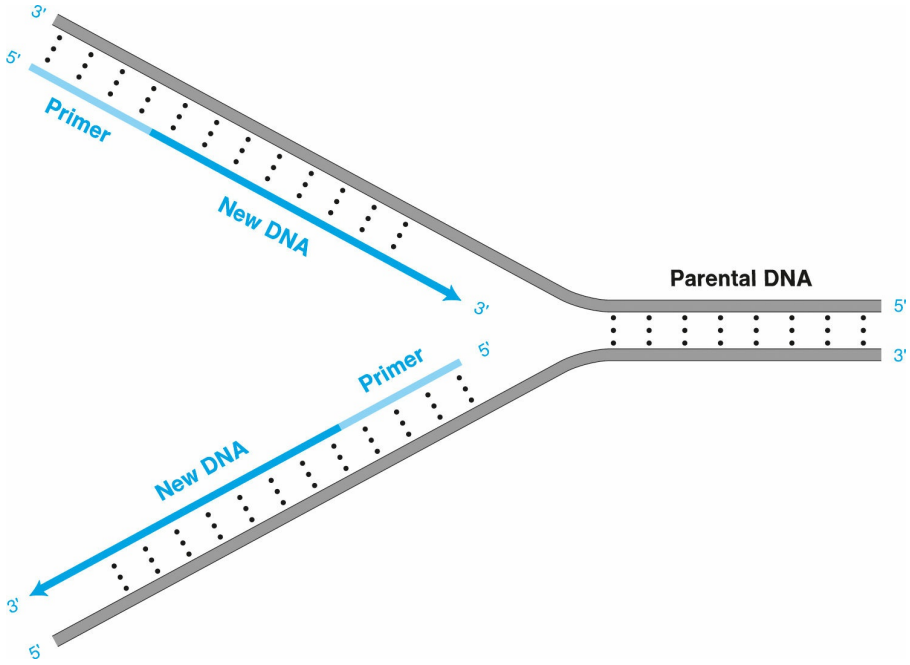


Figure 4.14 **Replication fork.** As helicase unwinds the parental DNA, DNA polymerase copies each strand in the 3' → 5' direction, forming a new strand in the 5' → 3' direction.

In the end of replication, one parental DNA (or chromosome) gives rise to two daughter DNA (or chromosomes). Each daughter DNA contains one strand from the parental DNA and one new strand (figure 4.15). We express this fact by referring to DNA replication as **semiconservative**.

In DNA replication, a duplex DNA yields two identical duplex DNA.

When each of the two chromosomes—and each member of the other pairs of identical chromosomes formed by replication—has been enclosed in separate daughter cells (at the end of mitosis), the genome of the parent cell has been equally divided between them. You may see this process at the microscopic level in figure 4.16.

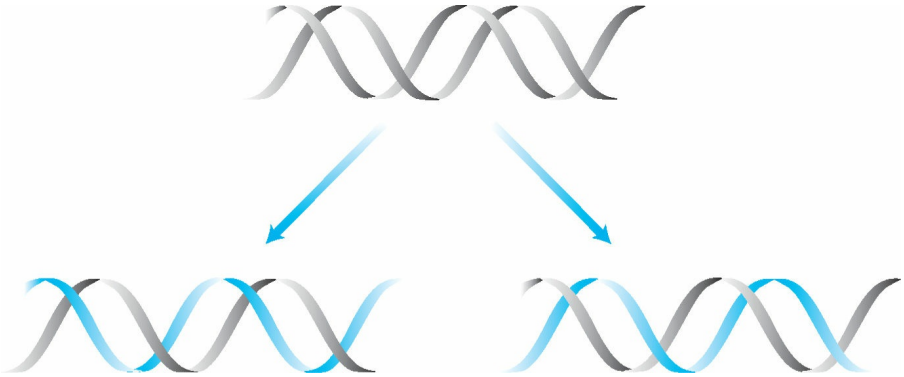
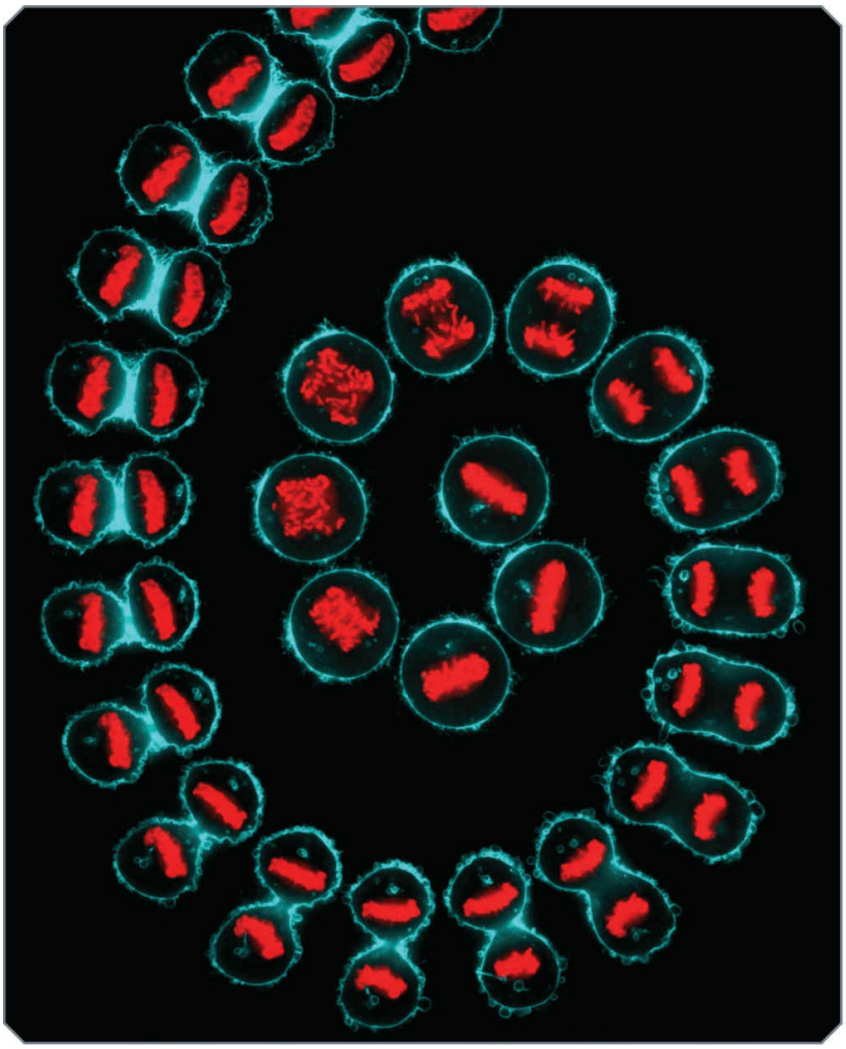


Figure 4.15 Semiconservative DNA replication. A parental chromosome (top) yields two daughter chromosomes, each of which contains one parental strand (gray) and one complementary new one (color).



Adapted by permission from Kuan-Chung Su, London Research Institute, Cancer Research UK; http://www.wellcomeimageawards.org/2012/cell-division

Figure 4.16 **Division of the genome.** Mitosis of a human cancer cell is shown in a digital collage of time-lapse photographs that were gradually displaced relative to each other to provide a spiral arrangement. The 27 stills were taken within an hour through the technique of confocal microscopy. DNA is shown in color, whereas the plasma membrane is shown in cyan. Starting at the center, the duplicated chromosomes migrate progressively toward opposite ends of the cell. This action is followed by compression of the plasma membrane in the middle until two daughter cells ensue.

4.8 Mutations

DNA replication is exceptionally but not completely faithful. DNA polymerase, although extremely accurate, is not infallible. Thus, once in a while, it adds a base that is not complementary to the one in the template, causing a base substitution. Base insertions (that is, addition of extra bases) and deletions (omissions of bases) are also possible, though less frequent. If

such mistakes escape an inherent proofreading mechanism (a rare occurrence), then the newly formed strand will not be completely complementary to the parental one. The frequency of mistakes ranges from one in ten million to one in ten billion, depending on the organism. Such changes in DNA base sequence are called **mutations**.

Mutations can also result from external causes, such as ultraviolet light, X-rays, extreme heat, and certain substances. These factors, known collectively as **mutagens**, may cause changes in DNA structure that "fool" DNA polymerase into adding a wrong base during replication. Mutations on a larger scale can be caused by viruses that insert their genetic material into the genome of the cells they infect and by **mobile genetic elements**. These large pieces of DNA tend to either move from one place in the genome to another or have copies inserted into various chromosomal places while the original element remains in place.

A mutation can modify the genetic information contained in a gene in such a way that the gene dictates a defective protein. A protein that does not function properly may cause disease or even death. There can also be **silent mutations**; that is, mutations can take place at a point containing no genetic information or can modify the genetic information in a way that makes no difference to the organism.

One of the best-known examples of disease caused by a mutation is sickle cell disease, in which a single A-to-T change in the β-globin gene results in synthesis of abnormal hemoglobin. For more on sickle cell disease, see problem 9 at the end of this chapter.

Less often, a mutation may render a protein more efficient in its biological role or even endow it with a new property. A mutation of this kind may confer an advantage to the individual that carries it, relative to the rest of the population, as far as survival is concerned. By improving their prospects of survival, such individuals increase their chances of reproducing and passing advantageous mutations on to their offspring (if the mutation is present in the cells used for reproduction like an animal's germ line). Because the offspring too will have a higher probability of reproducing, the proportion of individuals bearing the mutation will keep rising, generation after generation,

until they finally prevail and the novel genetic feature becomes a trait of the entire population. Eventually, the accumulation of new traits may give rise to a new biological species. We can therefore consider *mutations the prime vehicle of evolution.*

4.9 RNA

Let's turn now to the other nucleic acid, RNA. Its building blocks, the ribonucleotides, resemble the deoxyribonucleotides of DNA (as suggested by the similarity in the names), differing in just two aspects (figure 4.17). First, ribonucleotides contain not thymine (with one exception that I will present in section 4.16) but **uracil**, a structurally related compound. (For this reason, we do not need the prefix *deoxy-* in the deoxyribonucleoside thymidine, as mentioned in section 4.3.) Second, ribonucleotides contain **β-D-ribose** in place of β-D-2-deoxyribose; hence the name *ribonucleic acid* and the initial *R* in *RNA*. In contrast to deoxyribose, ribose possesses oxygen at position 2'. For the sake of simplicity, I will omit β-D-2- when referring to β-D-2-ribose in most cases from now on.

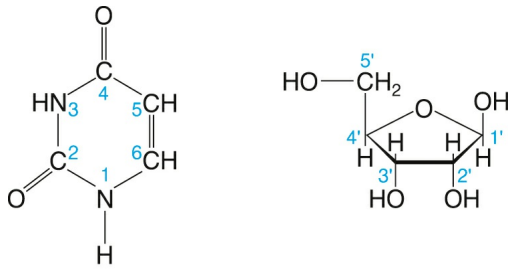


Figure 4.17 Unique features of RNA. RNA shares three bases (adenine, guanine, and cytosine) with DNA but contains uracil instead of thymine as the fourth. (How do these two differ?) In addition, RNA has ribose in place of DNA's deoxyribose.

By analogy to DNA, each RNA base is linked to ribose, and ribose is linked to either one, two, or three phosphoryl groups to form ribonucleotides specified as **ribonucleoside monophosphates**, **ribonucleoside diphosphates**, and **ribonucleoside triphosphates**, or **NMP**, **NDP**, and **NTP**, respectively. Note the new term **ribonucleoside**, which differs from

ribonucleotide by just one letter. A ribonucleoside consists of a base tied to ribose. In other words, *a ribonucleoside is a ribonucleotide without its phosphoryl group(s)*. The specific ribonucleosides of RNA are named **adenosine**, **guanosine**, **uridine**, and **cytidine**.

Also note that this is not your first encounter with ribonucleotides: In chapter 2, we considered adenosine monophosphate, adenosine diphosphate, and adenosine triphosphate (AMP, ADP, and ATP) as the main participants in energy exchange in biological systems. At the end of the present chapter, we will examine the significance of the fact that our energy currency is a ribonucleotide.

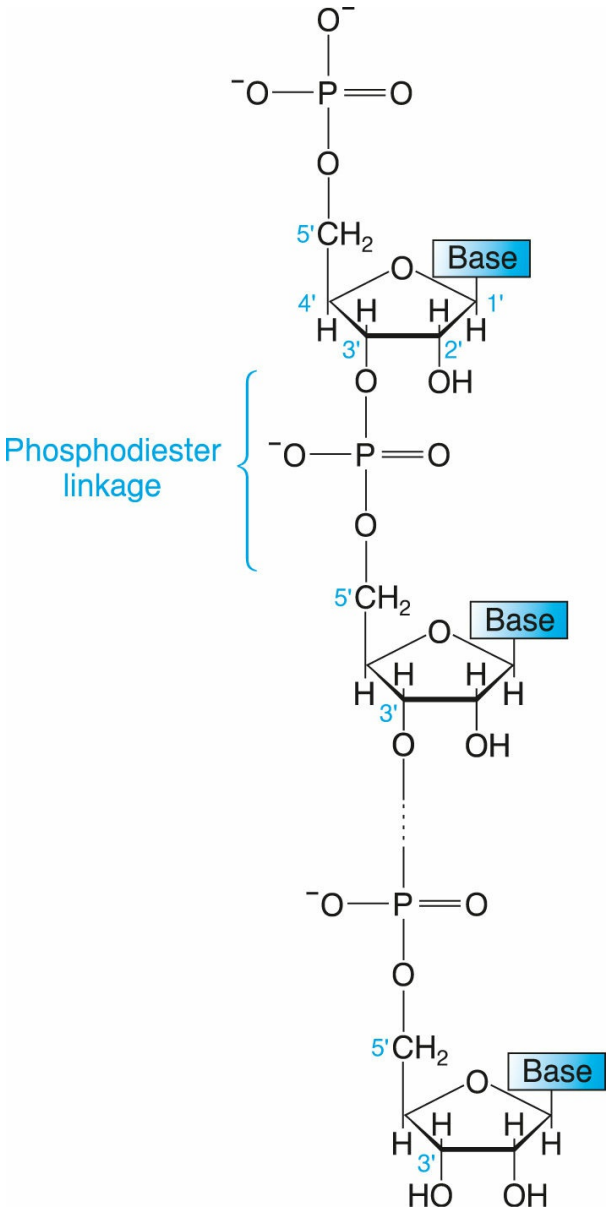


Figure 4.18 **RNA chain.** An RNA molecule is a chain of ribonucleotide residues.

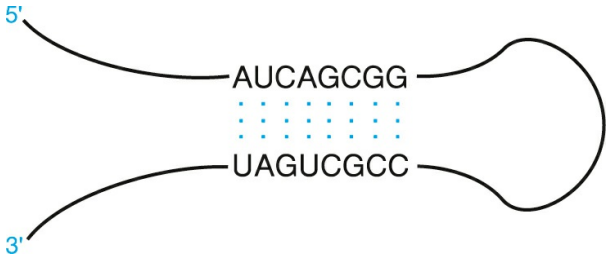


Figure 4.19 **Base pairing within an RNA strand.** Short complementary base sequences in an RNA molecule may be attracted to each other by hydrogen bonds to form a double helix. Such helices may confer specific shapes that are important for RNA function.

An RNA molecule (figure 4.18) is made of ribonucleoside monophosphate residues joined in a row by phosphodiester linkages identical to those in DNA. The conventional direction of an RNA chain is 5' → 3' too, and its ribonucleotide sequence is signified by the initials of the bases: A, G, U, and C.

RNA molecules vary in size and secondary structure. They are mostly single stranded but may form short double helices intramolecularly (figure 4.19) if complementary base sequences of opposite direction happen to exist at different sites in a molecule. The complementary base pairs in RNA are A–U and G–C. The size of RNA molecules ranges from about 20 residues to 100,000. This heterogeneity in structure is the basis for diversity in function. In the following sections, we will explore several kinds of RNA and their biological roles.

4.10 Transcription

Having made the acquaintance of RNA, we can now begin examining how genetic information flows from DNA to RNA to proteins. As a first step, the information recorded in the DNA base sequence is transmitted to RNA by the process of **transcription**, which differs from DNA replication in that it takes place ceaselessly during a cell's lifetime. In contrast (as we saw in section 4.7), DNA replication takes place only once in a cell's lifetime, when it is about to divide. Another difference between these processes is that replication encompasses the entire DNA, whereas transcription is performed only on parts of the DNA.

In transcription, RNA is synthesized on the basis of genetic information contained in DNA.

RNA polymerase stars in transcription, much as DNA polymerase stars in replication. RNA polymerase catalyzes the synthesis of RNA using DNA as the template. In this reaction, ribonucleoside triphosphates serve as substrates. As with replication, the two DNA strands need to unfold locally, at the site where transcription is to begin. RNA polymerase possesses this ability in itself—it does not require the action of helicase. Moreover, RNA

polymerase does not require a primer strand—that is, it does not require the action of primase.

The way RNA polymerase acts (figure 4.20) is similar to the way DNA polymerase acts: While one of the separated DNA strands lies in the active site of the enzyme, two NTP having bases complementary to two adjacent bases in the template are attached to them by hydrogen bonding. A covalent bond is then formed between the oxygen at 3' of one NTP and the α phosphorus of the other. As a result, the two ribonucleotides are linked, and PP_i is released.

Repetition of the previous reaction, with ribonucleotides being added one after another opposite the next bases of DNA, leads to the formation of an RNA molecule that is called a **transcript** and has a base sequence complementary to that of the transcribed DNA strand, or **template strand**. Moreover, as is evident in figure 4.20,

In transcription, RNA is formed that is complementary to a DNA strand.

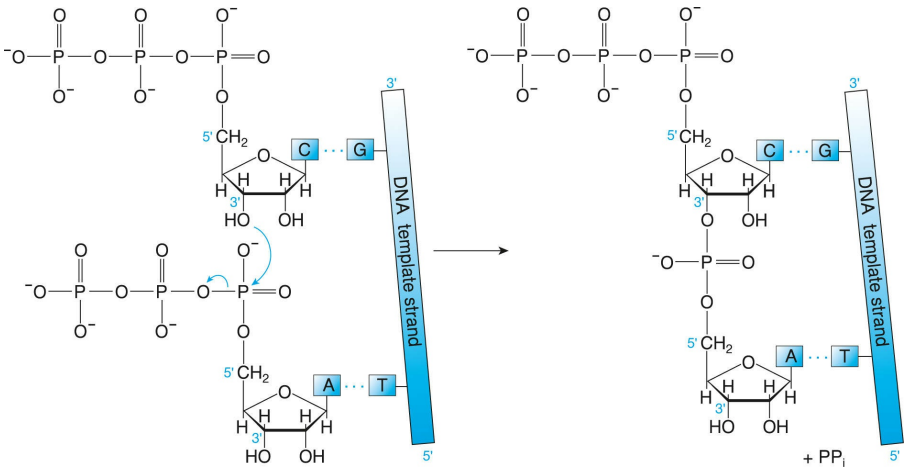


Figure 4.20 **Transcription.** A single strand of DNA, acting as a template, is transcribed into RNA by the sequential joining of ribonucleotides having bases complementary to the ones in the template. Look for differences between this figure and figure 4.13.

while DNA is transcribed in the 3' → 5' direction, RNA grows in the 5' → 3' direction. Because of this complementarity and direction, *the RNA base sequence is that of the other DNA strand in the DNA duplex, the one that is not transcribed.* This is the case because both the RNA strand and the DNA

strand that is not transcribed are complementary to the template strand and both have a direction that is opposite to the direction of the template strand (figure 4.21). The only difference in base sequence is the presence of uracil in RNA wherever thymine is present in DNA.

Figure 4.21 An easy way to deduce the RNA base sequence. RNA formed from duplex DNA by transcription has the base sequence of the strand that is not transcribed (with U in place of T). In the example shown, the one transcribed is the lower strand, termed the *template strand* or *antisense strand*. Therefore, RNA has the base sequence of the upper strand, which is termed the *coding strand* or *sense strand*.

Because of its near-identity to the base sequence of the transcript, the DNA strand that is not transcribed is known as the **coding strand**, or **sense strand**, thus leaving the unfair name **antisense strand** for the template strand. The complete set of transcripts in an organism, tissue, cell type, or biological fluid is its **transcriptome**, whereas the large-scale, comprehensive study of the transcriptomes in terms of RNA identity, quantity, and function constitutes the field of **transcriptomics**.

4.11 Delimiting Transcription

Since, as noted in the previous section, transcription is performed only on parts of the DNA, the following question arises: How are these parts selected along a continuous, vast DNA strand? The answer is that signposts for initiation of transcription are provided by DNA base sequences named **promoters**. A promoter lies near the transcription start site, upstream on the coding strand (left-hand side of figure 4.21). It is recognized by one or more proteins that bind to it and serve as docking sites for RNA polymerase (figure 4.22), which then begins transcribing by moving upstream along the template strand.

Recruitment of RNA polymerase at many transcription start sites is facilitated by **enhancers**, which are DNA regions attracting proteins that activate RNA polymerase. Enhancers differ from promoters in lying farther away from the transcription start site and in either direction (upstream or downstream). Even though the enhancer and promoter may lie thousands of bases apart, the coiling of DNA in the chromosomes can bring enhancer-bound proteins in contact with promoter-bound ones (including RNA polymerase). This meeting is like bringing together amino acid residues that lie far apart along a polypeptide chain because of chain folding (see section 3.5), though on a larger scale.

Promoters and enhancers are ubiquitous in the genome. According to **ENCODE** (short for **Encyclopedia of DNA Elements**, a project aimed at delineating all functional elements in the human genome), there are about 70,000 regions with promoter-like features and 400,000 regions with enhancer-like features. The main proteins that bind to these regions are known collectively as **transcription factors**. Also according to ENCODE, some 1,800 transcription factors were known as of 2012, making them the largest family of proteins in the human proteome.

> The term *factor* next to a biological process is used to describe a protein controlling the process (see section 13.2 regarding growth factors).

In contrast to the wealth of knowledge about transcription initiation, little is known about transcription termination in eukaryotes, including us humans. In general, termination is signaled by DNA base sequences aptly called **terminators**, which lie downstream of the transcription initiation site along the coding strand. When RNA polymerase passes over a terminator and transcribes it, the newly synthesized RNA base sequence is recognized by a number of proteins, which terminate transcription in a way that remains unclear.

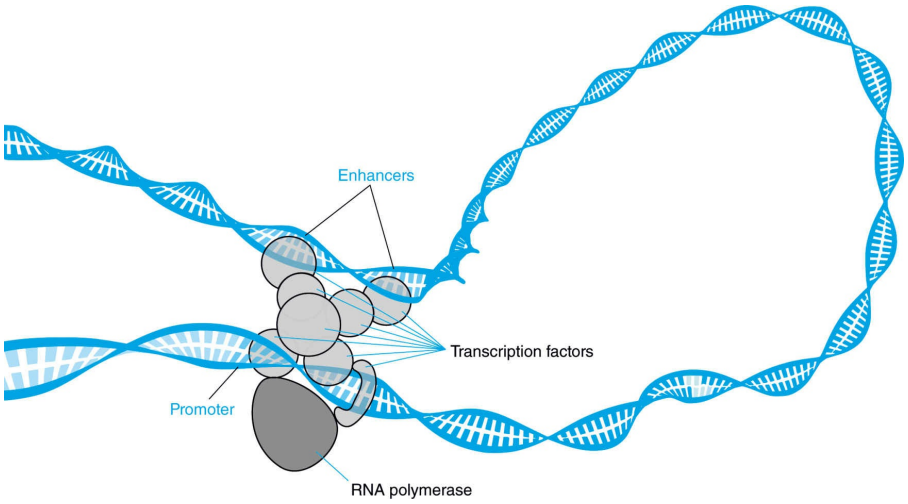


Figure 4.22 **Transcription initiation.** Transcription begins thanks to the cooperation of two kinds of DNA segments, specifically promoters and enhancers, and two kinds of proteins, specifically RNA polymerase and transcription factors. A promoter, lying just upstream of the transcription start site on the DNA coding strand, offers its base sequence as a recognition site for transcription factors, which bind and attract RNA polymerase. An enhancer, lying far away from the transcription start site (either upstream or downstream), attracts additional transcription factors, thus enhancing the binding of RNA polymerase.

4.12 Genes and Gene Expression

In section 4.2, I defined the gene roughly as a piece of DNA containing a unit of genetic information. Now, after our acquaintance with RNA and transcription, we are able to define the gene more accurately as *every region of DNA that is transcribed into functional RNA* (that is, RNA serving a biological function).

The number of genes in an organism relates to its complexity. *E. coli* has only 4,000 to 5,000 genes (depending on the bacterial strain), whereas we humans have about 39,000. Human genes can be divided into two classes: genes containing information for protein synthesis (thus taking the flow of genetic information all the way to the end) and genes containing information only for RNA synthesis. The former number about 21,000 and the latter about 18,000.

The process through which RNA or protein is synthesized according to the information contained in a gene is called **gene expression**, which is synonymous to the flow of genetic information (introduced in section 4.2).

When gene expression takes place, we say that *a gene is expressed* and we refer to RNA and protein as **gene products**. Only part of the gene repertoire of a multicellular organism is expressed in a single cell. In fact, Jingyi Jessica Li and associates estimate that about 40% of an organism's genes are not expressed in a given cell. This fact results in the multitude of cell types mentioned in section 1.15: A cell type differs from another just because not all cells express the same genes.

Differences in gene expression can be likened to differences in recipe selection from a cookbook. Although many cooks may have the same book, each one selects and prepares the recipes that suit his or her idiosyncrasies, budget, family or client preference, and circumstances (e.g., holiday, season). Similarly, although all cells in an organism have the same genome, each one expresses genes according to type, energy status, signals from other cells, and the current phase of the cell itself or the organism as a whole.

Gene expression is controlled primarily, though not exclusively, at the level of transcription. Attesting to this fact are the huge numbers of promoters, enhancers, and transcription factors mentioned in the previous section. In chapter 13, we will discuss how exercise modifies gene expression, thus causing many of the known adaptations of the body to training.

Genes for Performance and Fitness

Although the overwhelming majority of the genome is identical across all humans of the same sex, approximately 0.1% varies because of the gradual appearance and accumulation of different mutations in different populations over thousands of generations of evolution in *H. sapiens*. Differences in **genotype** (i.e., the base sequence of specific genes or other functional elements in the genome) may be responsible for differences in **phenotype** (i.e., visible or measurable traits, such as facial characteristics, colors, stature, and blood group).

As mentioned in section 4.8, harmful mutations are responsible for hereditary diseases. Are mutations also responsible for reduced physical performance or fitness in some individuals? Do other

mutations predispose some individuals for increased performance or fitness? Cautious positive answers to both of these questions are supported by a rapidly growing body of evidence. This line of study now constitutes the field of **exercise genomics**, which can be defined as the large-scale, comprehensive study of the genomic determinants of phenotypic responses to exercise.

Since 2001, an international group of researchers led by Claude Bouchard have been publishing yearly reports, in which they summarize the discoveries of genes and other functional elements in our genome that exhibit **polymorphisms** (variations in DNA base sequence) associated with performance and fitness. In simple terms, a certain form, or **allele**, of a gene may dictate an RNA or protein that is responsible for higher performance or fitness than another form is. See the paper by Molly Bray and colleagues for the most recent gene map and the paper by Mark Sarzynski and colleagues for the latest update before this book went to print.

The approximately 250 elements of the human genome associated with performance and fitness can be divided into the following categories of phenotypes:

- Physical performance
  - Cardiorespiratory (or aerobic) endurance
  - Muscle strength and anaerobic performance
- Health-related fitness
  - Hemodynamic traits including exercise heart rate, blood pressure, and heart morphology
  - Anthropometry and body composition
  - Insulin and glucose metabolism
  - Blood lipids and lipoproteins
  - Hemostatic factors
  - Inflammation
  - Plasma hormone levels
  - Exercise intolerance

- Physical activity

We will consider several of these categories in parts III and IV.

Discovering gene polymorphisms that can predict an exercise-related trait is of great scientific importance, because it can show previously unknown effects of a gene product on an aspect of performance or fitness. In practical terms, such discoveries could help us use genetic tests to foresee the ability of, for example, an athlete to excel in a sport or of an individual to reap the health benefits of an exercise training program. However, caution is warranted because none of the common gene variants (those in which the minor allele is present in more than 1% of the population under consideration) discovered so far can, alone, explain more than 1% of the variation in an exercise-related phenotype in the sedentary state (though evidence does show that some variants exert larger effects on health-related responses to exercise training).

In simpler terms, a single advantageous allele would contribute little to sport performance or fitness because of the multitude of other (genetic and nongenetic) factors that influence the same phenotype. By contrast, rare gene variants (those in which the minor allele is present in less than 1% of a population), such as the one described in problem 10 and in the Growth Boosters as Doping Substances sidebar in chapter 13, may exert strong influence on exercise-related phenotypes. However, they are very difficult to uncover and, hence, to study.

Thus, despite the large volume of data that have accumulated over the past two decades, exercise genomics has not yet generated meaningful scientific information that can be transferred to either athletes or the general population. As a result, the various genetic tests for sport performance that are being marketed lack scientific background and diagnostic value. Colin Moran and Yannis Pitsiladis note that the small portion of the variance in performance-related phenotypes that can be explained by common gene variants contrasts with the findings of many studies on twins and families, which attribute about 50% of this variance to genetic variation, leaving the remaining 50% to factors such as training, diet, lifestyle, and access to facilities.

The researchers propose that this discrepancy may be resolved by investigating rare gene variants and epigenetics (which we will explore in section 13.7) or by altering study designs through increased collaboration in order to pool existing data. Clearly, more high-quality research is needed before we arrive at the point of safely predicting athletic talent from genetic information.

4.13 Messenger RNA

Many of the RNA molecules produced through transcription carry the information to build proteins. However, RNA synthesized by RNA polymerase in eukaryotes needs to undergo extensive processing inside the nucleus before it becomes ready to transmit this information. Processing involves mainly the excision of segments from the interior of the so-called **primary transcript** (the transcript produced by RNA polymerase) and the splicing of the segments that are left (figure 4.23). We call the segments that are excised **introns** and those that are left **exons**. Exons form **messenger RNA (mRNA)**,

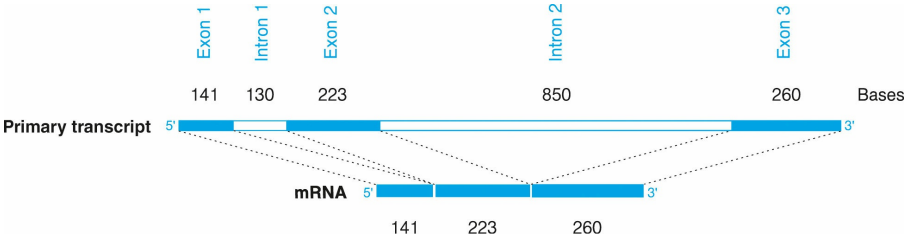


Figure 4.23 RNA splicing. The genetic information carried by a primary transcript destined to dictate the amino acid sequence of a protein in eukaryotes is discontinuous. The ribonucleotide sequence contains introns that must be removed accurately for the information-containing pieces, the exons, to be ligated. Shown are the primary transcript and mRNA of the gene directing the synthesis of human β-globin (see section 3.11).

which contains the precise information on the amino acid sequence in a protein. A primary transcript may have many introns; in fact, the total length of the introns may be greater than that of mRNA. Exons that form mRNA correspond to a mere 1.2% of the human genome.

Two other modifications of the primary transcript involve the addition of a modified ribonucleoside residue (7-methylguanosine) to its 5' end and the addition of a monotonous chain of 80 to 250 adenosine monophosphate residues to its previously shortened 3' end. These modifications have been aptly named the **5' cap** and the **poly(A) tail**. Both extend the life of mRNA molecules by delaying their hydrolysis and thus their degradation through the action of enzymes known as **ribonucleases** (like the one depicted in figure 3.10). In addition, the 5' cap helps position the mRNA in ribosomes, the protein-synthesizing machinery that we will meet in the next section.

After being formed, mRNA molecules cross the pores of the double nuclear membrane, called the **nuclear envelope**, and transfer the information about protein synthesis to the cytosol and endoplasmic reticulum.

Readers who are careful with numbers may have noted the following contradiction: How can the number of human proteins range from 100,000 to 200,000 (section 3.3) when the genes containing information for protein synthesis number only 21,000 (section 4.12)? The answer lies mainly in the **alternative splicing** of exons, that is, in the fact that different introns may be excised from a primary transcript (figure 4.24). This splicing results in the production of more than one mRNA species from a single gene.

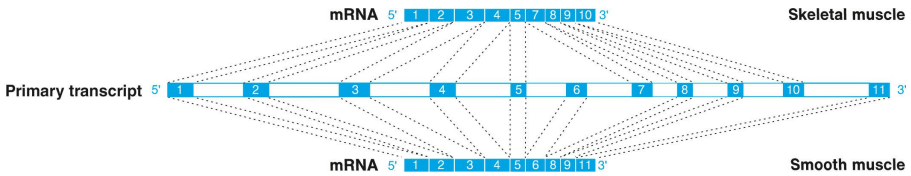


Figure 4.24 **Alternative splicing.** A primary transcript may produce more than one mRNA if its exons are spliced in different ways. The example shown is a gene directing the synthesis of tropomyosin (a muscle protein that we will consider in section 8.10) in a chicken. The primary transcript contains 11 exons that are spliced differently in skeletal and smooth muscles. In skeletal muscle, exons 6 and 11 are omitted, whereas in smooth muscle exons 7 and 10 are omitted. The size of the exons has been exaggerated for diagrammatic purposes. In reality, their total size is only 1/11 the size of the introns.

Another reason for the existence of more proteins than genes is that the protein dictated by an mRNA may be cut after translation, thus producing two or more new proteins. In addition, both DNA strands are transcribed (in opposite directions) in certain genes, thus doubling the available genetic

information. Finally, **RNA editing**, the changing of the RNA base sequence after transcription by processes other than splicing, is an effective means of varying the genetic information contained in a gene.

4.14 Translation

The synthesis of proteins according to the base sequence of mRNA is termed **translation** and takes place in the cytosol and endoplasmic reticulum. Translation is performed by **ribosomes**, which are huge (in molecular terms) complexes of RNA and protein having a mass of 4,200 kDa. Four RNA molecules occupy the bulk of a eukaryotic ribosome, which is why it is called a *ribosome* (*ribo-* stemming from *ribonucleic acid* and *-some* stemming from the Greek *sóma* for body). The RNA species in ribosomes are classified as **ribosomal RNA (rRNA)** and serve both ribosome coherence and functionality. In addition to rRNA, each ribosome contains more than 80 proteins.

In translation, proteins are synthesized on the basis of genetic information contained in mRNA.

Ribosomes consist of two **subunits**—a large one and a small one (figure 4.25)—which join to translate an mRNA molecule in the 5' → 3' direction, synthesizing a protein in the *N* → *C* direction. The two subunits dissociate when translation is complete. Now, to follow the entire process of translation in detail, we need to first consider the genetic code and the molecules serving as translators.

Do not confuse ribosomal subunits with protein subunits (introduced in section 3.7). The former are much larger and contain many proteins (along with RNA).

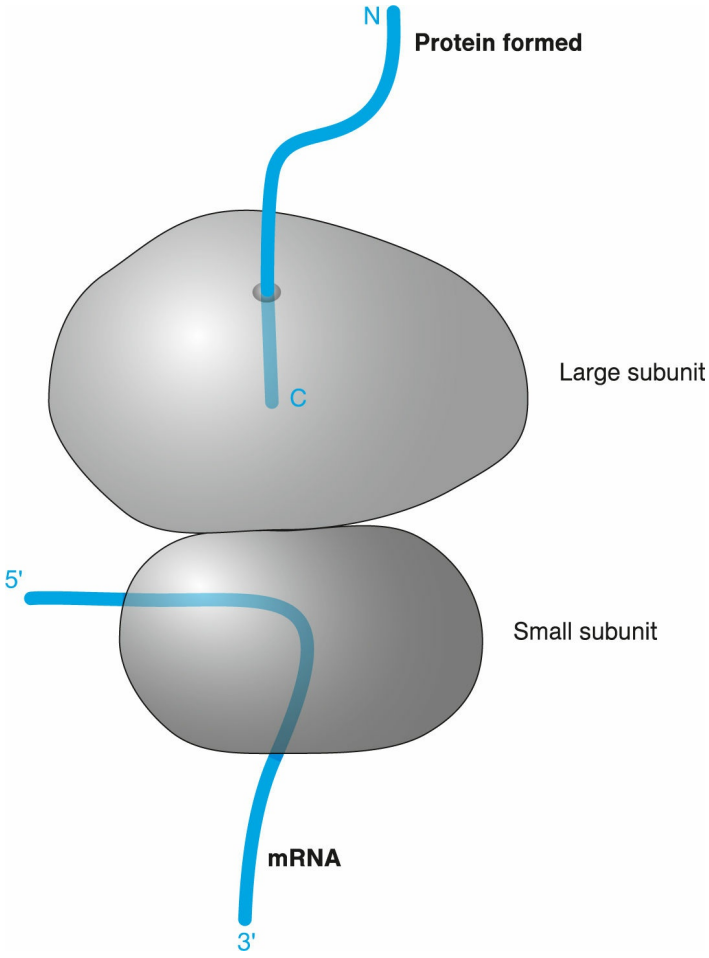


Figure 4.25 Ribosome. Ribosomes are complex intracellular machines synthesizing proteins based on information kept in mRNA. A eukaryotic ribosome consists of two subunits and measures about 35 nm tall (1 nm = 10 Å).

4.15 The Genetic Code

How does the four-letter alphabet of the RNA bases dictate the 20 amino acids of proteins? Evidently, there cannot be a one-to-one correspondence between bases and amino acids. How about a correspondence of two bases to one amino acid? The number of possible permutations of two items out of a pool of four is 16 (4^2), which is also insufficient. Therefore, three bases need to correspond to one amino acid, even though the resulting number of permutations (64, or 4^3) is much larger than necessary. The quest for the correspondence between all possible triplets of RNA bases and the amino acids has been one of the most exciting adventures in the history of biological

research. The fruit of this quest was the discovery of the **genetic code**.

A permutation is a combination in which order matters. In the case of mRNA, the order of bases matters because of its two distinct ends (5’ and 3’). The following 16 permutations of two bases are possible: AA, AG, AU, AC, GA, GG, GU, GC, UA, UG, UU, UC, CA, CG, CU, and CC.

To discuss the genetic code, we need to introduce some terminology. The triplets of bases are called **codons**. To convey that a specific amino acid corresponds to a specific codon, we say that *the codon encodes the amino acid*. By extension, we say that a gene or an mRNA encodes a protein. Other relevant terms will follow later in this section.

The genetic code, which is orthogonal in its classical form and circular in a handier modern version (figure 4.26), lets us find which amino acid a codon encodes. To do so, we must locate the bases of the codon one after another. Suppose we are interested in (5')AGC(3'). We seek the first base (A) inside the inner circle and find it in the lower left quadrant. Next, we seek the second base (G) within the zone surrounding this quadrant; it is located a little before 9 o’clock. Finally, we seek the third base (C) within the segment of the outer zone delimited by the preceding G and find that it corresponds to *Ser* (a three-letter abbreviation of *serine*; see table 3.1), written outside the circles.

Because the number of codons is more than three times the number of amino acids, most amino acids are encoded by more than one codon. Thus, there is not a one-to-one correspondence between codons and amino acids. We express this fact by saying that the genetic code is **degenerate**. The code is also **universal**: It is, with few exceptions, the same for all living organisms. *The universality of the genetic code provides strong evidence for the evolution of all forms of life on Earth from a common ancestor.*

In everyday—and even scientific—speech and writing, the genetic code is often confused with the genome. For example, the sequencing of the human genome in 2003 was heralded as the “discovery of the human genetic code.” However, the genetic code is the same for humans, plants, and bacteria alike, and its discovery dates to 1966.

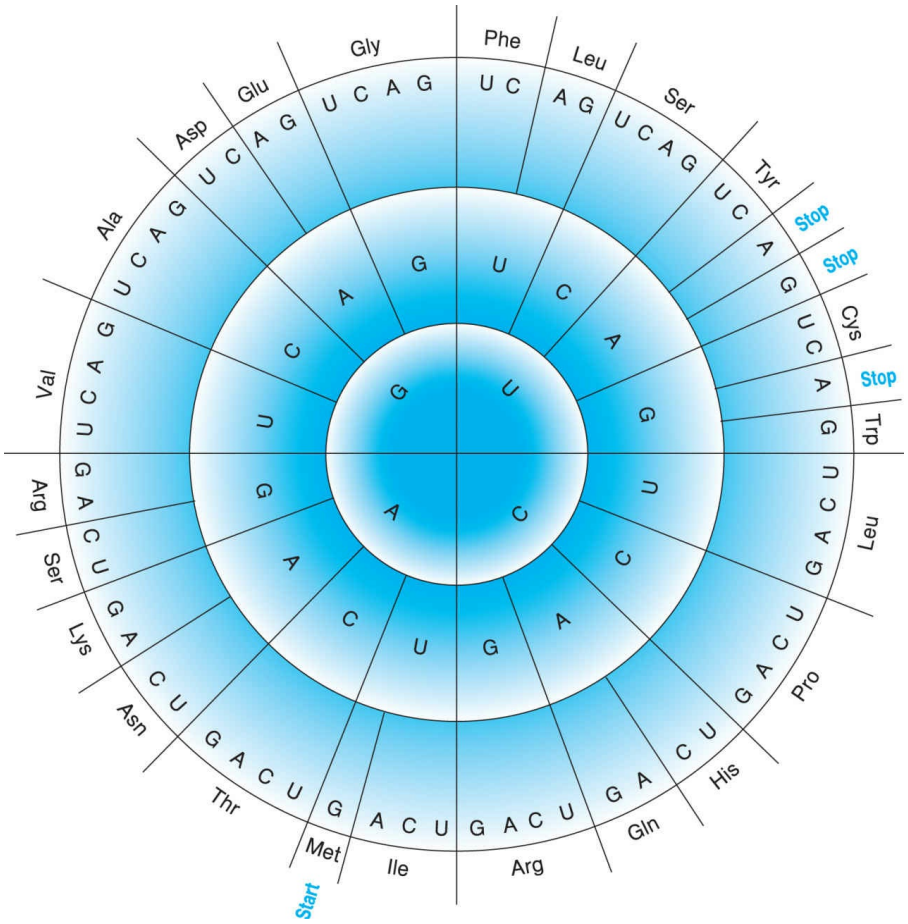


Figure 4.26 **The genetic code.** The genetic code relates every possible triplet of RNA bases to an amino acid or signal to stop translation. Look for the first base (5' end) of a triplet in the inner circle, the second base in the middle zone, and the third base in the outer zone. Then find the amino acid (as a three-letter abbreviation) outside. One initiation codon (AUG, encoding Met) signifies the start of translation, whereas three termination codons (UAA, UAG, and UGA, encoding no amino acid) signify the end of translation.

Of the 64 codons, one signals the initiation of translation for all proteins. This **initiation codon** is AUG, and it encodes methionine. The second amino acid in a polypeptide chain is encoded by the next triplet of bases in mRNA (there is no "punctuation" between codons) and so on until one of three **termination codons** appears. These codons are UAA, UAG, and UGA; they encode no amino acid and signal the termination of translation.

An example of a short peptide encoded by an imaginary sequence of mRNA bases follows. (This is purely for practice; in reality, mRNA encodes longer polypeptide chains.)

5'...AGGAGGUAUGCCAGUUAGCCUACCCAAGUGAAUC...3'
Met -Pro- Val -Ser- Leu -Pro- Lys

What you need to do—and what a ribosome does—to translate an mRNA is, first, locate the initiation codon by scanning the base sequence from the 5' to the 3' end. Once you find it, write down Met. Then continue translating based on the genetic code until you reach a termination codon. Write down nothing; translation is over!

4.16 Transfer RNA

Amino acids and codons recognize each other indirectly, by the intervention of yet another kind of RNA, **transfer RNA (tRNA)**, so called because it transfers amino acids. At least one tRNA exists for each amino acid. To specify the various tRNA, we write the amino acid (by its three-letter abbreviation) as superscript. For example, $tRNA^{Ala}$ is the tRNA specialized in transferring alanine.

Transfer RNA molecules contain roughly 80 ribonucleotide residues, including several unusual ones, which are formed by modification of standard ribonucleotide residues after transcription. For example, thymine, which is otherwise absent from RNA, is present in some tRNA. A tRNA molecule has the characteristic shape shown in figure 4.27. Its chain folds to bring together complementary bases, which form short stretches of double helix.

The resulting L-shaped molecule has two distinct ends. One (upper right in figure 4.27) contains both the 3' and 5' ends of the polynucleotide chain. The amino acid carried by tRNA is attached to the 3' end by a covalent bond between the carboxyl group of the amino acid and the hydroxyl group at position 2' or 3' of the terminal ribose of tRNA. Formation of such **aminoacyl-tRNA**, or simply **charged tRNA**, is catalyzed by highly specific **aminoacyl-tRNA synthetases**. At least one of these enzymes exists for each amino acid.

The other end of the L corresponds approximately to the middle of the polynucleotide chain. Here a triplet of bases is complementary to that mRNA codon, which, according to the genetic code, encodes the amino acid carried by the specific tRNA. This triplet of bases in tRNA is called **anticodon**.

Thus, tRNA, with an amino acid at one end and the ability to recognize the cognate codon at the other, bridges the world of ribonucleotides with that of amino acids.

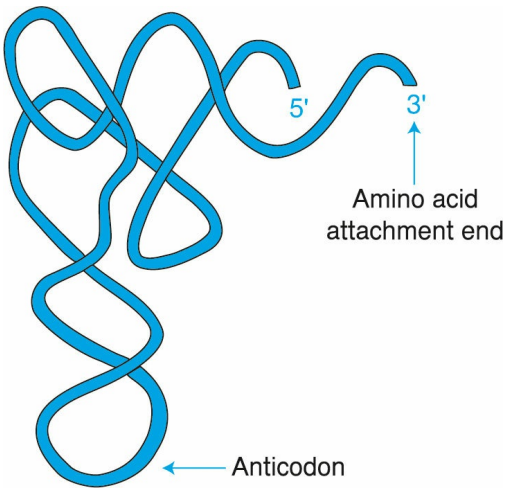


Figure 4.27 **Transfer RNA.** Transfer RNA translates the language of nucleic acids into the language of proteins. Its three-dimensional structure is usually depicted as an inverted L. Shown in this simplified diagram are the ends of amino acid attachment and codon recognition (the anticodon).

4.17 Translation Continued

When mRNA, ribosomes, and charged tRNA are available, translation may begin. The steps of this process (in simplified form) are as follows.

1. A tRNA charged with methionine binds to the small subunit of a ribosome. This tRNA is specialized for the initiation of translation and is abbreviated as **Met-tRNA$_i$**, where *i* stands for *initiation* (not for *inorganic*, as in the case of P_i and PP_i). The ribosomal subunit then binds to the 5' end of mRNA and proceeds along it toward the 3' end. When the ribosomal subunit reaches the initiation codon AUG, the anticodon of Met-tRNA$_i$ recognizes it (since they have complementary base sequences) and binds to it by hydrogen bonding. As in other cases of complementary binding between strands of nucleic acids, the chains of mRNA and tRNA have opposite directions. Finally, the large ribosomal subunit joins the small subunit, and everything is ready for

translation to continue (figure 4.28*a*).

2. Ribosomes have three binding sites for tRNA, spanning both subunits and distinguished by the initials P (for peptide), A (for amino acid), and E (for exit). The P site is occupied by Met-$tRNA_i$ when translation begins and is the site where the polypeptide chain grows, residue by residue. The A site is occupied by a tRNA charged with the amino acid encoded by the next codon in mRNA (figure 4.28*b*) and is the site where charged tRNA enter to provide the amino acids for protein synthesis. We will talk about the E site in a moment.
3. The two amino acids are now ready to be linked: The amino group of the second amino acid attacks the carboxyl group of methionine and removes it from Met-$tRNA_i$, forming a peptide bond (figure 4.28*c*). Thus, the second tRNA acquires a dipeptide (becoming a **peptidyl-tRNA**) and occupies the P site of the large subunit, though the area around its anticodon is still in the A site of the small subunit.
4. The ribosome moves by one codon toward the 3' end of mRNA (figure 4.28*d*). As a result, $tRNA_i$ moves to the E site and exits the ribosome, while the peptidyl-tRNA occupies the P site entirely.

A repetition of steps 2 to 4 follows. That is, a new tRNA, charged with the amino acid encoded by the third codon, enters the vacant A site. This amino acid is then added to the dipeptide of the tRNA located in the P site. The ribosome moves by yet another codon, and the uncharged tRNA in the P site moves to the E site (leaving the ribosome), while the tRNA carrying the tripeptide occupies the P site. With each cycle of this process, the polypeptide chain is elongated by one residue.

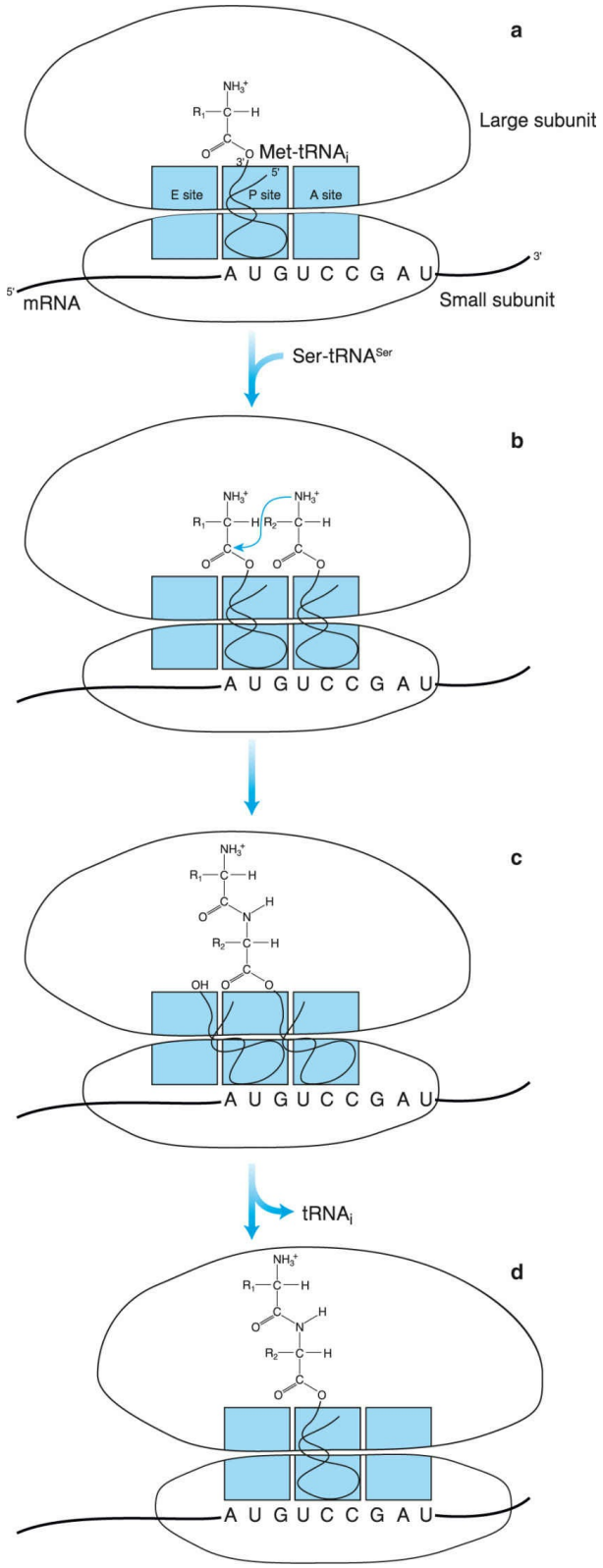


Figure 4.28 **Translation.** Follow the synthesis of a protein by a ribosome on the basis of instructions from mRNA in four stills. Synthesis begins when a specific initiation tRNA,

charged with methionine (Met-$tRNA_i$), enters the P site in the ribosome and the ribosome slides along the mRNA until it meets the initiation codon (AUG) along the mRNA base sequence *(a).* A tRNA charged with the amino acid encoded by the second codon enters the A site *(b)*. This amino acid is serine in the hypothetical case of the figure. Next, serine forms a peptide bond with methionine *(c)*. The ribosome moves by one codon along mRNA in the 5' → 3' direction *(d),* resulting in $tRNA_i$ being dislodged to the E site (through which $tRNA_i$ leaves the ribosome) and Met-Ser-$tRNA^{Ser}$ occupying the P site. Notice, by comparing stills *a* and *d* vertically, that, while mRNA did not move, the ribosome moved to the right. Also note the similarity of stills *a* and *d:* Both have charged tRNA in the P site, whereas the A site is vacant. R_1 and R_2 are the side chains of Met and Ser, respectively. For diagrammatic purposes, the proportions of the ribosome's parts have not been retained in this representation.

When any of the three termination codons appears in the A site, addition of amino acids stops, as there is no tRNA with an anticodon complementary to these codons. The polypeptide chain is liberated from tRNA by hydrolysis of the bond between the carboxyl group of the last residue and ribose. Transfer RNA and mRNA detach from the ribosome, which then dissociates into its subunits and becomes available for the synthesis of another protein molecule.

It is evident from the preceding description that the synthesis of a polypeptide chain proceeds from the free amino group to the free carboxyl group, that is, in the *N* → *C* direction. I should also stress that protein synthesis in the ribosomes requires a substantial input of energy, which is supplied by the hydrolysis of ATP and **GTP (guanosine triphosphate)** into AMP and **GDP (guanosine diphosphate)**, respectively. (You may practice writing down the equation of GTP hydrolysis.) Approximately four ~P are broken down per amino acid added to a polypeptide chain, although this rather brief presentation of translation did not allow me to pinpoint which endergonic processes are propelled by the energy released from ~P hydrolysis.

It is worth noting that the end of translation is usually not the end of protein synthesis. Rather, newly synthesized polypeptide chains usually undergo extensive processing (just like newly synthesized RNA does) before they become functional. Processing of a polypeptide chain includes modification of some of its residues and excision of parts of the chain. See section 13.1 for more on this matter.

4.18 Novel Kinds of RNA

Apart from the three classical kinds of RNA (mRNA, rRNA, and tRNA) presented thus far, several other kinds have been discovered in recent decades. In fact, the members of these categories seem to outnumber the members of the classical categories in humans. Here is a short presentation of the novel kinds of RNA.

- **Small nuclear RNA (snRNA)** participates in RNA splicing in the nucleus.

> Small nuclear RNA is 100 to 200 ribonucleotides long.

- **Small nucleolar RNA (snoRNA)** is essential for the processing of rRNA in the nucleolus (see section 1.15 and figure 1.9).
- **MicroRNA (miRNA)** is involved in the regulation of gene expression by preventing translation. It does so by binding to a complementary base sequence of mRNA, usually in its 3' untranslated region—that is, downstream of the termination codon. This binding blocks translation or leads to degradation of the bound mRNA. MicroRNA molecules are usually produced from longer RNA strands that fold on themselves to form double helices before they are cleaved by two ribonucleases specializing in hydrolyzing duplex RNA. One of the ribonucleases, called **Drosha**, is located in the nucleus; the other, called **Dicer**, is found in the cytosol. The resulting short RNA duplex is then separated into its strands, with one (or sometimes both) acting as miRNA. A single miRNA species can have multiple targets, thus enabling simultaneous regulation of the expression of many genes involved in specific metabolic pathways or biological processes. MicroRNA is thus the key player in a natural process known as **gene silencing** through **RNA interference**.

> MicroRNA is about 22 ribonucleotides long.

- **Small interfering RNA (siRNA)** is also involved in gene silencing by RNA interference, but its origin is external rather than internal. That is, siRNA is produced by the action of Dicer on long duplex RNA introduced into the cytosol through either infection by a virus having RNA (rather than DNA) as its genetic material or manipulation in the laboratory. Because it can silence specific genes of interest, RNA interference holds much therapeutic potential, especially in regard to viral infections and cancer.

The impressive variety of kinds and functions of RNA provides evidence for its fundamental role in the evolution of life on Earth, which is the topic of the next section.

4.19 In the Beginning, RNA?

The origin of life has puzzled humans for thousands of years. Throughout the centuries, the human mind has strived to provide answers based on the available knowledge. However, scientifically founded views on the descent of living organisms began to appear only during the second half of the 19th century. The theory of *evolution by natural selection,* founded at that time by Charles Darwin, is nowadays supported by a huge body of evidence and is universally accepted by scientists. Our current view of the origin of life is that modern complex, multicellular organisms are descendants of simpler ones, which in turn originated from unicellular organisms that appeared in the primordial lakes and seas of our planet about four billion years ago.

To put life in perspective, Earth is believed to be 4.5 billion years old, and the universe is estimated to be 13.8 billion years old.

The deeper we travel into the past, the fuzzier the picture becomes. How did the first cell arise? Did it have the features of modern cells? Going even further, we leave the realm of **biological evolution** and enter **chemical evolution**, which is believed to have taken place on Earth between 3.8 and 4.1 billion years ago. What was the first molecule of life? “Protein!” one might answer, since almost no biological process can occur without it. But

how was the first protein formed? The instructions for protein synthesis reside in DNA. Even if the first proteins were put together in a way different from the one we know today—that is, in some way that did not necessitate DNA—how did they reproduce? Polypeptide chains lack the ability to serve as templates for the formation of like chains. On the other hand, DNA (which does possess this ability) is unable to duplicate without the aid of enzymes. Thus, we enter a vicious cycle with no obvious way out (figure 4.29*a*).

A probable solution to the conundrum appeared in the 1980s, when the Canadian and American molecular biologist Sidney Altman and the American chemist Thomas Cech independently discovered biological catalysts that were not proteins but RNA—a discovery that earned them the Nobel Prize in Chemistry in 1989. Today we know of more than 100 catalytic RNA, which have been aptly named **ribozymes** to reflect the fact that they are ribonucleic acids acting as enzymes. Ribozymes are endowed with the ability to cut, splice, and transpose RNA segments. There is also evidence that they can recognize and handle amino acids directly. The coexistence of the catalytic activity of enzymes and the reproductive ability of nucleic acids in one molecule overcomes the dilemma of whether DNA or proteins preexisted: Earth at the dawn of life could have been a world of ribonucleic acids where every bit of genetic information was contained in RNA and every reaction in cells was catalyzed by RNA.

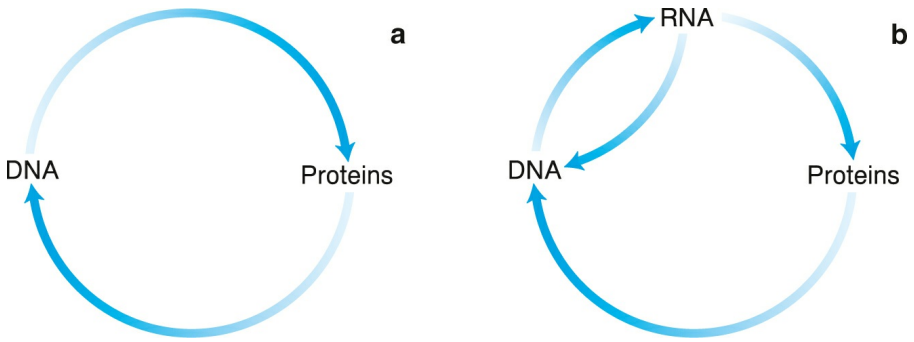


Figure 4.29 **A chicken-and-egg problem.** Which was the first biomolecule? *(a)* DNA cannot be synthesized without proteins, but proteins cannot be synthesized without instructions from DNA. *(b)* DNA and proteins may have been preceded by a primitive RNA capable of both reproducing itself and catalyzing reactions. RNA may then have driven the formation of DNA and proteins, relinquishing the functions of genetic information storage to the former and of catalysis to the latter while reserving the role of go-between.

A primary role of RNA in the chemical evolution of life is supported by the intense presence of the same and its components (the ribonucleotides) in biological processes, as summarized in the following points:

- RNA is omnipresent in the transmission of genetic information from DNA to protein: It carries the instructions from the genes (mRNA), forms the bulk of ribosomes (rRNA), and matches ribonucleotides with amino acids (tRNA). Moreover, the very formation of peptide bonds during translation is catalyzed by an rRNA in the large ribosomal subunit.
- As just presented in the previous section, recently discovered types of RNA (snRNA, snoRNA, miRNA, and siRNA) modulate gene expression in an impressive variety of ways.
- Bacteria and some eukaryotes possess mRNA molecules that form special secondary structures, termed **riboswitches**, that are capable of binding metabolites. Metabolite binding aborts transcription, blocks translation initiation, or regulates intron splicing—all of which are ways of controlling gene expression without the need for proteins.
- A ribonucleotide (ATP) is the universal energy currency of cells.
- Another ribonucleotide, GTP, participates in metabolic processes as a high-energy compound (sections 4.17, 10.12, and 10.24) and in signal transduction pathways (section 10.6).
- Of the remaining ribonucleoside triphosphates, UTP is used in the activation of biomolecules participating in carbohydrate synthesis (section 10.3), and CTP plays a similar role in lipid synthesis.
- An unusual ribonucleotide, cyclic AMP, mediates the transduction of the messages of many hormones (section 10.6). A similar function is served by cyclic GMP.
- Some compounds holding key positions in metabolism contain ribonucleotide residues in their structures. Such compounds include NAD, NADP, and FAD (section 2.5), as well as coenzyme A (section 10.10).

It is thus possible that the first chemical system meeting the definition of

life (p. 1) was based on RNA (figure 4.29*b*). Later, with small modifications (of ribose to deoxyribose and of uracil to thymine), DNA emerged as a more appropriate depository of genetic information because of its higher stability than RNA. Parallel to that development, catalytic RNA molecules acquired protein segments, which improved their functions, until, in the end, proteins deposed RNA in this area as better and more versatile catalysts. The ability of RNA to recognize amino acids rendered it the link between DNA and proteins, granting it a variety of roles in the transmission of genetic information. Finally, the participation of ribonucleotides in a multitude of other cellular functions shows that some primitive solutions to the problems of life might have been so good that they withstood the trial of evolution.

How was the first RNA molecule created? Laboratory experiments have been aimed at discovering what kinds of substances could have formed on Earth under the conditions that—we assume—prevailed before the emergence of life, that is, 4.5 to 4 billion years ago. According to available evidence, those conditions were quite different from the contemporary ones and utterly unsuitable for modern forms of life. The atmosphere contained hydrogen, methane, ammonia, and water, but hardly any oxygen. It was stunned by lightning, thunder, and volcanic explosions; it was stormed by ultraviolet radiation from space. Therefore, there must have been ample energy for the ingredients of the atmosphere to react.

Experiments simulating Earth's primordial atmosphere have yielded many of today's cellular components, including amino acids, nucleic acid bases, simple carbohydrates, fatty acids, peptides, and RNA-like compounds. Thus, it appears that circumstances on **prebiotic Earth** (that is, Earth before the emergence of life) favored a chemical evolution from simple to complex organic compounds capable of supporting two fundamental functions of contemporary biomolecules: catalysis and reproduction. This evolution gave life on Earth the needed spark. Finally, it is also possible that the chemical precursors of life arrived on Earth from space, on board meteorites bombarding the planet at the time that our solar system was being formed.

Summary

Nucleic acids are biological macromolecules consisting of nucleotide residues. Each residue of deoxyribonucleic acid (DNA) contains one of the four bases adenine (A), guanine (G), thymine (T), and cytosine (C); the carbohydrate deoxyribose; and a phosphoryl group. Each residue of ribonucleic acid (RNA) contains one of the four bases adenine, guanine, uracil (U), and cytosine; the carbohydrate ribose; and a phosphoryl group. Nucleotide residues are linked through phosphodiester linkages between positions 5' and 3' of ribose or deoxyribose to form strands numbering as few as tens (in the case of RNA) to as many as hundreds of millions of monomers (in the case of DNA).

DNA is the genetic material of cells, is organized in chromosomes, and usually has the shape of a double right-handed helix, in which two strands of opposite directions (5' → 3' and 3' → 5') are held together by hydrogen bonding. The bonds develop between the base pairs A–T and G–C; these bases face each other in the two strands. Thus, the DNA strands have complementary base sequences. This complementarity is essential for the semiconservative DNA replication. During replication (which precedes division of a parent cell into two daughter cells) the enzyme DNA polymerase uses each strand as a template to construct a new strand with a complementary base sequence. Each daughter chromosome contains a parental and a newly formed strand. In this way, daughter cells ensure an exact copy of the parental DNA. Errors in replication happen less frequently than once in a million and result in mutations. Mutations are also caused by environmental agents, or *mutagens*, and by mobile genetic elements. Mutations may elicit disease or evolution.

The genetic information contained in DNA is transmitted to RNA through transcription. RNA polymerase, using a DNA strand as a template, constructs an RNA molecule of complementary base sequence. DNA is transcribed in pieces called *genes*. Humans have about 39,000 genes, 250 of which

have been associated with physical performance and fitness through studies in exercise genomics—a field devoted to translating robust observations into a better understanding of exercise biology, the potential for human performance, and the health benefits of exercise. Some of the synthesized RNA molecules, called *messenger RNA* (*mRNA*), carry the instructions for protein synthesis. The instructions are "read" by ribosomes, which are complexes of proteins and another kind of RNA, ribosomal RNA.

Protein synthesis based on the mRNA base sequence is called *translation* and completes the flow of genetic information, or gene expression (figure 4.30). Translation obeys the genetic code, which matches every possible triplet of RNA bases with one amino acid or a termination signal. Of the 64 possible triplets, or codons, one signals the initiation of translation and encodes the amino acid methionine. Three codons signal the termination of translation. The remaining 60 codons encode the other 19 amino acids. The matching of codons and amino acids is mediated by transfer RNA molecules, which bind an amino acid at one end and recognize the codon at their other end, or *anticodon*. Messenger RNA is translated in the 5' → 3' direction, and a protein is synthesized in the *N* → *C* direction. Novel kinds of RNA include small nuclear RNA, small nucleolar RNA, microRNA, and small interfering RNA, all of which regulate gene expression by binding to other RNA molecules. The primary role of RNA in the flow of genetic information, the participation of nucleotides in a multitude of metabolic processes, and the existence of RNA molecules with catalytic properties all provide evidence for the appearance of RNA in the history of life on Earth before DNA and proteins.

Figure 4.30 **Flow of genetic information.** The genetic information contained in a cell's DNA

is copied to daughter cells through DNA replication. Genes in DNA are transcribed into RNA, and RNA (in particular, mRNA) is translated into proteins.

Problems and Critical Thinking Questions

1. (Integrative problem) Fill in the following table to show the analogies between nucleic acids and proteins.

Feature	Protein	Nucleic acid
Building block (monomer)		
Number of different monomers		
What links monomers		
Conventional direction of chain		
Periodic secondary structure		
What stabilizes the secondary structure		

2. Twenty-three percent of the bases of a DNA duplex are cytosine. What are the percentages of the other bases?
3. Biochemists often study metabolism in a living system (for example, cells in culture) by adding a metabolite that contains one or more radioactive isotopes in its structure and examining which other compounds become radioactive through metabolic reactions. Suppose that a researcher wants to study DNA replication in multiplying cells by labeling DNA but not RNA. Which radioactive compound may she add to the culture medium?
4. Does the following base sequence belong to DNA or RNA? Write its complementary sequence, including the notation of the ends.

   5' ACUAGCGCUA 3'
5. Consider the following piece of DNA:

   5' … ACGACATGTT … 3' (template strand)

   3' … TGCTGTACAA … 5'

Write the RNA base sequence resulting from the transcription of the template strand.

6. How many mRNA, rRNA, and tRNA molecules are involved in the synthesis of a human protein molecule having 200 amino acid residues?
7. Consider the following piece of synthetic RNA:

   5' … GAGCGAUGCGCAGUUACCCUACCCAAUGA-AUGAA … 3'

   Write the peptide resulting from its translation.
8. A mutation involving a single base (called a point mutation) in the previous sequence results in the production of the following peptide.

   Met–Arg–Cys–Ala–Val–Thr–Leu–Pro–Asn–Glu

   Which mutation is it?
9. (Integrative problem) In sickle cell disease, the A in what is normally a GAG codon in the β-globin gene is mutated to T. How is the amino acid sequence of β-globin changed? Why is this change so important as to cause disease?
10. Eero Antero Mäntyranta (1937-2013) was a Finnish cross-country skier who won a total of seven medals in the 1960, 1964, and 1968 Winter Olympic Games, as well as five more in World Championships. He had familial erythrocytosis, an inherited condition characterized by abnormally high erythrocyte mass in blood. As a result, his blood hemoglobin concentration was up to 23.6 $g \cdot dL^{-1}$ (compare that with a typical concentration in section 3.11), thus endowing him with extraordinary aerobic capacity. Three decades after his astonishing achievements, Mäntyranta and many members of his greater family were reported by Albert de la Chapelle and coworkers to possess a rare polymorphism in the erythropoietin receptor gene. In particular, the middle G in what is normally a TGG codon was changed to A. How is the amino acid sequence of the erythropoietin receptor changed?

How could this mutation be connected to erythrocytosis? (*Hint:* The erythropoietin receptor transmits the signal of erythropoietin, a hormone that causes erythrocyte formation, as discussed in section 16.1.)

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

deoxyribonucleic acid, DNA
ribonucleic acid, RNA
nucleotide
deoxyribonucleotide
ribonucleotide
genome
chromosome
gene
adenine
guanine
purine
pyrimidine
thymine
cytosine
β-D-2-deoxyribose
deoxyadenosine monophosphate, dAMP
deoxyadenosine diphosphate, dADP
deoxyadenosine triphosphate, dATP
deoxyribonucleoside monophosphate, dNMP
deoxyribonucleoside diphosphate, dNDP
deoxyribonucleoside triphosphate, dNTP
deoxyribonucleoside

deoxyadenosine
deoxyguanosine
thymidine
deoxycytidine
polynucleotide chain
phosphodiester linkage
primary structure
5' end
3' end
upstream
downstream
secondary structure
double helix
B-DNA
duplex
strand
base pair, bp
complementary strands
ϕX174
Escherichia coli
diploid cell
autosome
sex chromosome
haploid cell
Human Genome Project
genomics
chromatin
histone
nucleosome
nucleoid

mitosis
DNA replication
S phase
DNA polymerase
helicase
template
primer
primase
semiconservative DNA replication
mutation
mutagen
mobile genetic element
silent mutation
uracil
β-D-ribose
ribonucleoside monophosphate, NMP
ribonucleoside diphosphate, NDP
ribonucleoside triphosphate, NTP
ribonucleoside
adenosine
guanosine
uridine
cytidine
transcription
RNA polymerase
transcript
template strand, antisense strand
coding strand, sense strand
transcriptome
transcriptomics

promoter
enhancer
Encyclopedia of DNA Elements, ENCODE
transcription factor
terminator
gene expression
gene product
genotype
phenotype
exercise genomics
polymorphism
allele
primary transcript
intron
exon
messenger RNA, mRNA
5' cap
poly(A) tail
ribonuclease
nuclear envelope
alternative splicing
RNA editing
translation
ribosome
ribosomal RNA, rRNA
(ribosomal) subunit
genetic code
codon
degenerate genetic code
universal genetic code

initiation codon
termination codon
transfer RNA, tRNA
aminoacyl-tRNA, charged tRNA
aminoacyl-tRNA synthetase
anticodon
Met-$tRNA_i$
peptidyl-tRNA
guanosine triphosphate, GTP
guanosine diphosphate, GDP
small nuclear RNA, snRNA
small nucleolar RNA, snoRNA
microRNA, miRNA
Drosha
Dicer
gene silencing
RNA interference
small interfering RNA, siRNA
biological evolution
chemical evolution
ribozyme
riboswitch
prebiotic Earth

References and Suggested Readings

Berg JM, Tymoczko JL, Gatto GJ, Stryer L (2015). *Biochemistry* (Freeman, New York), pp. 105-134.

Bray MS, Hagberg JM, Pérusse L, Rankinen T, Roth SM, Wolfarth B, Bouchard C (2009). The human gene map for performance and health-related fitness phenotypes: The

2006-2007 update. *Medicine and Science in Sports and Exercise* 41: 34-72.

Collins FS, Green ED, Guttmacher AE, Guyer MS, U.S. National Human Genome Research Institute (2003). A vision for the future of genomics research. *Nature* 422: 835-847.

de la Chapelle A, Träskelin AL, Juvonen E (1993). Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. *Proceedings of the National Academy of Sciences of the United States of America* 90: 4495-4499 (cited in problem 10).

ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature* 489: 57-74.

Garrett RH, Grisham CM (2012). *Biochemistry* (Cengage, Boston), pp. 309-377.

Li JJ, Bickel PJ, Biggin MD (2014). System wide analyses have underestimated protein abundances and the importance of transcription in mammals. *PeerJ* 27: e270.

Mathews CK, van Holde KE, Appling DR, Anthony-Cahill SJ (2013). *Biochemistry* (Pearson, New York), pp. 84-125.

Moran CN, Pitsiladis YP (2017). Tour de France Champions born or made: where do we take the genetics of performance? *Journal of Sports Sciences* 35: 1411-1419.

Nelson DL, Cox MM (2017). *Lehninger Principles of Biochemistry* (Freeman, New York), pp. 281-312.

Sarzynski MA, Loos RJ, Lucia A, Pérusse L, Roth SM, Wolfarth B, Rankinen T, Bouchard C (2016). Advances in exercise, fitness, and performance genomics in 2015. *Medicine and Science in Sports and Exercise* 48: 1906-1916.

CHAPTER 5

Carbohydrates and Lipids

Learning Objectives

After reading this chapter, you should be able to do the following:

- Distinguish the classifications of carbohydrates used in biochemistry and nutrition.
- Describe the salient features of monosaccharides, list some examples, and draw structural formulas of the glucose isomers.
- Explain how disaccharides are built from monosaccharides, name the linkage that joins monosaccharide residues, and list some examples of disaccharides.
- Describe what the three polysaccharides—cellulose, starch, and glycogen—have in common and differentiate them in terms of both structure and function.
- Provide examples of the hydrophilicity of carbohydrates.
- Name the main categories of lipids found in humans and describe the structure of each category.
- Draw a saturated, a monounsaturated, and a polyunsaturated fatty acid.
- Distinguish the classes of lipids in terms of miscibility with water.

- Discuss the structure and properties of a cell membrane.

Our acquaintance here with carbohydrates and lipids will be briefer than it was with proteins and nucleic acids in chapters 3 and 4, respectively—not because they are less important but because we will deal with them extensively in part III, which addresses exercise metabolism. One can distinguish proteins and nucleic acids from carbohydrates and lipids in that the former are not considerable energy sources, whereas the latter are. Since exercise is interwoven with increased energy demand, we will discuss how energy is extracted from carbohydrates and lipids in part III. In the present chapter, we will limit ourselves to examining their structure and some aspects of their utility.

5.1 Carbohydrates

Carbohydrates are compounds that contain at least two hydroxyl groups and either share the molecular formula $C_nH_{2n}O_n$ or derive from compounds of that formula. If one takes n as a common factor, the formula becomes $(CH_2O)_n$ and gives the impression that carbon atoms and water molecules are present in equal numbers. This coincidence is the source of the name *carbohydrate*. However, there are no water molecules inside a carbohydrate molecule.

Carbohydrates serve a multitude of functions in living organisms:

- They provide energy, as mentioned in section 2.6. Such is the role of sugar (probably the most familiar and popular carbohydrate) and glucose (the most common energy source of cells).
- They offer cells external protection. Cellulose supports (literally) the entire plant kingdom.
- Attached to proteins and lipids, they help cells to recognize molecules or other cells in their surroundings.
- They are part of every building block of nucleic acids (as ribose or deoxyribose).

A protein bearing a covalently attached carbohydrate residue is a **glycoprotein**, whereas a lipid bearing a covalently attached carbohydrate residue is a **glycolipid**. As we will see, the prefixes *glyco-* and *gluco-* dominate in carbohydrate nomenclature. They derive from the Greek word *glykýs,* meaning “sweet,” which is what many carbohydrates are.

Depending on the size of carbohydrates, biochemists divided them into three categories: monosaccharides, oligosaccharides, and polysaccharides.

5.2 Monosaccharides

Monosaccharides are the simplest of carbohydrates. A monosaccharide molecule usually contains three to seven carbon atoms and is called specifically a **triose** (3 C), **tetrose** (4 C), **pentose** (5 C), **hexose** (6 C), or **heptose** (7 C). Let’s meet some monosaccharides.

Trioses

The trioses **glyceraldehyde** and **dihydroxyacetone** (figure 5.1) are the smallest monosaccharides. The two compounds are isomeric, since they share the molecular formula $C_3H_6O_3$. The way they differ is in the position of their **carbonyl groups**.

Organic compounds bearing a carbonyl group at a terminal position are called **aldehydes**, whereas those bearing a carbonyl group at an intermediate position are called **ketones**.

Like amino acids, monosaccharides, except for dihydroxyacetone, exhibit the particular isomeric forms (*enantiomers*) characterized by the prefixes D- and L- (cf. section 3.1). In contrast to amino acids, however, the carbohydrates that prevail in biological systems are of the D form.

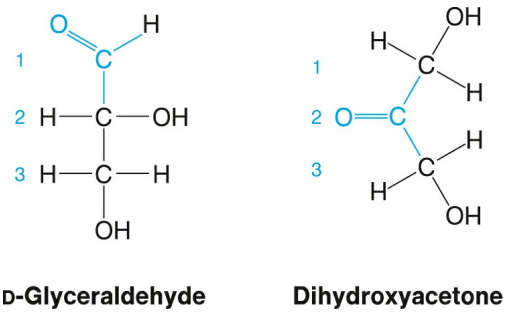


Figure 5.1 Trioses. Glyceraldehyde and dihydroxyacetone are the simplest of carbohydrates. Note the numbering of the carbon atoms. The carbonyl groups are marked in color.

Pentoses

We move now from trioses to a familiar pentose: ribose (the component of ribonucleotides). Ribose has the molecular formula $C_5H_{10}O_5$ and can be either open chain or cyclic (figure 5.2). In cyclic ribose—the predominant form in biological fluids—the oxygen of C4 forms a covalent

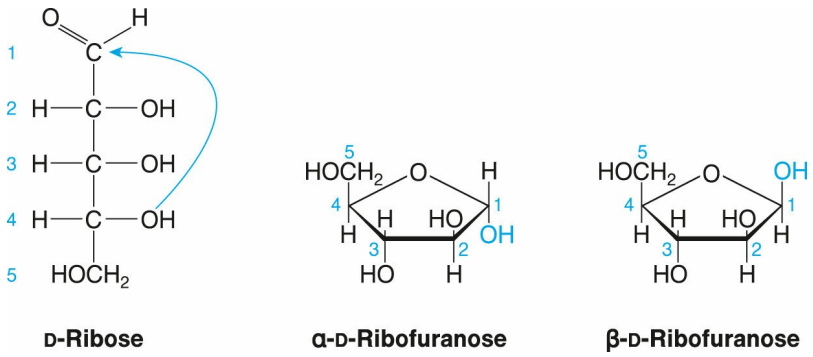


Figure 5.2 Ribose. D-Ribose exists in open-chain and cyclic forms. The arrow shows how the former is converted into the latter. We distinguish the cyclic form from the open-chain form by inserting the term *furano*. There are two cyclic isomers, α and β, which differ in the position of the hydroxyl group at C1 (in color).

bond with C1, and the aldehyde oxygen of the open-chain form is converted into hydroxyl. The resulting five-membered ring, bearing one oxygen and four carbons, is called a **furanose**. C5 stays outside the ring. If we need to distinguish the cyclic from the open-chain form, we call the former **ribofuranose**.

The cyclization of ribose gives rise to two possible positions of the newly

formed hydroxyl group at C1: one above the ring and the other below it. Thus, there are two possible isomers. As we will see shortly, discerning them is important; that is why we introduce two additional prefixes, α- and β-. When looking at the formula from above (so that the numbering of the carbons follows a clockwise direction), we have α-ribose if the hydroxyl group is below the plane of the ring and β-ribose if the hydroxyl group is above the plane.

Hexoses

We conclude our introduction to monosaccharides by examining two hexoses, **glucose** and **fructose**, which occur in fruits and account for their sweetness. Both have the molecular formula $C_6H_{12}O_6$ and, like ribose, appear in both open-chain and cyclic forms; the latter predominates in biological systems. Open-chain glucose cyclizes when the O of C5 attacks C1 (figure 5.3). The aldehyde oxygen of C1 is then converted into hydroxyl. The resulting six-membered ring with one O and five C is called a **pyranose**, and the cyclic glucose is called **glucopyranose** (if we need to distinguish it from the open-chain form). C6 stays outside the ring. Again, depending on the position of the hydroxyl group at C1, we have two isomers, α and β.

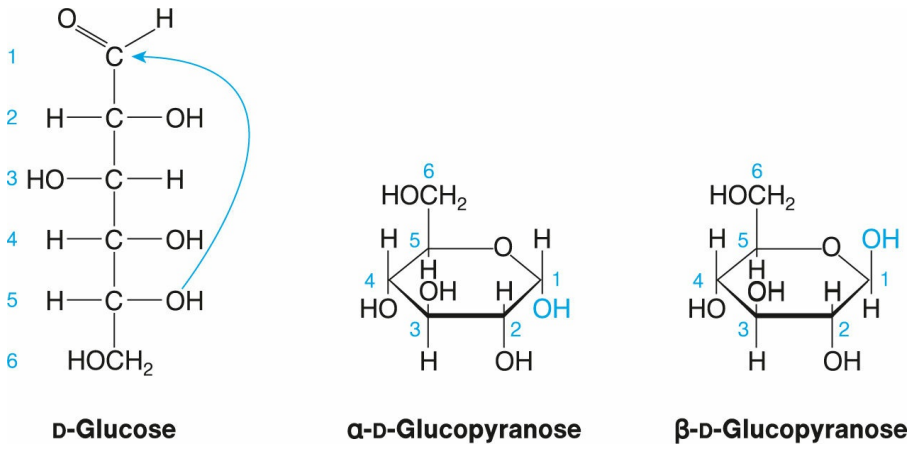


Figure 5.3 **Glucose.** D-Glucose, a fundamental energy source for cells, exists in open-chain and cyclic forms. We distinguish the latter from the former by inserting the term *pyrano*. There are two cyclic isomers, α and β, which differ in the position of the hydroxyl group at C1 (in color).

Fructose bears a carbonyl group at position 2 in its open-chain form. As a result, a five-membered furanose ring forms when the O at C5 attacks C2 (figure 5.4). Two carbon atoms (C1 and C6) are left outside the ring. Alternatively, the O at C6 may attack C2, yielding a six-membered pyranose ring.

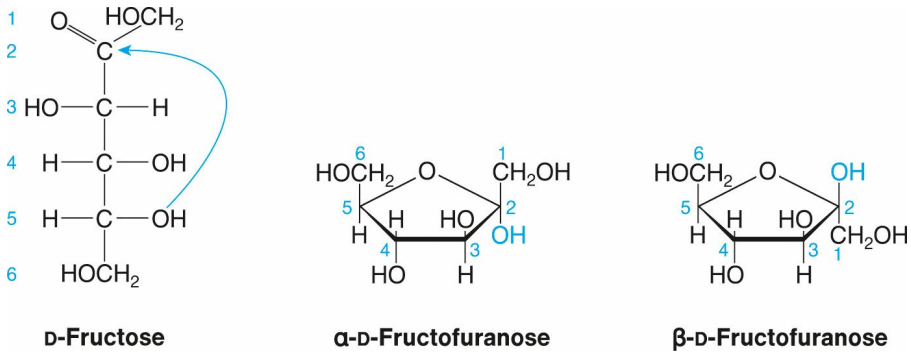


Figure 5.4 Fructose. D-Fructose differs from D-glucose in having a carbonyl group at position 2 rather than at position 1. As a result, the closure of the O at C5 on C2 produces a furanose ring. Assignment of the α or β configuration to fructofuranose is based on the position of the hydroxyl group at C2 (in color). Try to draw fructopyranose, produced when the O at C6 closes on C2.

5.3 Oligosaccharides

When 2 to 10 monosaccharides are linked covalently, **oligosaccharides** result. The linkages between monosaccharide residues in an oligosaccharide are called *glycosidic*. A **glycosidic linkage** consists of an oxygen atom joined to two carbon atoms and is formed from two hydroxyl groups upon removal of a water molecule (figure 5.5).

Let's meet three **disaccharides**, that is, oligosaccharides containing two monosaccharide residues. **Maltose** (figure 5.5) is a breakdown product of starch, a polysaccharide to be presented shortly, and consists of two α-D-glucose residues linked at C1 and

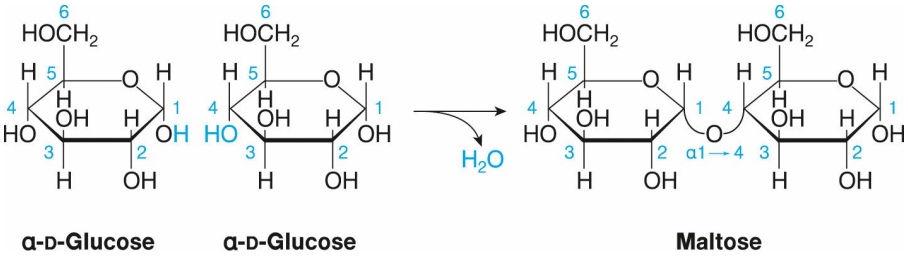


Figure 5.5 **Glycosidic linkage.** Two monosaccharides can be linked through their hydroxyl groups when they shed water. In this example, two molecules of α-D-glucose are joined by an α1 → 4 linkage to form the disaccharide maltose. The bonds between O and C in the glycosidic linkage have been bent for diagrammatic purposes (that is, to allow drawing the two monosaccharide residues side by side). In reality, the bonds are straight, just like every covalent bond.

C4. That is why the glycosidic linkage in maltose is characterized as α1 → 4. **Sucrose** (figure 5.6) is the common sugar. It consists of an α-D-glucose residue and a β-D-fructose residue linked at C1 and C2, respectively. Therefore, the glycosidic linkage is α1 → β2. Finally, **lactose** (figure 5.7) is the first carbohydrate we all taste, because it is contained in milk. Lactose consists of a β-D-galactose and an α-D-glucose residue linked at C1 and C4, respectively. Therefore, the glycosidic linkage is β1 → 4.

Galactose is a hexose differing from glucose in the position of the hydroxyl group at C4.

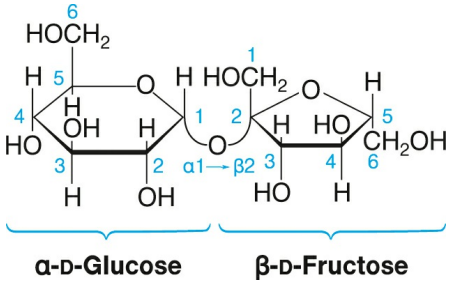


Figure 5.6 **Sucrose.** Our most familiar sweetener is a disaccharide of glucose and fructose. Note that the β-D-fructose residue has been turned upside down relative to figure 5.4.

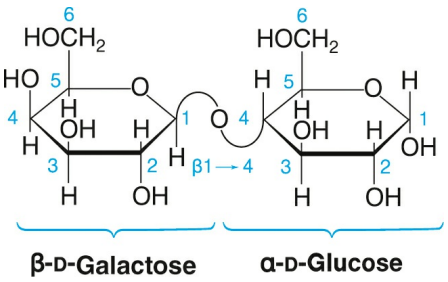


Figure 5.7 **Lactose.** The sweetener of milk is a disaccharide of galactose and glucose.

5.4 Polysaccharides

Polysaccharides are the most abundant category of carbohydrates. They contain more than 10 monosaccharide residues and are divided into **homopolysaccharides** and **heteropolysaccharides**. The former are polymers of a single monosaccharide, whereas the latter are composed of different monosaccharide residues.

We will deal here with three homopolysaccharides (and no heteropolysaccharide) consisting of hundreds to tens of thousands of D-glucose residues. These three polysaccharides are cellulose, starch, and glycogen. Cellulose serves a structural role in plants, whereas the role of starch and glycogen is to store energy (starch does so in plants, and glycogen does so in animals). Starch and glycogen differ from cellulose in that they consist of α-D-glucose residues, as opposed to the β-D-glucose residues in the latter. As a result, the chains of starch and glycogen assume curved, compact shapes, which render them suitable for energy storage, whereas the chains of cellulose are straight and thus suitable for supporting cells.

Cellulose

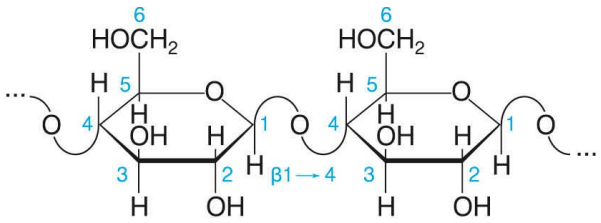
Cellulose is a polymer of β-D-glucose residues linked in a row by β1 → 4 linkages (figure 5.8). Thus, one end of a cellulose molecule has a free hydroxyl group at C1 and a bound hydroxyl group at C4, whereas the reverse happens at the other end. The end with free C1 is referred to as **reducing**, because it has the ability to reduce other substances; the end with free C4 is referred to as **nonreducing**. Remember that proteins and nucleic acids have distinct ends too.

Cellulose is found in plants and is the main solid constituent of their leaves and wood. It helps support and protect plant cells because its glycosidic linkages give rise to straight chains, which form bundles held together through hydrogen bonding. Cellulose is the most abundant organic compound on Earth, amounting to approximately 1 to 1.5 trillion tons. In terms of human nutrition, cellulose is a constituent of **dietary fiber**. Large quantities of it are found in cereals, fruits, and vegetables. Cellulose is also the main component of the book you are reading (unless you are reading the ebook) and, no doubt, some of your clothing, since paper and cotton are almost pure cellulose.

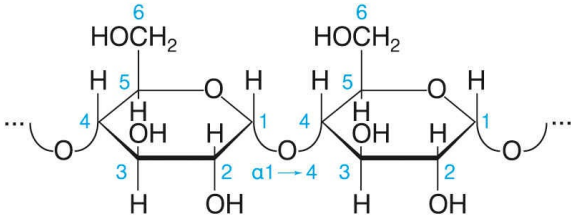
Starch

Starch is normally the major form of carbohydrate in our diet. It is found in the seeds of plants, among which cereals, legumes, and nuts are the most important for human nutrition. Flour consists mainly of starch, which can also be found in potatoes.

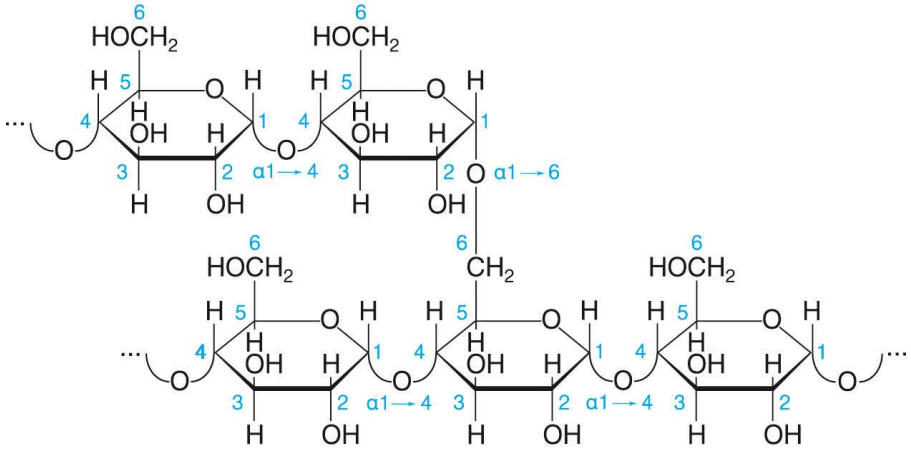
Starch is a mixture of two substances, **amylose** and **amylopectin**. Amylose is unbranched, with an average of 600 glucose residues linked in an α1 → 4 configuration (figure 5.8). In amylopectin, on the other hand, a branch appears every 20 to 30 glucose residues. The glycosidic linkage is α1 → 6 at the branch point but remains α1 → 4 inside the branches. It is common to have a branch point in a branch, another branch point in the new branch, and so on, so that an amylopectin molecule acquires a bushy appearance and a size about 10 times that of amylose. As a result, although amylose has one reducing end and one nonreducing end (just like cellulose), amylopectin has one reducing end but many nonreducing ends.



Cellulose



Amylose



Amylopectin, glycogen

Figure 5.8 **Polysaccharides.** Cellulose, starch, and glycogen—three polysaccharides of glucose—differ in the glucose isomers they consist of and in how their monomers are connected. Only a few glucose residues from each polymer are presented here, because the glycosidic linkages are repeated throughout the molecules. Cellulose is unbranched with β1 → 4 linkages. Starch is a mixture of amylose and amylopectin. Amylose is unbranched with α1 → 4 linkages (note the similarity with maltose in figure 5.5). Amylopectin, in addition to α1 → 4 linkages, has branches with α1 → 6 linkages at the branch points. Glycogen resembles amylopectin but is bushier. Reducing ends are on the right-hand side of the structural formulas.

Glycogen

Glycogen is found mainly in the liver and skeletal muscle. Its presence in these organs is crucial for energy metabolism, as we will see in chapter 10. Nevertheless, liver and meat are negligible sources of carbohydrate in our diet for reasons that I will explain in section 10.2. Glycogen (figures 5.8 and 5.9) is similar in structure to amylopectin: It is branched, having the same glycosidic linkages as amylopectin, but its branches appear more frequently (every 8 to 12 glucose residues) and are shorter (12 to 14 residues).

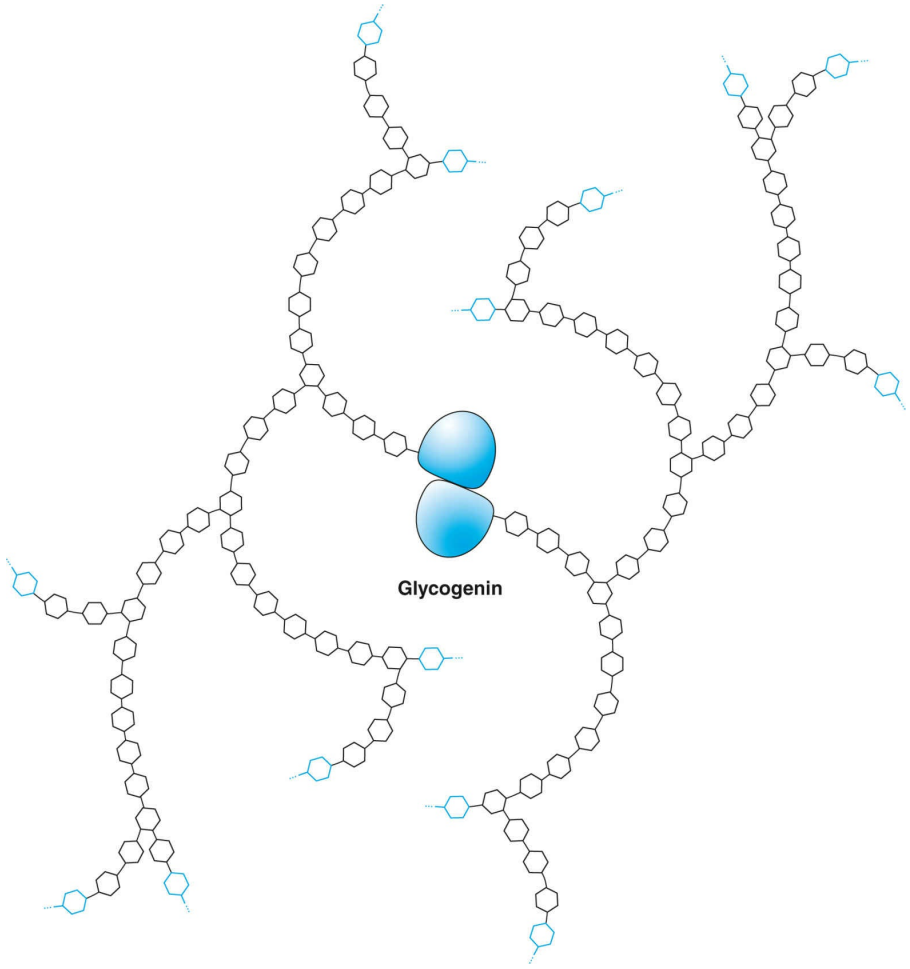


Figure 5.9 **Glycogen structure.** Glycogen has relatively dense branches, which render it suitable for rapid degradation and rapid energy production. In this representation of a small part of its structure, hexagons depict α-D-glucose residues. All ends are nonreducing and available for removal of glucose residues (in color). What would have been the two reducing ends are instead covalently attached to **glycogenin**, a protein that forms the core of glycogen molecules. Can you spot the α1 → 6 linkages?

Therefore, a glycogen molecule has more nonreducing ends than an

amylopectin molecule of equal mass (although glycogen molecules are usually larger than amylopectin molecules). The presence of many branches enables fast breakdown for energy production, because glycogen is degraded by the sequential removal of glucose residues from its nonreducing ends (see section 10.4). The potential for fast breakdown contributes to the prominent place of glycogen in exercise metabolism, which we will explore in part III.

The Hydrophilicity of Polysaccharides

The presence of so many hydroxyl groups in the molecules of polysaccharides allows extensive hydrogen bonding with water. This capacity renders them hydrophilic, although they do not dissolve in water, because of their large sizes. As a result of this hydrophilicity, cotton and paper are water absorbent, and flour mixes readily with water to form dough or batter. See problem 2 in this chapter for another enjoyable effect of the hydrophilicity of starch. As for glycogen, it is stored in tissues in conjunction with a roughly threefold mass of water, as recently confirmed by Valentín Fernández-Elías and coworkers.

The other categories of carbohydrates (monosaccharides and oligosaccharides) are not only as hydrophilic as polysaccharides but also water soluble, thanks to their small molecular sizes. Just think of how easily sugar dissolves in water!

5.5 Carbohydrate Categories in Nutrition

Nutritionists use a different classification of carbohydrates than do biochemists, one that discerns two categories: **simple** and **complex carbohydrates**. Monosaccharides and disaccharides are considered simple carbohydrates, whereas larger carbohydrates are considered complex. The differences between the two classification systems are clarified in table 5.1.

Table 5.1 Carbohydrate Classification in Biochemistry and Nutrition

In biochemistry	Monosaccharide residues		In nutrition
Monosaccharides	1		Simple carbohydrates
Oligosaccharides	2-10	2	
		3-10	Complex carbohydrates
Polysaccharides	> 10		

5.6 Lipids

Lipids present a great variety of structures and functions. This class of biomolecules encompasses the main constituent of a large tissue (adipose tissue), membrane components, hormones, vitamins, and a plethora of other substances possessing important biological properties. The common feature of these compounds is their low solubility in water. Considered rather inert as chemical entities up until a few decades ago, they seem now to have acquired the reputation they deserve—that of active participants in a multitude of biochemical processes.

In this chapter, we will consider four lipid categories: fatty acids, triacylglycerols, phospholipids, and steroids. Then, in the next chapter, we will meet the vitamins that belong to the lipid class.

5.7 Fatty Acids

Fatty acids are organic acids containing relatively large numbers of carbon atoms (usually 12 to 26). A fatty acid consists of a long carbon chain with hydrogen atoms all around (a chain that organic chemists call **aliphatic**) and a carboxyl group at one end (figure 5.10). As with amino acids, the carboxyl group lacks a proton at physiological pH; thus, it carries one negative charge. This is why we prefer to call fatty acids *-ates* rather than *-ic acids* (for example, *palmitate* rather than *palmitic acid*; see below in this section).

The carboxyl group is hydrophilic, whereas the aliphatic chain is hydrophobic. The two features combine in a fatty acid to produce a molecule that is **amphipathic** (meaning “passionate for both” in Greek) or **amphiphilic** (“loving both”). Amphipathic compounds are poorly soluble in

water.

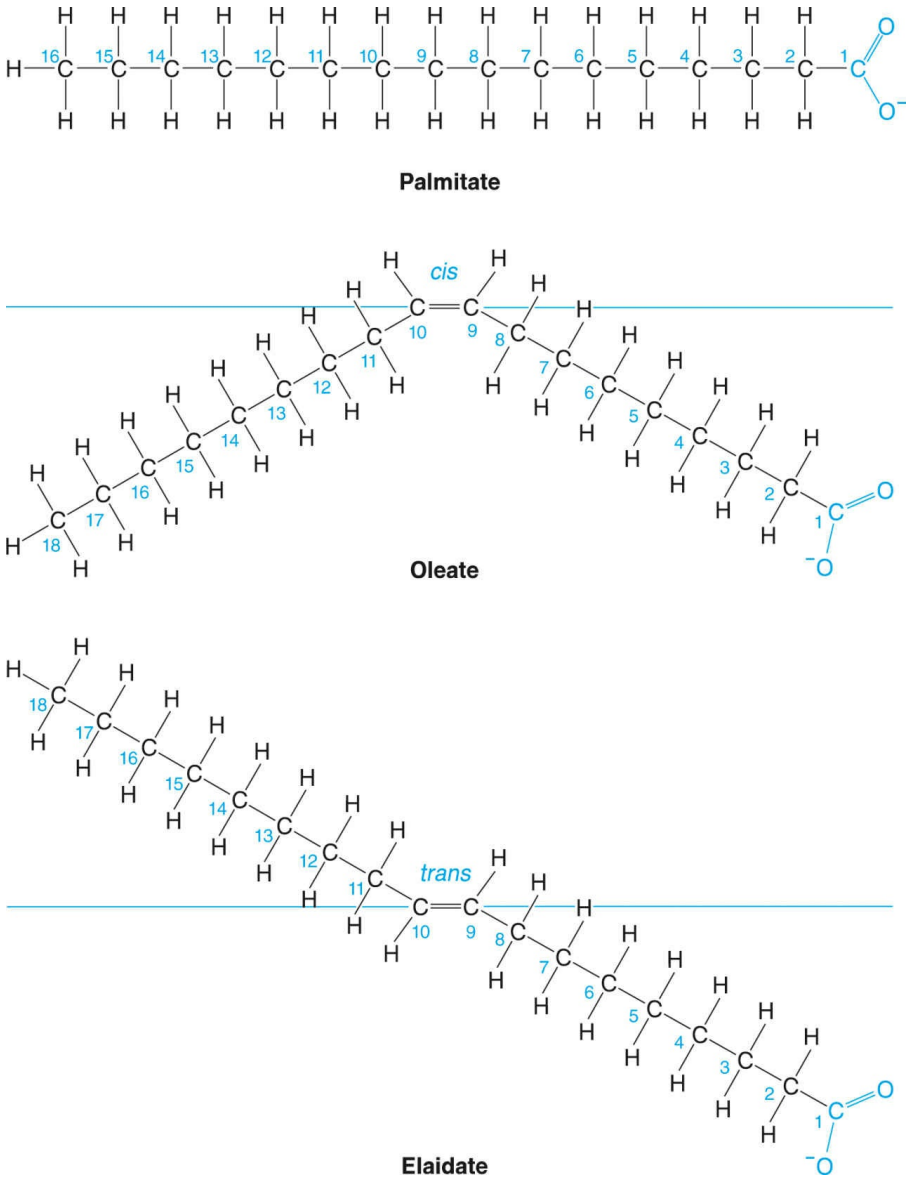


Figure 5.10 **Saturated and monounsaturated fatty acids.** A fatty acid contains an aliphatic chain (in black) and a carboxyl group at the end (in color). Carbon numbering starts at the carboxyl end. A fatty acid, such as palmitate, may have only single bonds between its carbon atoms, in which case it is called *saturated*. If two of its carbon atoms are linked by a double bond, it is characterized as *monounsaturated*. Depending on where the two parts into which a double bond separates the carbon chain lie relative to the line passing through the carbon atoms of the double bond, double bonds are assigned a *cis* or *trans* configuration. Oleate is a *cis* fatty acid, whereas elaidate is a *trans* fatty acid. Palmitate and oleate are among the most abundant fatty acids, whereas elaidate is a minor fatty acid.

Most fatty acids have even numbers of carbon atoms because they are synthesized by the joining of acetyl groups, which have two carbons (see section 11.13). Apart from length, fatty acids differ in the number of double bonds. If all carbon atoms in a fatty acid are linked by single bonds, it is characterized as **saturated** because there is no room to add more hydrogen atoms. Conversely, if there are double bonds between some of the carbons, the fatty acid is called **unsaturated** because a double bond may receive two H to become single. If we wish to distinguish the fatty acids with one double bond from those with more double bonds, we use the terms **monounsaturated** and **polyunsaturated**, respectively. Double bonds in a fatty acid do not exceed six and are almost invariably spaced three carbons apart in the polyunsaturated fatty acids. There are no triple bonds in fatty acids.

Figure 5.10 presents a saturated fatty acid with 16 C, named **palmitate**, and two monounsaturated fatty acids with 18 C, named **oleate** and **elaidate**. A double bond divides the carbon chain into two (not necessarily equal) parts, which may lie on either the same side or opposite sides of the imaginary line passing through the two carbons participating in the double bond. The former configuration (appearing in oleate) is called *cis;* the latter (appearing in elaidate and being reminiscent of an offside alignment in soccer) is called *trans*.

Figure 5.10 illustrates the remarkable change that a *cis* double bond introduces in the shape of a fatty acid: Whereas the molecule of a saturated fatty acid is straight, that of an unsaturated fatty acid is bent. This difference affects the physical properties of fatty acids and of those lipid categories that contain fatty acid units, because the straight molecules of saturated fatty acids can lie closer to each other than can the crooked molecules of unsaturated fatty acids. Thus, more hydrophobic interactions (section 3.5) develop among the molecules of saturated than of unsaturated fatty acids, and more heat is needed to break the former apart. As a result, saturated fatty acids have higher melting points than do unsaturated fatty acids with the same number of carbon atoms, thus being solid at room temperature, as opposed to the liquid form of most unsaturated fatty acids in the same conditions.

In contrast to a *cis* double bond, a *trans* bond does not bring about much

of a change in the shape of a fatty acid. Indeed, as you can see in figure 5.10, a *trans* fatty acid is nearly as straight as a saturated one. Since unsaturated fatty acids are synthesized from saturated ones (as we will see in section 11.13), it seems that cells produce them if that is going to make a difference. The absence of any essential difference in shape between saturated and *trans* unsaturated fatty acids is probably why the overwhelming majority of unsaturated fatty acids in nature have double bonds of the *cis* configuration. Thus, when referring to *unsaturated fatty acids* from now on, I will mean unsaturated fatty acids with *cis* double bonds.

Having multiple double bonds, a polyunsaturated fatty acid is even more crooked than a monounsaturated one, as shown with the example of **linoleate**, a fatty acid with 18 C and two double bonds, in figure 5.11. An alternative carbon numbering is introduced here—one that counts from the methyl end rather than the carboxyl end (the latter being the systematic way of numbering). The methyl end is considered the ω (omega) end, with ω being the ultimate letter in the Greek alphabet. Thus, carbon atoms are numbered ω1, ω2, and so on, or $n - 1$, $n - 2$, and so on. If, moving from the methyl to the carboxyl end, one encounters the first double bond after carbon ω6, as in the case of linoleate, one characterizes the fatty acid ω6. This approach gives rise to the classification of unsaturated fatty acids into ω3, ω6, and so on—a classification that is particularly common in the fields of nutrition and food science. Practice the ω numbering in problem 4! Some of the most common fatty acids in our bodies are presented in table 5.2.

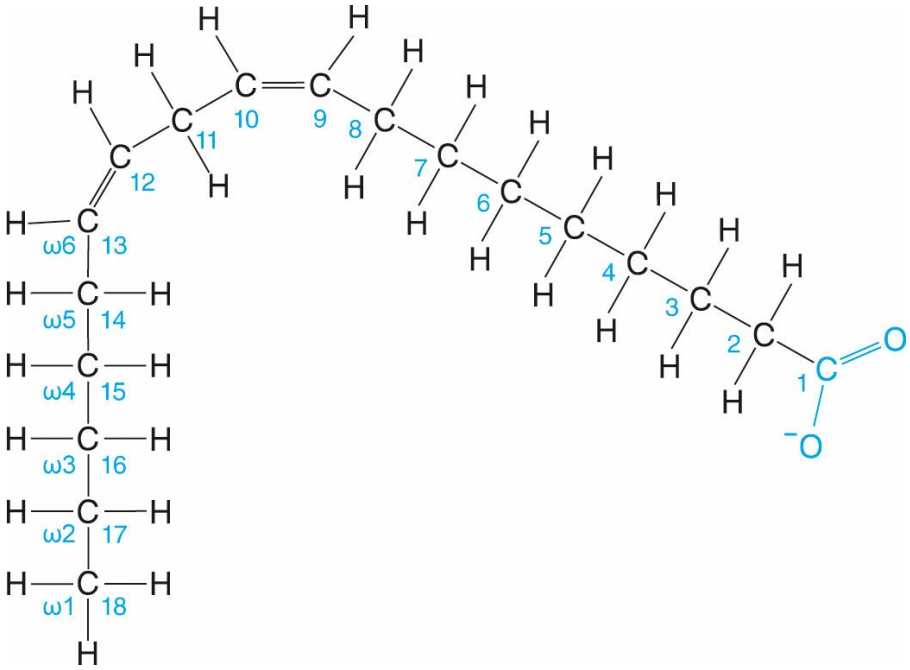


Figure 5.11 **Polyunsaturated fatty acid.** Linoleate is a common polyunsaturated fatty acid with double bonds at positions 9 and 12. One of its possible conformations is shown here. Linoleate is an ω6 fatty acid, since, counting from the methyl (or ω) end, one finds the first double bond after the sixth carbon.

The main utility of fatty acids comes in energy production both at rest and during exercise, as we will discuss in chapters 11 and 14. The concentration of fatty acids in free form is generally low in biological fluids. In contrast, much higher quantities of fatty acids are enclosed in other lipid categories, such as triacylglycerols and phospholipids.

Table 5.2 Common Fatty Acids in Human Tissues

Name	Carbon atoms	Double bonds	Position of double bonds[a]
Laurate	12	0	
Myristate	14	0	
Palmitate	16	0	
Palmitoleate	16	1	9
Stearate	18	0	
Oleate	18	1	9
Linoleate	18	2	9, 12
α-Linolenate	18	3	9, 12, 15
Arachidonate	20	4	5, 8, 11, 14
Eicosapentaenoate	20	5	5, 8, 11, 14, 17

Docosahexaenoate	22	6	4, 7, 10, 13, 16, 19

[a]Numbers indicate the carbon atom after which there is a double bond.

5.8 Triacylglycerols

Triacylglycerols, or **triglycerides**, are the most abundant lipid category. They are the main components of animal (including human) fat, most of which is concentrated in adipose tissue; they are also the main components of vegetable oils. Because of this abundance, they constitute 90% to 95% of dietary fat. In both animals and plants, triacylglycerols serve mainly as energy depots.

A triacylglycerol consists of a glycerol unit and three fatty acid units. **Glycerol** (figure 5.12), also known as *glycerin* or *glycerine*, is a compound of three carbons and three hydroxyl groups, each of which connects with the carboxyl group of a fatty acid to form an **ester linkage**. Thus, every triacylglycerol (figure 5.12) contains three ester linkages, which makes it a **triester**.

Because there is a great variety of available fatty acids, and because any of them can be linked to any of the hydroxyl groups of glycerol, we end up with an even greater variety of triacylglycerols. Chances are that there will be both saturated and unsaturated fatty acids present in a single triacylglycerol. Therefore, it is not accurate to state that a certain food contains only saturated or unsaturated fat. Instead, one should state that a certain food contains *primarily* saturated or unsaturated fatty acids.

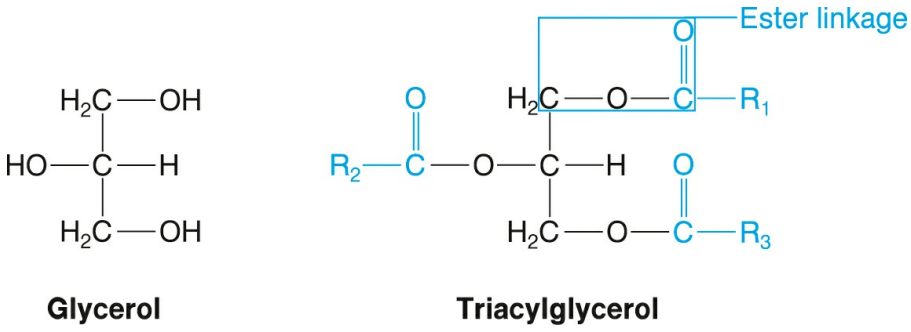


Figure 5.12 Glycerol and triacylglycerol. Triacylglycerols, the largest energy depot in living organisms, are triesters of glycerol and fatty acids. R_1, R_2, and R_3 represent the aliphatic chains of the fatty acids, which usually differ. Acyl groups are shown in color.

Triacylglycerols are hydrophobic, which is evident by the immiscibility of fats or oils with water. Moreover, triacylglycerols have low thermal conductivity, rendering the subcutaneous fat of animals an efficient insulator of their internal organs against cold exposure (figure 5.13).

The part of a fatty acid connected to an oxygen of glycerol in a triacylglycerol is called an **acyl group**. This is where the term *tri-acyl-glycerol* comes from; hence it is more accurate than *triglyceride*. The acyl groups derive from the ion of a fatty acid by removal of O^-, and they bear the name of the fatty acid with the ending *-oyl* in place of *-ate*. Thus, the acyl group of palmitate is called the **palmitoyl group**.

The difference in melting point between saturated and unsaturated fatty acids described in the previous section is reflected in triacylglycerols: The more saturated acyl groups they contain, the higher their melting point is. Triacylglycerols of animal origin have a high content of saturated acyl groups, which is why animal fat is solid at room temperature. Conversely, plant triacylglycerols have a high content of unsaturated acyl groups, which is why vegetable oils are liquid in the same conditions.

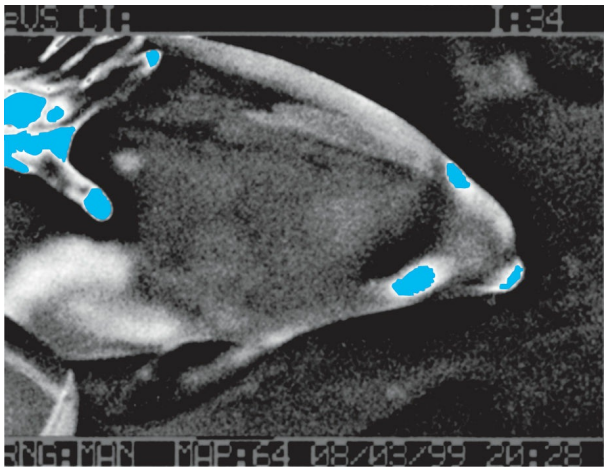


Figure 5.13 **A great insulator.** This infrared photograph of a harbor porpoise, the smallest cetacean of the North Atlantic, reveals what an effective insulator fat is. Body heat is emitted only through the blowhole, snout, and eye (colored dots). Heat is also lost from the nails and a large part of the palm of the person holding the animal.

D. Ann Pabst. University of North Carolina.

5.9 Phospholipids

Phospholipids are a category of lipids presenting a remarkable structural variety. The most common phospholipids have a glycerol backbone (like the one in triacylglycerols) and are called **glycerophospholipids** or **phosphoglycerides**. The simplest glycerophospholipid has a phosphoryl group attached to the terminal hydroxyl group of glycerol and an acyl group attached to each of the remaining groups (figure 5.14). It is called **phosphatidate** and is the parent compound of glycerophospholipids. Phosphatidate—like all glycerophospholipids—is not a single compound but a group of compounds, because its two acyl groups may come from a variety of fatty acids.

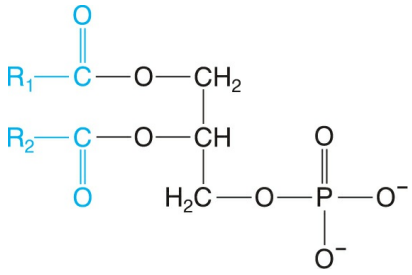


Figure 5.14 Phosphatidate. Glycerophospholipids, the main group of phospholipids, are derivatives of phosphatidate. Acyl groups are shown in color. How does this compound differ from a triacylglycerol?

Phosphatidate is a minor glycerophospholipid of cells and body fluids. The major glycerophospholipids derive from phosphatidate by the attachment of an alcohol to its phosphoryl group. The alcohols most frequently encountered in glycerophospholipids are **choline**, **ethanolamine**, the amino acid serine (considered an alcohol because of the hydroxyl group at its side chain), and **inositol**. These alcohols are presented in the left-hand column of figure 5.15. The corresponding glycerophospholipids are **phosphatidyl choline**, **phosphatidyl ethanolamine**, **phosphatidyl serine**, and **phosphatidyl inositol** (right-hand column of figure 5.15).

Alcohols are organic compounds with hydroxyl groups.

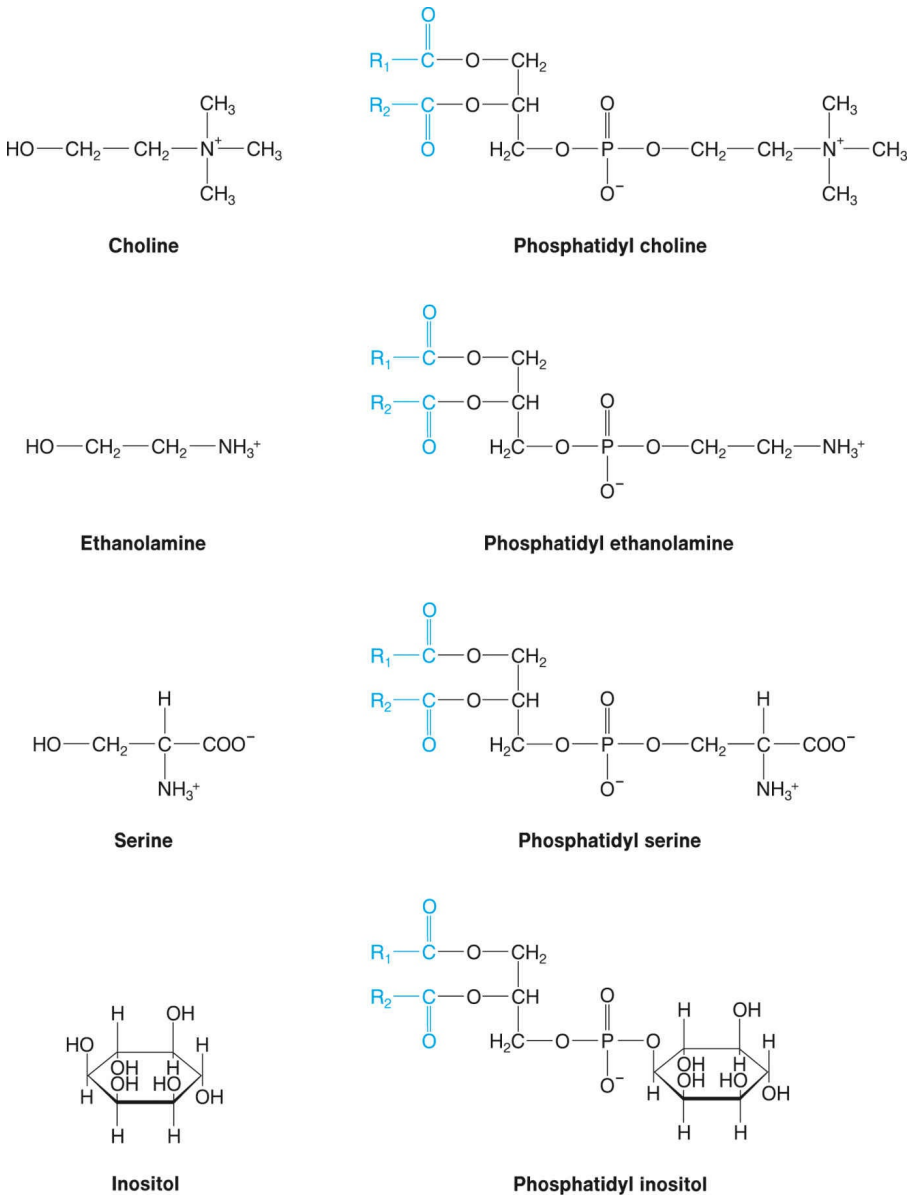


Figure 5.15 **Glycerophospholipids.** The linking of phosphatidate to choline, ethanolamine, serine, or inositol yields the most common glycerophospholipids of animals. Acyl groups are shown in color.

Animal tissues contain small amounts of a phospholipid that does not belong to the group of glycerophospholipids. **Sphingomyelin** (figure 5.16) contains **sphingosine**, an amino alcohol with a long aliphatic chain, rather than glycerol. One acyl group and a phosphoryl choline unit are attached to sphingosine to produce sphingomyelin.

Sphingomyelin's name is a combination of fact and fiction. Fact: It abounds in the myelin sheath, the wrapping around many nerve cells, which I will present in section 7.1. Fiction: When the German physician and chemist Johann Thudichum discovered it in the 1880s, its biological role seemed so enigmatic that he named it after the Sphinx.

Like fatty acids, phospholipids are amphipathic. Their aliphatic chains are hydrophobic, whereas the remainder of their molecules are hydrophilic. The hydrophilic part of a phospholipid is frequently referred to as the **polar head group**. In fact, the size of the polar head group (much larger than the size of the carboxyl group in fatty acids) makes the amphipathic character of phospholipids more balanced than that of fatty acids. This balance renders phospholipids ideal constituents of cell membranes, as we will see in section 5.11. To form membranes is thus the main function of phospholipids.

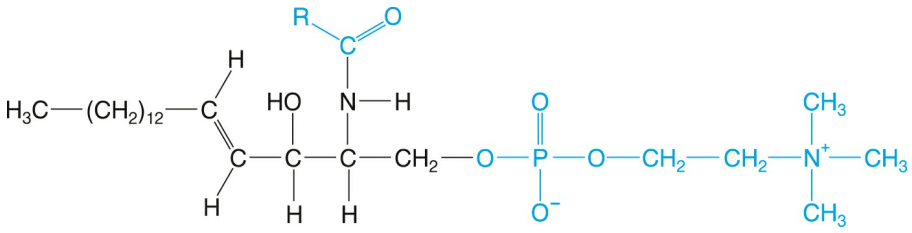


Figure 5.16 **Sphingomyelin.** A minor phospholipid of animals is composed of a sphingosine unit (in black), a single acyl group, a phosphoryl group, and a choline unit.

5.10 Steroids

Steroids are another complex lipid category, encompassing hormones (see section 14.10), a vitamin (see the discussion of vitamin D in section 6.2), and other compounds. All steroids are derivatives of **cholesterol**, a compound containing 27 carbon atoms and 4 rings (figure 5.17). The arrangement of these rings is the "trademark" of steroids. Like phospholipids, cholesterol is amphipathic: Most of its structure is hydrophobic, but the hydroxyl group at its end is hydrophilic; this is also called a *polar head group*. Thanks to its amphipathic character and its size, cholesterol participates in membrane formation, side by side with phospholipids.

If cholesterol is linked to a fatty acid by an ester linkage between the hydroxyl group of the former and the carboxyl group of the latter, a

hydrophobic **cholesterol ester** forms. Cholesterol esters serve as a cholesterol reserve. Cholesterol and its esters are found mainly in animal tissues. Plants contain little of cholesterol or cholesterol esters, though they do have other **sterols**, that is, steroids with hydroxyl groups.

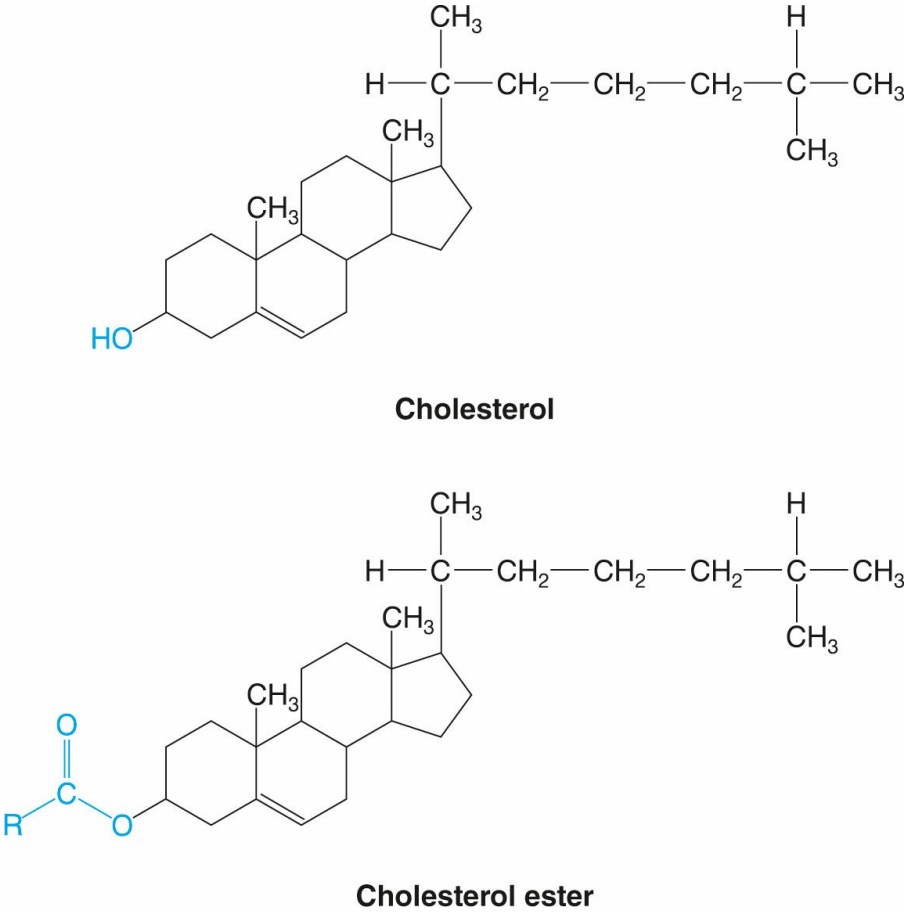


Figure 5.17 Cholesterol and cholesterol ester. Cholesterol is the parent compound of steroids and a component of the membranes of animal cells. The hydroxyl group (in color) is its only hydrophilic part; the rest of the molecule is hydrophobic. A cholesterol ester contains an acyl group (also in color) in place of the hydroxyl group and is entirely hydrophobic.

5.11 Cell Membranes

Cell membranes are wonders of molecular architecture. Remember that they surround cells and intracellular organelles such as the nucleus and mitochondria (section 1.15). Membranes separate aqueous solutions—for example, the cytosol from the extracellular fluid or the cytosol from the

nuclear content. Because they are in touch with water, they need to have some affinity for it. At the same time, however, they need to be insoluble in water or they will vanish. Living organisms overcame this apparent contradiction by evolving membranes that consist of two phospholipid layers placed in such a way that their hydrophobic parts are in contact with each other, being attracted by hydrophobic interactions and excluding water. Their hydrophilic parts, on the other hand, face outward, interacting with water (figure 5.18). This arrangement creates a **bilayer** that is about 5 nm thick.

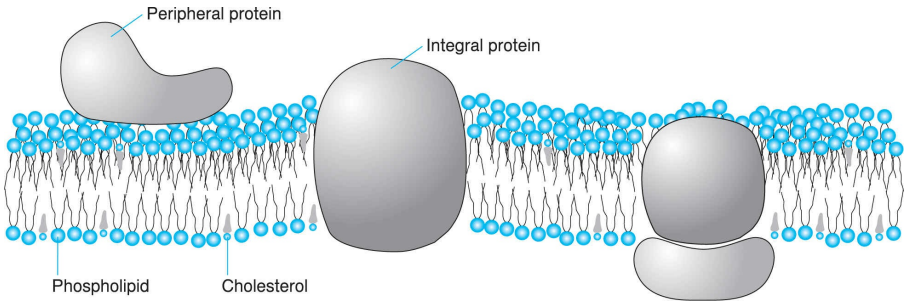


Figure 5.18 **Cell membrane.** Cell membranes are bilayers of phospholipids and cholesterol. The polar head groups of these amphipathic lipids (depicted as colored orbs) face the aqueous environment, whereas their hydrophobic tails (depicted as two waving lines in phospholipids and a wedge in cholesterol) are hidden inside the bilayer. The coherence of a membrane derives primarily from hydrophobic interactions among the tails. Membranes also contain peripheral and integral proteins.

Apart from phospholipids, membranes (especially the plasma membrane of animal cells) contain cholesterol. The cholesterol is placed parallel to the phospholipids so that its small polar head group is aligned with their polar head groups and its hydrophobic body is aligned with their acyl groups and attracted to them by hydrophobic interactions.

Finally, membranes are studded with proteins, which control the communication of the two sides and permit the recognition of cells by biomolecules or other cells. Many of these proteins are bound tightly to a membrane, usually spanning the bilayer and appearing on both faces of it. Such proteins, referred to as **integral**, are held in membranes through hydrophobic interactions of the parts that span the membrane bilayer with the hydrophobic parts of phospholipids and cholesterol.

Other proteins adhere less tightly to membranes by binding to just one

face of them. These proteins, termed **peripheral**, are held on membranes by forming hydrogen and electrostatic bonds with the polar head groups of membrane lipids or with polar residues on the surface of integral proteins. Integral and peripheral proteins may add such bulk to a membrane that its thickness may double.

Summary

Carbohydrates are diverse biomolecules divided into monosaccharides, oligosaccharides, and polysaccharides. Monosaccharides, such as glucose, are joined by glycosidic linkages to form oligosaccharides (with up to 10 monosaccharide residues) and polysaccharides (with more than 10 monosaccharide residues). The best-known oligosaccharide is the disaccharide sucrose (sugar), which consists of a glucose and a fructose residue. The polysaccharides cellulose, starch (a mixture of amylose and amylopectin), and glycogen contain hundreds to thousands of glucose residues in their molecules. Cellulose is the main structural component of plants, whereas starch and glycogen are energy depots in plants and animals, respectively. Glucose residues are joined by β1 → 4 linkages in cellulose and by α1 → 4 and α1 → 6 linkages in starch and glycogen. An alternative classification of carbohydrates, used in the field of nutrition, recognizes two categories—simple and complex—depending on whether they have up to two or more than two monosaccharide residues, respectively. Carbohydrates are hydrophilic.

Lipids comprise a large class of biomolecules, dominated by hydrophobic aliphatic groups. Fatty acids are the simplest lipids and components of other lipid categories. They can be saturated (with no double bonds between their carbon atoms) or unsaturated (containing as many as six double bonds). The most abundant lipid category is that of triacylglycerols, which are the main constituent of adipose tissue and the largest energy depot

in the body. Another lipid category, phospholipids, features an amphipathic (hydrophilic and hydrophobic) character, which makes it suitable for cell membranes. Membranes also contain sterols (such as cholesterol) and proteins. In addition to being a membrane component, cholesterol is the parent compound of steroids.

Problems and Critical Thinking Questions

1. What are the differences between the three natural fibers—cotton, wool, and silk—used in clothing?
2. How does corn become popcorn?
3. What do the three polysaccharides—cellulose, starch, and glycogen—have in common?
4. What is the omega notation of each of the unsaturated fatty acids in table 5.2? That is, are they ω3, ω6, or something else?
5. Shortenings are made from vegetable oils through a chemical process that turns them from being liquid into being solid at room temperature. What might that process be?
6. Rank triacylglycerol, phosphatidyl inositol, sphingomyelin, and cholesterol ester in order of increasing number of acyl groups.
7. Rank glycogen, triacylglycerol, phosphatidate, and cholesterol in order of increasing hydrophobicity.
8. Oil and water do not mix, yet they get along perfectly in mayonnaise, which is made with vegetable oil; vinegar or lemon juice or both; and eggs or egg yolks or both. How does the egg reconcile the oil with the water of vinegar or lemon juice?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

carbohydrate
glycoprotein
glycolipid
monosaccharide
triose
tetrose
pentose
hexose
heptose
glyceraldehyde
dihydroxyacetone
carbonyl group
aldehyde
ketone
furanose
ribofuranose
glucose
fructose
pyranose
glucopyranose
oligosaccharide
glycosidic linkage
disaccharide
maltose
sucrose
lactose
polysaccharide
homopolysaccharide

heteropolysaccharide
cellulose
reducing end
nonreducing end
dietary fiber
starch
amylose
amylopectin
glycogen
glycogenin
simple carbohydrate
complex carbohydrate
lipid
fatty acid
aliphatic
amphipathic
amphiphilic
saturated fatty acid
unsaturated fatty acid
monounsaturated fatty acid
polyunsaturated fatty acid
palmitate
oleate
elaidate
linoleate
triacylglycerol, triglyceride
glycerol
ester linkage
triester
acyl group

palmitoyl group
phospholipid
glycerophospholipid, phosphoglyceride
phosphatidate
choline
ethanolamine
inositol
phosphatidyl choline
phosphatidyl ethanolamine
phosphatidyl serine
phosphatidyl inositol
sphingomyelin
sphingosine
polar head group
steroid
cholesterol
cholesterol ester
sterol
bilayer
integral protein
peripheral protein

References and Suggested Readings

Berg JM, Tymoczko JL, Gatto GJ, Stryer L (2015). *Biochemistry* (Freeman, New York), pp. 315-366.

Fernández-Elías VE, Ortega JF, Nelson RK, Mora-Rodriguez R (2015). Relationship between muscle water and glycogen recovery after prolonged exercise in the heat in humans. *European Journal of Applied Physiology* 115: 1919-1926.

Garrett RH, Grisham CM (2012). *Biochemistry* (Cengage,

Boston), pp. 193-308.

Mathews CK, van Holde KE, Appling DR, Anthony-Cahill SJ (2013). *Biochemistry* (Pearson, New York), pp. 278-357.

Nelson DL, Cox MM (2017). *Lehninger Principles of Biochemistry* (Freeman, New York), pp. 243-280, 357-383.

CHAPTER 6

Vitamins and Minerals

Learning Objectives

After reading this chapter, you should be able to do the following:

- List all vitamins in the human diet.
- Distinguish water-soluble from fat-soluble vitamins.
- Identify the main food sources of each vitamin.
- Explain the major role(s) of each vitamin in human metabolism.
- Describe the possible health consequences of vitamin deficiency.
- List the main minerals in the human diet.
- Identify the main food sources of each mineral.
- Explain the major role(s) of each mineral in human metabolism.
- Describe the potential health consequences of mineral deficiency.

This chapter will complete your acquaintance with biochemistry basics by examining **vitamins** and **minerals**, which complement carbohydrates, fats, and proteins (examined in previous chapters) in forming the quintet of nutrient classes introduced in section 1.14. Vitamins and minerals are not energy providers; thus, they do not serve the first purpose of food mentioned

in section 1.14. However, both are vital—metaphorically and literally—regulators of numerous bodily functions, including energy provision in exercise, thus serving the third purpose of food. Some minerals also contribute to the second utility of food, that of providing raw materials to build tissues. The following sections examine each vitamin and mineral, with a focus on structure, role in metabolism, and consequences of deficiency.

6.1 Water-Soluble Vitamins

Vitamins are a diverse group of organic compounds, which are present in food in minuscule amounts and, as mentioned earlier, participate in metabolic regulation. Their name stems from the Latin word for life (*vita*), with an ending that signifies amines, since many, though not all, vitamins carry amino groups. Most vitamins are not synthesized in the human body, because we lack the genes that encode enzymes catalyzing certain reactions in their biosynthetic pathways.

As you will see when we examine each vitamin separately, a vitamin may be not a single compound but a group of compounds of similar structure and function. These compounds are connected by metabolic reactions, and usually one stands out as possessing the highest biological activity.

Currently, 13 vitamins are recognized universally, and they are divided into two categories: **water soluble** (9) and **fat soluble** (4). The former are examined in this section and the latter in the next. Water-soluble vitamins are polar compounds and are widespread in foods, since most foods contain large amounts of water.

Water-soluble vitamins include the following:

- Vitamin B_1, or thiamine
- Vitamin B_2, or riboflavin
- Niacin
- Vitamin B_6, or pyridoxine
- Vitamin B_{12}, or cobalamin
- Folate
- Pantothenate

- Biotin
- Vitamin C, or ascorbate

The first eight vitamins in the preceding list are said to belong to the **B complex**, a term of historical, rather than factual, bearing: Because they occur together in several foods, they were gradually isolated from what was known originally as vitamin B.

Vitamin B_1

Vitamin B_1, or **thiamine**, can be found in high quantities in legumes, whole-grain cereals, pork, and liver. Thiamine is converted in the body into **thiamine pyrophosphate**, a coenzyme participating in the cleavage of bonds lying next to carbonyl groups. Thiamine pyrophosphate is found in the active sites of several enzymes, two of which—pyruvate dehydrogenase and α-ketoglutarate dehydrogenase—we will encounter in chapter 10. The former participates in carbohydrate catabolism and the latter in the citric acid cycle. Thiamine pyrophosphate is also found in the active site of an additional enzyme, branched-chain α-ketoacid dehydrogenase, which is involved in amino acid metabolism and will be considered in chapter 12.

The nervous system is particularly sensitive to thiamine deficiency, for a reason that I will explain in section 10.10. Symptoms include mental confusion, apathy, depression, limb pain, muscle weakness, and edema (that is, swelling of tissues). Prolonged thiamine deficiency results in **beriberi**, a disease manifested by damage to the nervous system and the heart—damage that may ultimately lead to death.

One distinct cause of beriberi has been the practice of milling and polishing rice to increase its shelf life and make it more palatable. This process, which converts naturally brown rice into white rice, removes the husks, a prime source of thiamine. Human populations whose diet was based on white rice and who consumed little else were prone to beriberi until the connection was discovered in the late 19th century. Then it became possible to prevent and treat the disease by supplementing the diet with inexpensive bran (prepared from the husks of cereal grains).

Vitamin B_2

Vitamin B_2, or **riboflavin**, is present in substantial amounts in liver, meat, eggs, milk, other dairy products, legumes, and dark-green vegetables. Riboflavin is a precursor of FAD and FMN (section 2.5), which serve as coenzymes for numerous enzymes that catalyze redox reactions. We will encounter such enzymes in chapters 10 and 11, when we examine carbohydrate and lipid metabolism. Riboflavin deficiency causes cracks at the corners of the mouth, glossitis (tongue lesions), and dermatitis (skin lesions).

In its pure form, riboflavin is a yellow-to-orange powder, which is responsible for the bright yellow color of the urine of persons who take vitamin supplements containing a lot of vitamin B_2.

Niacin

Food sources of **niacin**, or **nicotinate**, include organ meats, red meat, poultry, fish, nuts, whole-grain cereals, and legumes. Niacin can also be synthesized in our bodies from excess amounts of the amino acid tryptophan. Because niacin is a precursor of NAD and NADP (section 2.5), it is important to cells' ability to carry out redox reactions. Niacin deficiency causes skin rashes, mental confusion, and muscle weakness. Prolonged deficiency leads to **pellagra**, which is characterized by severe dermatitis, diarrhea, and dementia.

Niacin carries the double distinction of being the first vitamin to be synthesized under laboratory conditions simulating the environment of prebiotic Earth (section 4.19) and the only vitamin that has been detected in meteorites that have fallen on Earth. These findings, as pointed out by Danielle Monteverde and coworkers, suggest that some vitamins may have preceded and even facilitated the emergence of life.

Vitamin B_6

Vitamin B_6, or **pyridoxine**, can be found in organ meats, red meat, poultry, fish, egg yolk, pistachios, whole-grain cereals, legumes, bananas, and potatoes. Pyridoxine yields **pyridoxal phosphate**, a coenzyme specializing in the transfer of amino groups between compounds and present in the active sites of enzymes known collectively as aminotransferases. These enzymes are

involved in amino acid metabolism and will be presented in chapter 12. In another, unusual, role, pyridoxal phosphate participates in glycogen breakdown by being present in the active site of phosphorylase, to be discussed in section 10.4.

Pyridoxal phosphate also serves as a coenzyme for enzymes involved in the biosynthesis of compounds that transmit signals in the nervous system. These enzymes are known collectively as **neurotransmitters** and will be dealt with in chapter 7. The neurotransmitters in question are all produced from amino acids; they are **γ-aminobutyrate** (from glutamate), **histamine** (from histidine), **serotonin** (from tryptophan), and **dopamine** (from tyrosine). Two more neurotransmitters are then produced from dopamine in sequence—**norepinephrine** and **epinephrine**, which, together with dopamine, form the group of **catecholamines** (figure 6.1). Finally, pyridoxal phosphate is needed for heme biosynthesis.

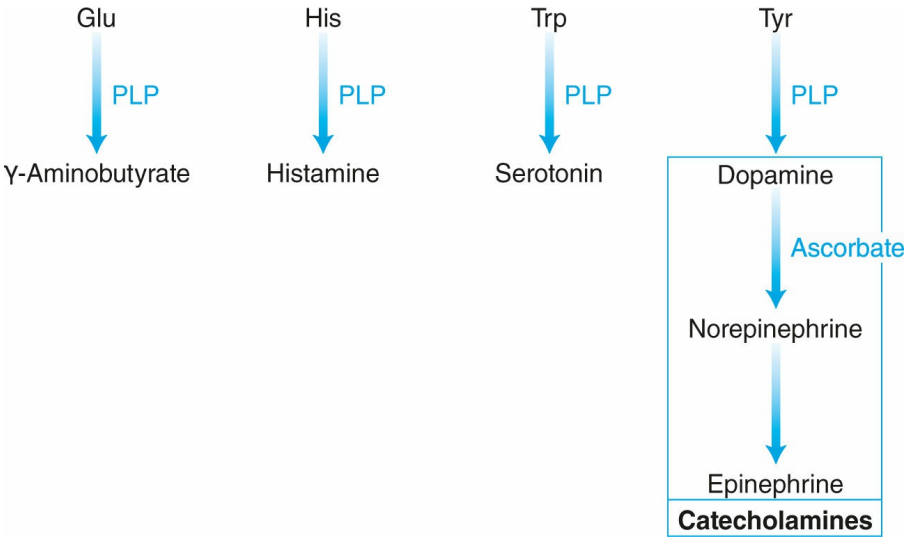


Figure 6.1 A specialty in neurotransmitter biosynthesis. Pyridoxal phosphate (PLP) works as a coenzyme for enzymes that catalyze the biosynthesis of four neurotransmitters from amino acids. One of these neurotransmitters, dopamine, is further converted into norepinephrine and epinephrine with the help of ascorbate.

Vitamin B_6 deficiency is rare. Symptoms include skin disorders, weakness, depression, confusion, and **anemia** due to impaired heme biosynthesis. When not enough heme is available for hemoglobin synthesis and erythrocyte formation, the bone marrow (the spongy tissue inside some

of our bones that produces blood cells) makes erythrocytes that are smaller and have lower hemoglobin concentration than normal. This condition is characterized as **microcytic hypochromic anemia** (meaning anemia with small cells and low color).

Anemia involves a deficiency of erythrocytes and hemoglobin in blood. Symptoms include weakness, low aerobic capacity, and pallor. For more on this condition, see sections 16.1 and 16.7.

Vitamin B_{12}

Vitamin B_{12} has a complex structure resembling heme, with a **cobalt** cation (Co^{3+}), rather than Fe^{2+}, at its center (figure 6.2). The presence of Co^{3+} is responsible for the vitamin's alternative name, **cobalamin**, and makes it the only vitamin that contains a metal.

Cobalamin participates as a metallocoenzyme in two reactions in the human body. One is part of the pathway that processes the few fatty acids with odd numbers of carbon atoms, as we will see in section 11.11. The other is the synthesis of the amino acid methionine from **homocysteine** (a nonprotein amino acid) and a derivative of folate. We will return to this reaction after we meet folate in the next subsection.

Vitamin B_{12} is found only in animal-based foods, such as clams, liver, meat, fish, milk, other dairy products, and egg yolk. When food gets into the stomach, a glycoprotein, known as **intrinsic factor**, binds vitamin B_{12} and escorts it to the small intestine, where the complex of the two is absorbed into blood. Through this process, vitamin B_{12} becomes available to the body.

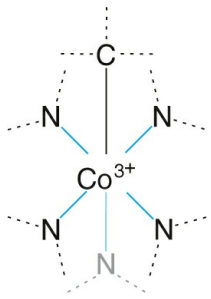


Figure 6.2 **Cobalamin cobalt.** Cobalt sits in the middle of the vitamin B_{12} molecule, forming five coordinate bonds (in color) with nitrogen atoms that belong to a complex organic structure

(not shown in its entirety). One of the coordinate bonds is almost vertical to the plane formed by the other four (which is the plane of the page) and is depicted in faint print to create the illusion that it lies below the plane. Co^{3+} forms a sixth bond above the plane, which links it covalently with the reactive part of the vitamin through carbon.

People who do not produce sufficient amounts of the intrinsic factor suffer nerve damage. One possible cause for this damage, based on the role played by cobalamin in the degradation of odd-number fatty acids, is the accumulation of unusual fatty acids in the nervous tissue. Another possible cause, based on the interplay of cobalamin and folate metabolism, will be explained in the next subsection. Lack of the intrinsic factor also results in a severe form of anemia, **pernicious anemia**, the causes of which will be explained in the next subsection. Both the nerve damage and pernicious anemia are treated with vitamin B_{12} supplements or injections.

Pernicious anemia was so named because most of the people who developed the disease died from it before the discovery that it could be treated with vitamin B_{12}.

Folate

Folate is present in dark-green leafy vegetables, as reflected in its name (*folium* is Latin for “leaf”). Other good sources include yeast, beans, liver, nuts, whole grains, and some fruits, such as papayas and oranges. In the body, folate is reduced to **tetrahydrofolate** by the addition of four H. Tetrahydrofolate specializes in the transfer of one-carbon groups, such as methyl (—CH_3) and methylene (—CH_2—), to a variety of substrates. Thus, tetrahydrofolate participates in the synthesis of adenine, guanine, and, especially, thymine nucleotides, all of which are used in the synthesis of DNA.

The demand for DNA building blocks is high in the bone marrow, where erythrocytes are produced through the division of precursor cells. Folate deficiency limits the availability of nucleotides, which prevents DNA replication and mitosis. As a result, the cells continue to grow and become large, immature, and dysfunctional erythrocytes, called *megaloblasts*. This development results in a type of anemia called **megaloblastic anemia**.

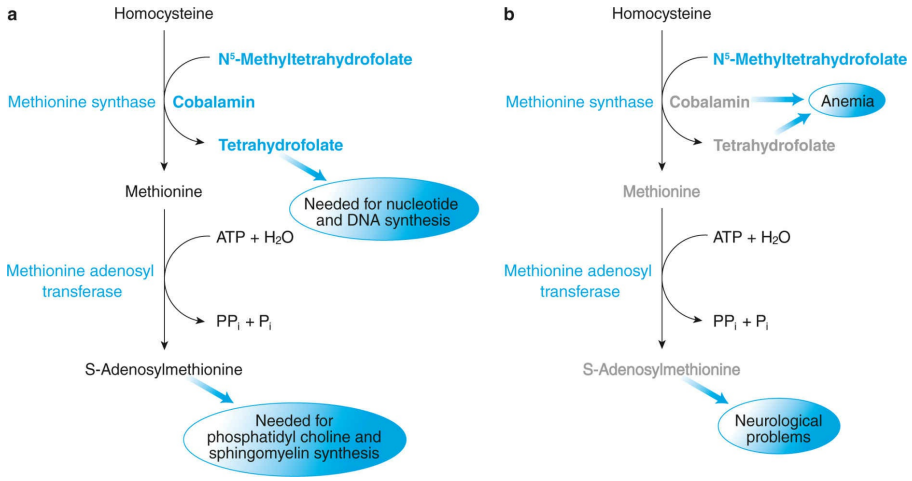


Figure 6.3 **Devastation by vitamin B_{12} and folate deficiency.** *(a)* Vitamins and their derivatives (in bold color) play key roles in the synthesis of methionine from homocysteine: Vitamin B_{12} (cobalamin) is a coenzyme for methionine synthase, and two derivatives of folate (*N* ^{5}-methyltetrahydrofolate and tetrahydrofolate) are substrate and product, respectively, in the reaction. Tetrahydrofolate is needed for nucleotide and DNA synthesis. Methionine then produces *S*-adenosylmethionine, which is needed for the synthesis of phosphatidyl choline and sphingomyelin. *(b)* Folate deficiency limits the availability of tetrahydrofolate (in gray, as are all biomolecules in deficiency) for DNA synthesis in the bone marrow, thus resulting in anemia. Cobalamin deficiency has the same effect, because it prevents *N* ^{5}-methyltetrahydrofolate from replenishing tetrahydrofolate. In addition, cobalamin deficiency limits the availability of methionine and, hence, *S*-adenosylmethionine, thus resulting in neurological problems.

Even with adequate folate present, megaloblastic anemia can still occur if there is a deficiency of vitamin B_{12}, due to the involvement of cobalamin in methionine synthesis. Methionine is produced from homocysteine by the addition of a methyl group (figure 6.3). The methyl donor is ***N*^{5}-methyltetrahydrofolate**, but the methyl group is first transferred to cobalamin, which acts as a coenzyme in the active site of **methionine synthase** to produce **methylcobalamin**. Methylcobalamin then transfers the methyl group to homocysteine. In the absence of cobalamin, tetrahydrofolate is "stuck" with the methyl group and cannot be converted into other forms (such as ***N*^{5},*N*^{10}-methylenetetrahydrofolate**), which are needed for nucleotide and DNA synthesis. Hence, anemia ensues, and it is characterized as either megaloblastic (to indicate the morphology of the erythrocytes) or pernicious (to indicate that it results from vitamin B_{12} deficiency).

Blockade of the reaction catalyzed by methionine synthase because of

vitamin B_{12} deficiency deprives cells not only of tetrahydrofolate but also of methionine. This deprivation carries additional health consequences because methionine reacts with ATP to produce ***S*-adenosylmethionine**, an important methyl donor in a variety of metabolic reactions. These reactions include the ones that convert phosphatidyl ethanolamine into phosphatidyl choline by the sequential addition of three methyl groups to the ethanolamine nitrogen (figure 5.14). Phosphatidyl choline then serves to synthesize sphingomyelin, which also contains choline (figure 5.15). Because sphingomyelin abounds in the myelin sheath (as mentioned in section 5.11), vitamin B_{12} deficiency may create a domino effect ending in abnormal myelin sheath formation and function. This development may be the second cause of the neurological disorders associated with vitamin B_{12} deficiency (following the first purported cause, that is, the accumulation of unusual fatty acids in the nervous tissue, mentioned in the previous subsection).

Folate deficiency during pregnancy is linked to **spina bifida** (Latin for "split spine"), a birth defect characterized by incomplete closing of the backbone around the spinal cord, which causes neurological problems in the newborn. Although it is not known why folate deficiency causes spina bifida, supplementing the mother's diet with this vitamin before and during pregnancy reduces the frequency and severity of this defect.

Pantothenate

Pantothenate abounds in all foods, as reflected in its name (*pantóthen* is Greek for "from everywhere"). Particularly rich sources are liver, red meat, poultry, fish, milk, other dairy products, egg yolk, legumes, and whole grains. Pantothenate is part of the structure of coenzyme A, a key molecule in the metabolism of carbohydrates, lipids, and proteins (figure 2.11). Coenzyme A specializes in the transfer of acyl groups. Thanks to the widespread presence of pantothenate in foods, no deficiency effects have been observed.

Biotin

Good sources of **biotin** are liver, red meat, fish, poultry, eggs, cheese, nuts, and some vegetables. It is also synthesized in considerable quantities by

bacteria in the gut and then absorbed into the body, thus supplementing the biotin contained in the diet. Biotin acts as a coenzyme for enzymes involved in biosynthesis of glucose and fatty acid, as well as degradation of some amino acids. These enzymes are all **carboxylases**: They use biotin in their active sites to add CO_2 to a substrate and create a product with a carboxyl group through a process termed **carboxylation**.

Biotin deficiency is rare. However, it can be caused by high consumption of raw egg whites, which contain **avidin**, a protein that binds biotin with high affinity, thus preventing it from being absorbed through the intestine. The deficiency can cause dermatitis, muscle pain, and depression. Cooking the egg denatures avidin and releases biotin.

Vitamin C

Vitamin C, or **ascorbate**, is found mainly in plant foods. Good sources include peppers, broccoli, citrus fruits, green leaves, potatoes, and tomatoes.

Ascorbate serves as a reducing agent (or antioxidant) for several enzymes. As we will see later, one of these enzymes is involved in the synthesis of collagen, the main protein of our connective tissue in skin, bones, teeth, tendons, ligaments, cartilage, and blood vessels. In addition, ascorbate aids in the absorption of nonheme iron (that is, iron not bound to heme) through the intestine. Ascorbate is also needed for the synthesis of norepinephrine (figure 6.1).

Scurvy has been documented repeatedly in human history, beginning with ancient Egyptians in the 16th century BCE. Hippocrates also described it in the 5th century BCE. Then came vivid, gruesome accounts of it by participants of the Crusades in the 13th century. The disease also plagued European sailors during expeditions conducted between the 15th and 18th centuries. In all cases, the cause was the absence of fresh fruits and vegetables from the diet. The first scientific evidence that scurvy can be treated and prevented through consumption of fruits or vegetables was presented by James Lind, a Scottish surgeon in the Royal Navy, in 1753.

Vitamin C deficiency can be caused either by avoidance of fruits and vegetables or by consumption of fruits and vegetables that are stale or have been overcooked, because prolonged storage or heating decomposes the

vitamin. Ascorbate deficiency causes **scurvy**, which involves decomposition of connective tissue in blood vessels, skin, gums, tendons, and cartilage. Scurvy is manifested by hemorrhages under the skin, falling gums, slow healing of wounds, weakness, and heart failure.

Why does vitamin C deficiency cause scurvy? Ascorbate is needed for the action of the enzyme **prolyl 4-hydroxylase**, which catalyzes the **hydroxylation** (that is, addition of a hydroxyl group) of proline residues in collagen. The product of this reaction, **4-hydroxyproline** (figure 6.4), enables collagen to assume its normal structure. Without it, collagen forms weak fibers, which are responsible for the fragility of skin and blood vessels seen in scurvy.

Formation of 4-hydroxyproline in collagen is a case of **post-translational modification**, so named because it occurs after the protein has been synthesized in the ribosomes by translation. Look out for more post-translational modifications in this and other chapters.

6.2 Fat-Soluble Vitamins

Fat-soluble vitamins are nonpolar and do not dissolve readily in water. However, they are contained in dietary fat, which is composed primarily of hydrophobic triacylglycerols, as we saw in section 5.8.

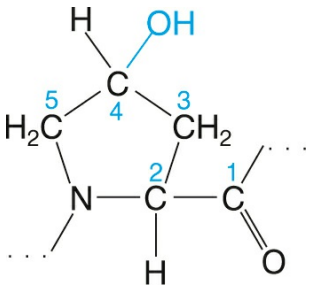


Figure 6.4 Hydroxyproline residue in a protein. This amino acid residue accounts for about 10% of the residues in collagen and needs vitamin C to be produced from proline (compare with figure 3.2) by means of hydroxylation (color). Without vitamin C, collagen is poor in 4-hydroxyproline and forms abnormal fibers, resulting in scurvy.

Fat-soluble vitamins include the following:

- Vitamin A, or retinol

- Vitamin D, or cholecalciferol
- Vitamin E, or α-tocopherol
- Vitamin K, or phylloquinone

Vitamin A

Vitamin A is present in foods in two distinct forms: **retinol** and **carotenoids**. Retinol can be found, in high amounts, in animal-based foods such as liver, butter, cheese, fortified milk, egg yolk, and fish liver oil. Carotenoids, represented by **β-carotene**, abound in plant foods such as carrots (which owe their very color to β-carotene), dark-green leafy vegetables, and oranges.

In the intestine, carotenoids can be converted initially into **retinal** (an aldehyde), which is then converted into retinol (an alcohol). The latter reaction (but not the former) is reversible (figure 6.5). Thus, retinal can be produced from both carotenoids and retinol, although the contribution of the latter is higher because of the poor absorption of carotenoids in the intestine.

Retinal serves as the prosthetic group of proteins located in the retinas of our eyes, which respond to light by initiating a nervous response that becomes vision in the brain (see section 7.6). A derivative of retinal, **retinoic acid**, turns on the transcription of genes that encode proteins essential for growth, differentiation, and development.

The terms *growth*, *differentiation*, and *development* carry distinct meanings in biology. Growth involves increase in size (of a cell, tissue, or organism), whereas differentiation involves a change in cell type; this is the process, for example, through which the initially identical cells of an embryo change as they multiply and become skin, muscle, bone, and organs. Finally, development integrates growth, differentiation, and other processes (such as organization and connection of different parts of the body) into a whole that reflects the qualitative change of an organism with age.

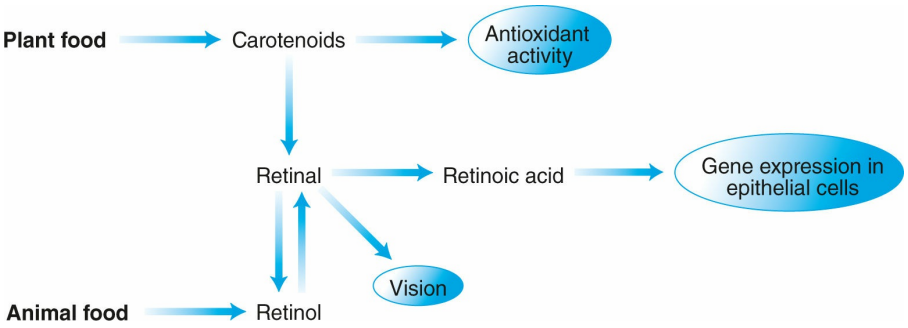


Figure 6.5 **Vitamin A metabolism and function.** Vitamin A can be obtained from plant and animal food sources in two forms—carotenoids and retinol, respectively. In the intestine, carotenoids are converted into retinol through retinal. Retinol is also converted into retinal, which is the light-sensing compound in the eyes. Furthermore, retinal is converted into retinoic acid, which regulates gene expression in epithelial cells. Carotenoids possess the capacity for antioxidant activity.

Thus, vitamin A is necessary for vision and for the development and maintenance of several tissues, especially the epithelial ones (those covering the body surface and lining body cavities). A final utility of vitamin A is that β-carotene acts as an antioxidant.

Vitamin A deficiency causes **nyctalopia**, or night blindness—that is, inability to see in dim light. Severe vitamin A deficiency results in **xerophthalmia** (the term means "dry eyes" in Greek). In this condition, the eyes are incapable of producing tears, which results in dryness of the conjunctiva and cornea. The cornea develops ulcers, ending in blindness. Vitamin A deficiency also retards development and causes problems in other epithelial tissues (for example, skin lesions).

Vitamin D

Vitamin D comes in two main forms: **ergocalciferol**, or **vitamin D_2**, and **cholecalciferol**, or **vitamin D_3**. Ergocalciferol is found naturally in mushrooms; it is also added to milk, margarine, and breakfast cereals. Cholecalciferol, on the other hand, is present in egg yolk and in oily fish (such as salmon, mackerel, and sardines). However, food normally provides only about one tenth of our vitamin D. The remaining nine tenths are synthesized in our bodies as cholecalciferol, provided that we are reasonably exposed to sunlight. Thus, vitamin D is one of the few vitamins that we can

synthesize and the only one of which we synthesize more than we eat.

Let's examine the remarkable process of vitamin D metabolism with the aid of figure 6.6. Biosynthesis of cholecalciferol begins when cholesterol loses two H to become 7-dehydrocholesterol, or provitamin D_3. In the skin, the energy of the sun's ultraviolet radiation converts provitamin D_3 into previtamin D_3 (notice the difference in just one letter), which isomerizes to vitamin D_3.

Like other vitamins, vitamin D itself (in either D_2 or D_3 form) is not biologically active but requires some conversion. It is initially transported by the bloodstream from the intestine or skin to the liver, where it receives a hydroxyl group to become 25-hydroxyvitamin D. This substance then is transported to the kidneys, where another hydroxyl group is added to yield 1,25-dihydroxyvitamin D.

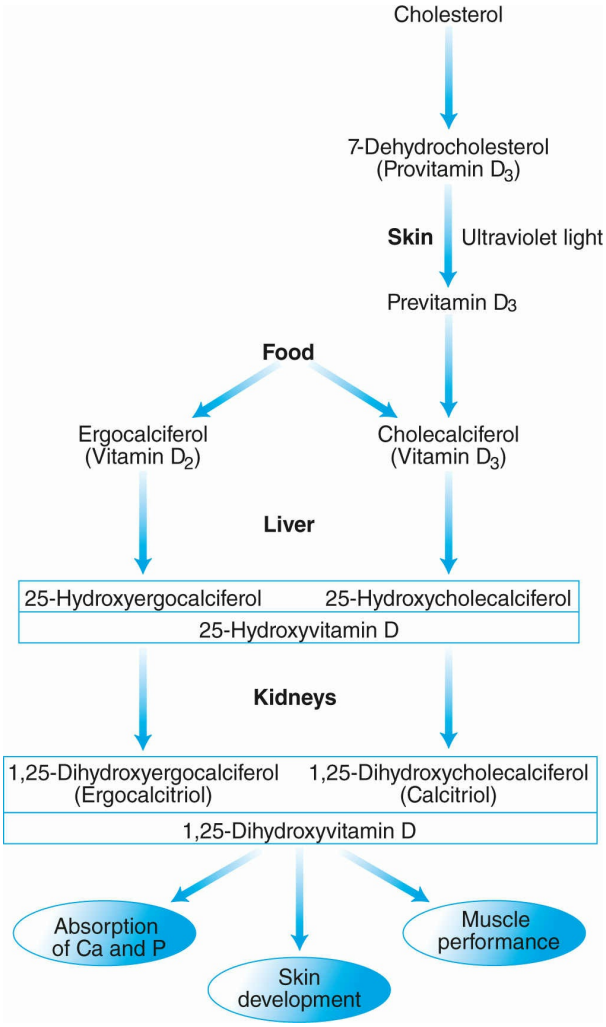


Figure 6.6 Vitamin D metabolism and function. The main source of vitamin D in the human body is synthesis from cholesterol in the skin with energy from the sun's ultraviolet light. We also obtain a small amount of vitamin D in two forms (D_2 and D_3) from food. These forms then undergo two hydroxylations, one in the liver and the other in the kidneys, to produce the biologically active 1,25-dihydroxyvitamin D, which is important for the absorption of calcium and phosphorus from the intestine and kidneys; skin development; and, possibly, muscle function and performance.

1,25-Dihydroxyvitamin D acts as a hormone to increase the transcription of genes encoding transport proteins that control the absorption of calcium from the intestine and kidneys, as reviewed by Sylvia Christakos and colleagues. In this way, it helps the body to maintain adequate calcium in plasma and the bones. In doing so, it collaborates with other hormones, particularly parathormone, which is secreted by the parathyroid gland. 1,25-Dihydroxyvitamin D also regulates phosphate levels in the body, mainly by enhancing the expression of a gene that encodes a phosphate-transporting protein in the intestine. Thus, adequacy of vitamin D is crucial for bone health. Vitamin D also aids in the development of the skin and may play a role in muscle function and performance. Vitamin D deficiency causes **rickets** in children and **osteomalacia** in adults.

Rickets is characterized by softening and weakening of bones in childhood, which can lead to bone deformities. In adults, the same condition is known as *osteomalacia*.

Vitamin E

Vitamin E abounds in a variety of foods, particularly vegetable oils, nuts, whole-grain products, egg yolk, and dark-green leafy vegetables. The active form, **α-tocopherol**, serves as an antioxidant protecting the polyunsaturated fatty acids in the phospholipids of cell membranes against oxidation and damage by radicals such as the hydroxyl radical (section 1.5). The hydrophobic nature of α-tocopherol enables it to insert itself into the lipid bilayer of a membrane, exactly where it is needed. Vitamin E deficiency, though rare because of the vitamin's widespread presence in foods, may result in anemia due to oxidative damage of the erythrocyte membranes and subsequent erythrocyte destruction. This condition is known as **hemolytic**

anemia (hemolysis is the process of erythrocyte rupture).

The term *tocopherol* derives from a combination of Greek words that, freely translated, mean “alcohol that brings birth.” The term was coined in 1936 after the observation that vitamin E was necessary in order for female rats to be fertile. This finding is often extrapolated to humans; however, there is no evidence that vitamin E deficiency causes infertility in women.

Vitamin K

Vitamin K is present in the diet mainly as **phylloquinone**. Good sources of it include green vegetables and vegetable oils. A related form, **menaquinone**, is found in meat and cheese, but in much lower quantities. Menaquinone is also synthesized by gut bacteria from phylloquinone.

Vitamin K is required in the production of the mature and functional form of proteins that mediate blood coagulation or regulate bone metabolism. It does so by serving as a coenzyme for **γ-glutamyl carboxylase**, an enzyme that catalyzes the post-translational carboxylation of many glutamate residues in these proteins. This modification produces **γ-carboxyglutamate** residues (figure 6.7). Because glutamate already possesses a carboxyl group (see figure 3.2), γ-carboxyglutamate has two carboxyl groups as close as they can get (attached to the same carbon).

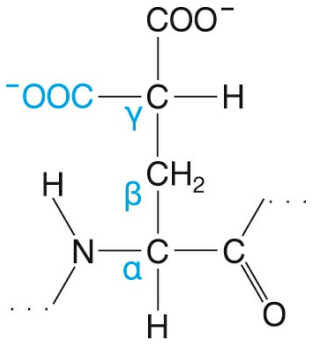


Figure 6.7 **γ-Carboxyglutamate residue in a protein.** This unusual amino acid residue is formed in some proteins by the addition of a carboxyl group (in color) to the γ carbon (according to the old numbering explained in section 3.1) of glutamate (compare with figure 3.2). Vitamin K is essential for this modification, which, in turn, is essential for proper functioning of the proteins, especially those involved in blood clotting.

Why is the conversion of glutamate into γ-carboxyglutamate important? Its two negatively charged carboxyl groups can attract calcium ions (Ca^{2+}) through coordinate bonds more strongly than the single carboxyl group of glutamate can. This binding of Ca^{2+} enables proteins involved in blood clotting to attach to phospholipids in cell membranes and initiate a cascade of events that leads to clot formation. For this reason, vitamin K deficiency causes hemorrhage. We have yet to clarify the role of Ca^{2+} binding by γ-carboxyglutamate in proteins regulating bone metabolism.

The very letter *K* is indicative of the vitamin's biological function in humans and other animals: It stems from *Koagulationsvitamin,* meaning "coagulation vitamin" in German, the language in which its discovery was first published.

6.3 Metal Minerals

The nutrient class of minerals includes elements that are considered essential in the human body. On the basis of their chemical character, they are divided into metals and nonmetals. The former are more numerous, as they are in nature overall.

Metal minerals in the human diet include the following:

- Sodium
- Potassium
- Calcium
- Magnesium
- Iron
- Copper
- Zinc
- Chromium
- Manganese
- Molybdenum

Sodium

Sodium is found in almost all foods as Na^+. Its simplest and richest source is table salt, which is practically pure sodium chloride (NaCl). Natural foods contain much less sodium than processed ones (such as canned food and cured meat products), because considerable quantities of salt are usually added to the latter during processing.

Na^+ is the main cation in extracellular fluids, such as the interstitial fluid (that is, the fluid surrounding tissue cells) and plasma. Na^+ is often called an **electrolyte**, a term that actually applies to all ions because they all conduct electricity when in solution. Na^+ is involved in maintaining the water content of the various compartments in the body and in maintaining blood pressure. It is also essential for the electrical transmission of nerve signals and for muscle activity (see part II). For this reason, sodium deficiency, though rare, is manifested by disturbances in neural function and by muscle cramps. There is also evidence that sodium deficiency causes hypertension, much as excessive sodium intake does.

A short-term sodium deficiency may appear during prolonged exercise as **hyponatremia** (meaning “low blood sodium” in Greek). This condition results from a combination of sodium loss in the sweat and excessive intake of fluids that contain no or little sodium. The resulting drop in the plasma sodium concentration may cause nausea, vomiting, headache, fatigue, and muscle cramps. Depending on the severity of the condition, treatment consists of stopping fluid intake (in mild cases), drinking salty fluids (in cases of moderate severity), or administering an NaCl solution intravenously (in severe cases).

Potassium

Potassium is present in most foods as K^+. Good sources include potatoes, bananas, citrus fruits, several vegetables, liver, red meat, poultry, fish, milk, and other dairy products. K^+ is the main electrolyte in the cytosol and acts together with Na^+ to maintain the balance of fluids and to allow the electrical excitation of nerve and muscle cells. Potassium deficiency, which is rare, causes muscle weakness and cardiac arrhythmia.

Calcium

Good sources of **calcium** include dairy products, sardines, dark-green leafy vegetables, legumes, and nuts. Calcium is a component of bones and teeth. Its cationic form, Ca^{2+}, regulates a multitude of biological processes, such as neural transmission, muscle activity, glycogen breakdown, and gene expression—all of which we will consider later (in chapters 7, 8, 10, and 13, respectively). Additionally, Ca^{2+} is involved in blood clotting, as mentioned in section 6.2 under vitamin K. Calcium deficiency causes rickets in children and **osteoporosis** in adults.

> Osteoporosis involves the loss of bone mass resulting in thin and weak bones that are susceptible to fractures. See section 15.11 for more.

Magnesium

Magnesium abounds in nuts, legumes, whole-grain products, dark-green leafy vegetables, and seafood. Mg^{2+} is located mainly in the bones and muscle. According to current bioinformatics databases (such as ExPASy, www.expasy.org, and MetaCyc, www.metacyc.org), magnesium serves as a cofactor for about two hundred characterized enzymes. Prominent among them are enzymes that use ATP as substrate (see section 8.5) or use nucleotides to synthesize DNA and RNA, such as DNA polymerase (section 4.7) and RNA polymerase (section 4.10). Magnesium deficiency is manifested by muscle weakness, muscle tremor, muscle cramps, and cardiac arrhythmia. For a detailed discussion of magnesium function in health and disease, see the review by Jeroen de Baaij and associates.

Iron

Main sources of **iron** in the diet include liver, red meat, poultry, seafood, legumes, nuts, and dark-green leafy vegetables. Iron is present in such foods in the form of two cations, ferrous (Fe^{2+}) and ferric (Fe^{3+}), both introduced in section 3.10. The former is absorbed readily through the gut, whereas the latter must be reduced to the former for absorption, as reviewed by James Collins and coworkers. However, once released to blood, Fe^{2+} is oxidized back to Fe^{3+} for transport to the tissues.

Only a minor part of dietary iron is taken up through the intestine. The key regulator of iron homeostasis in the body is **hepcidin**, a peptide synthesized in the liver that acts in a negative manner. That is, when there is an abundance of iron, the liver secretes more hepcidin, which curbs iron absorption from the gut. Conversely, when there is a lack of iron, the liver secretes less hepcidin, resulting in increased iron absorption.

In our bodies, iron is embedded in proteins that handle oxygen, such as myoglobin (section 3.10), hemoglobin (section 3.11), and proteins of the electron-transport chain in the mitochondria (to be discussed in section 10.14). The electron-transport chain involves redox reactions (section 2.6), in which iron takes part by alternating between the Fe^{2+} and Fe^{3+} states. Thus, iron is essential for the uptake, transport, storage, and utilization of O_2 for ATP production. In fact, iron is so important for health and sport performance that I devote the entirety of chapter 16 to it.

Iron deficiency is one of the most common dietary deficiencies, especially in women who lose substantial quantities of blood during menstruation. If left untreated, iron deficiency evolves into microcytic hypochromic anemia, as described earlier in the discussion of vitamin B_6.

Copper

Copper (Cu) is found in liver, shellfish, legumes, nuts, seeds, potatoes, and whole-grain products in either the cuprous (Cu^+) or cupric state (Cu^{2+}). As with iron, the latter must be reduced to the former for absorption. Copper cooperates with iron in the utilization of O_2 by serving as a cofactor for cytochrome *c* oxidase, a component of the electron-transport chain that we will meet in section 10.14. Copper alternates between the Cu^{2+} and Cu^+ states in the active site of the enzyme as it accepts and donates electrons. Copper is also needed for iron absorption through the gut. No effects of copper deficiency have been observed.

Zinc

Food sources of **zinc** (Zn) include oysters, liver, red meat, poultry, cheese, egg yolk, legumes, nuts, and whole-grain products. The zinc cation (Zn^{2+}) sits

in the active sites of about 200 enzymes. One of them is **carbonic anhydrase**, which catalyzes the reactions that help us dispose of most of the CO_2 produced by the burning of foodstuffs (section 3.12 and equation 3.3). An even larger class of zinc-containing proteins binds to DNA though structures called **zinc fingers**. Zn^{2+} in these proteins (many of which are transcription factors; section 4.11) is essential in shaping short stretches of amino acid residues in the form of fingers that touch the DNA double helix. Thus, zinc plays an important role in gene expression. Zinc deficiency depresses growth, immunity, and appetite.

Chromium

Chromium (Cr) is present in broccoli, processed meat, whole-grain products, nuts, potatoes, and certain fruits (for example, grapes). The function of chromium in the body remains uncertain. Its predominant form (Cr^{3+}) is believed to reinforce the action of the hormone insulin, although it is not known how this reinforcement happens. Scientific information is lacking in regard to the consequences of chromium deficiency.

Manganese

Manganese can be found in whole-grain products, beans, nuts, leafy vegetables, and teas. The Mn^{2+} cation is a cofactor of more than 100 enzymes involved in metabolism of carbohydrates, lipids, and amino acids, as well as antioxidant protection (for example, mitochondrial superoxide dismutase; figure 3.11). Signs of manganese deficiency have not been observed in the general population.

Molybdenum

Food sources of **molybdenum** (Mo) include liver, whole-grain products, legumes, and nuts. The Mo^{6+} cation is part of a complex metallocoenzyme termed the *molybdenum cofactor*, which can be found in enzymes involved in the oxidation of some amino acids and purines. Effects of molybdenum deficiency have not been reported.

6.4 Nonmetal Minerals

Nonmetal minerals in the human diet include the following:

- Chlorine
- Phosphorus
- Selenium
- Fluorine
- Iodine

Chlorine

Chlorine is present in almost all foods, much like sodium, with which it coexists in table salt. Chloride (Cl^-) is the main anion in extracellular fluids, where it collaborates with Na^+ in the maintenance of fluid balance and controls the balance of electrical charges across cell membranes. Chloride is also used in the production of hydrochloric acid in the stomach at a concentration high enough to reduce the pH of the gastric juice (the fluid secreted by glands in the stomach wall) to a range of 1.5 to 3.5. This acidic environment serves as a bacteriocide; denatures and unfolds proteins, thus making them more susceptible to digestion; and activates digestive enzymes, as we will see in section 12.1. Symptoms of chlorine deficiency are rarely seen; they include convulsions.

Phosphorus

Phosphorus is pervasive. Its best dietary sources include liver, red meat, poultry, seafood, egg yolk, dairy products, nuts, legumes, and grain products. Most of the phosphorus in the body is present as phosphate (PO_4^{3-}) in the bones and teeth, in conjunction with Ca^{2+}. In addition, hydrogen phosphate (HPO_4^{2-}, introduced in section 1.13 and referred to as P_i thereafter) is abundant in biological fluids; phosphoryl groups are covalently attached to a vast array of organic biological compounds, some of which we have encountered in previous chapters (nucleotides, DNA, RNA, and phospholipids); and others will be introduced in part III (phosphocreatine,

phosphoproteins, and sugar phosphates). Thanks to the widespread presence of phosphorus in foods, phosphorus deficiency is rare. When it does occur, its symptoms are similar to those of calcium deficiency.

Selenium

Food sources of **selenium** (Se) include Brazil nuts, organ meats, seafood, red meat, and poultry. In the human body, Se serves as part of some antioxidant enzymes, as well as enzymes involved in the metabolism of thyroid hormones (see the section on iodine later in this chapter). These proteins, termed **selenoproteins**, contain **selenocysteine** (abbreviated as *Sec*), a rare amino acid resembling cysteine except that it contains Se in place of S (figure 6.8). What is amazing about Sec is that it is not produced from post-translational modification of any amino acid residue (as in the case of 4-hydroxyproline and γ-carboxyglutamate); rather, it is incorporated in the protein during translation in the ribosomes.

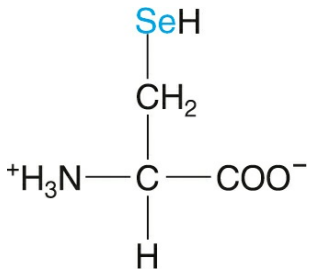


Figure 6.8 **Selenocysteine.** This rare amino acid, differing from cysteine in that it has Se (in color) in place of S (compare with figure 3.2), defies the genetic code by being encoded by a termination codon during the synthesis of some proteins.

How can this happen if the genetic code contains no codon corresponding to Sec? The answer is that Sec is encoded by UGA, which is normally a termination codon (figure 4.26). But how are the ribosomes "tricked" into inserting Sec where they should just terminate translation? This feat is achieved by a particular mRNA sequence of about 60 bases, named the SECIS (Secinsertion sequence) element, which directs the ribosomes to translate UGA as Sec. The SECIS element is present in the 3' untranslated region of the mRNA encoding a selenoprotein and assumes a characteristic

secondary structure that is critical for its function. This secondary structure results from intramolecular base pairing (as in figure 4.19). As if these oddities were not enough, the SECIS element also features an unorthodox base pair, A–G.

Selenium deficiency may contribute to a cardiac condition known as **Keshan disease**, which is characterized by enlargement and insufficiency of the heart in children and young women. It appears frequently in parts of China where people eat primarily foods deriving from plants grown locally in soil that is poor in Se. Another condition that is endemic among children in China, as well as in North Korea and southeastern Siberia, is **Kashin-Beck disease**, which is characterized by **osteoarthritis** (degeneration of the cartilage between joints) and results in joint deformities and dwarfism.

Fluorine

Fluorine occurs in living organisms as the fluoride anion (F^-). Its main dietary source in many parts of the world is fluoridated drinking water. Other sources include tea and seafood. Fluoridated dental products (such as toothpaste and mouthwash) also contribute to adequate fluoride intake.

Fluoride is incorporated into bones and teeth, both of which it hardens. In teeth, fluoride increases their resistance against erosion by acids produced in the mouth, thus helping to prevent **dental caries**. Fluorine deficiency increases the risk for dental caries.

Dental caries involves tooth decay caused by bacteria in dental plaque. The bacteria catabolize carbohydrates (which remain in the mouth after eating) into organic acids such as lactic acid. The resulting acidic environment causes the tooth enamel to dissolve. This effect allows the bacteria to penetrate deeper into the tooth, where they cause cavities and expose the soft pulp tissue at the tooth center. These conditions can elicit intense pain, local or even systemic infection, and tooth loss.

Iodine

Iodine (I) is found in the form of the iodide anion (I^-) in iodized salt, seafood, dairy products, potatoes, grains, poultry, and eggs. Iodine is part of the structure of two thyroid hormones, **triiodothyronine** and **thyroxine**, known

by the abbreviations **T_3** and **T_4**, respectively, in which *3* and *4* indicate the numbers of iodine atoms in their molecules. Thyroid hormones increase the expression of genes that encode key enzymes in catabolism. Hence, thyroid hormones regulate energy expenditure at rest. They also regulate growth and development.

Iodine deficiency initially causes **goiter**, which involves swelling of the thyroid gland and may cause anatomical problems in the neck. More severe iodine deficiency results in **hypothyroidism**, that is, impaired synthesis of T_3 and T_4. This condition slows down growth and development and exerts a particularly detrimental effect on the developing brain, resulting in mental retardation.

6.5 Elements in the Human Body

We have reached the end of part I. Before embarking on the next parts, it would be useful to sum up the elements we have considered as constituents of the human body. This summary is provided in table 6.1, which serves as an extension of table 1.1 (without its last column). You may use table 6.1 as a reference each time we encounter an element in the ensuing parts of the book.

In addition to the 21 elements included in table 6.1, boron (B), silicon (Si), vanadium (V), nickel (Ni), and arsenic (As) have been proposed as components of the human body. At present, however, no biological function is known for any of them; thus it is possible that their presence in the body is the mere result of consuming foods that contain them.

Table 6.1 Elements in the Human Body

Name	Symbol	Atomic number	Atomic mass (Da)
Hydrogen	H	1	1
Carbon	C	6	12
Nitrogen	N	7	14
Oxygen	O	8	16
Fluorine	F	9	19
Sodium	Na	11	23
Magnesium	Mg	12	24
Phosphorus	P	15	31
Sulfur	S	16	32

Chlorine	Cl	17	35.5
Potassium	K	19	39
Calcium	Ca	20	40
Chromium	Cr	24	52
Manganese	Mn	25	55
Iron	Fe	26	56
Cobalt	Co	27	59
Copper	Cu	29	63.5
Zinc	Zn	30	65
Selenium	Se	34	79
Molybdenum	Mo	42	96
Iodine	I	53	127

Summary

Vitamins serve as regulators of numerous processes in the body, although they provide neither energy nor raw materials with which to build tissues. On the basis of their solubility in water, they are divided into nine water-soluble vitamins and four fat-soluble ones. The water-soluble vitamins—vitamin B_1, vitamin B_2, niacin, vitamin B_6, vitamin B_{12}, folate, pantothenate, biotin, and vitamin C—are converted into coenzymes, are present unchanged in the active sites of enzymes, or are involved in some other way in the metabolism of one or more of the four major classes of biomolecules (carbohydrates, lipids, proteins, and nucleic acids). The fat-soluble vitamins—that is, A, D, E, and K—are required for other specialized functions, such as vision, antioxidant protection, mineral absorption, and blood clotting. Most vitamins are not synthesized in the human body and therefore need to be consumed in adequate amounts. Vitamin deficiency results in health problems of variable severity, affecting the nervous system, muscles, heart, connective tissue, eyes, bones, and blood. If left untreated, some of these problems may prove fatal.

Minerals serve as regulators of bodily functions and as raw materials with which to build tissues; like vitamins, they provide

no energy. Consensus holds that ten metals and five nonmetals are essential in the human body. The metals—sodium, potassium, calcium, magnesium, iron, copper, zinc, chromium, manganese, and molybdenum—carry out a variety of functions, such as maintaining the body's water content, transmitting nerve signals, forming bones and teeth, serving as cofactors of enzymes, and regulating gene expression. The nonmetals—chlorine, phosphorus, selenium, fluorine, and iodine—are involved in fluid balance, digestion, bone and tooth formation, antioxidant defense, growth, and development. Mineral deficiency may cause neurological, muscular, and cardiac problems, as well as weak bones, anemia, and impaired growth and development.

Problems and Critical Thinking Questions

1. Which vitamins can be synthesized in the human body, and how?
2. The adequacy of which vitamins may be at risk in a person who does not consume fruits and vegetables?
3. The adequacy of which vitamins may be at risk in a strict vegetarian?
4. Which vitamin is involved in each of the following biological processes? Draw a line between each vitamin and its associated process.

Vitamin	**Process**
vitamin B_1	a. breakdown of odd-number fatty acids
vitamin B_2	b. synthesis of FAD
niacin	c. antioxidant protection of polyunsaturated fatty acids
vitamin B_6	d. fatty acid synthesis
vitamin B_{12}	e. calcium absorption

pantothenate	f. synthesis of nucleotides
folate	g. carbohydrate catabolism
biotin	h. synthesis of coenzyme A
vitamin C	i. collagen synthesis
vitamin A	j. glycogen degradation
vitamin D	k. synthesis of NAD
vitamin E	l. blood clotting
vitamin K	m. vision

5. Fill in the following table to show which unusual amino acid residues are synthesized in which proteins with the aid of which vitamins.

Amino acid residue	Proteins	Vitamin

6. Which mineral corresponds to each of the following biological processes and characteristics? Draw a line between each mineral and its associated process or characteristic.

Mineral	**Process or characteristic**
sodium	a. component of thyroid hormones
potassium	b. regulation of transcription
calcium	c. metabolism of thyroid hormones
magnesium	d. cofactor for enzymes acting on ATP
iron	e. excitation of nerve and muscle cells; major extracellular cation

copper	f. oxygen utilization
zinc	g. component of nucleic acids
manganese	h. oxygen uptake, transport, stor-age, and utilization
molybdenum	i. maintenance of fluid balance; major extracellular anion
chlorine	j. excitation of nerve and muscle cells; major intracellular cation
phosphorus	k. hardening of the bones and teeth
selenium	l. oxidation of amino acids and purines
fluorine	m. cofactor of mitochondrial super oxide dismutase
iodine	n. regulation of glycogen break down

7. A deficiency of which vitamins and minerals can cause anemia? Characterize the types of anemia.
8. Which vitamins and minerals are involved in redox reactions, and how?
9. Which vitamins and minerals are needed for bone and tooth formation?
10. Deficiency of which vitamin or mineral may cause each of the following health problems? Draw a line from each vitamin or mineral to the associated health problem.

Vitamin or mineral	**Health problem**
vitamin B_2	a. scurvy
niacin	b. osteomalacia
vitamin C	c. hemorrhage
vitamin A	d. goiter
vitamin D	e. dental caries
vitamin K	f. skin rashes
calcium	g. osteoporosis

fluorine	h. nyctalopia
iodine	i. cracks at the corner of the mouth

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

vitamin
mineral
water-soluble vitamin
fat-soluble vitamin
B complex
vitamin B_1, thiamine
thiamine pyrophosphate
beriberi
vitamin B_2, riboflavin
niacin, nicotinate
pellagra
vitamin B_6, pyridoxine
pyridoxal phosphate
neurotransmitter
γ-aminobutyrate
histamine
serotonin
dopamine
norepinephrine
epinephrine
catecholamine
anemia
microcytic hypochromic anemia

vitamin B_{12}, cobalamin
cobalt
homocysteine
intrinsic factor
pernicious anemia
folate
tetrahydrofolate
megaloblastic anemia
N ^{5}-methyltetrahydrofolate
methionine synthase
methylcobalamin
N ^{5},*N* ^{10}-methylenetetrahydrofolate
S-adenosylmethionine
spina bifida
pantothenate
biotin
carboxylase
carboxylation
avidin
vitamin C, ascorbate
scurvy
prolyl 4-hydroxylase
hydroxylation
4-hydroxyproline
post-translational modification
vitamin A
retinol
carotenoid
β-carotene
retinal

retinoic acid
nyctalopia
xerophthalmia
vitamin D
ergocalciferol, vitamin D_2
cholecalciferol, vitamin D_3
rickets
osteomalacia
vitamin E
α-tocopherol
hemolytic anemia
vitamin K, phylloquinone
menaquinone
γ-glutamyl carboxylase
γ-carboxyglutamate
sodium
electrolyte
hyponatremia
potassium
calcium
osteoporosis
magnesium
iron
hepcidin
copper
zinc
carbonic anhydrase
zinc finger
chromium
manganese

molybdenum
chlorine
phosphorus
selenium
selenoprotein
selenocysteine
Keshan disease
Kashin-Beck disease
osteoarthritis
fluorine
dental caries
iodine
triiodothyronine, T_3
thyroxine, T_4
goiter
hypothyroidism

References and Suggested Readings

Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2016). Vitamin D: Metabolism, molecular mechanism of action, and pleiotropic effects. *Physiological Reviews* 96: 365-408.

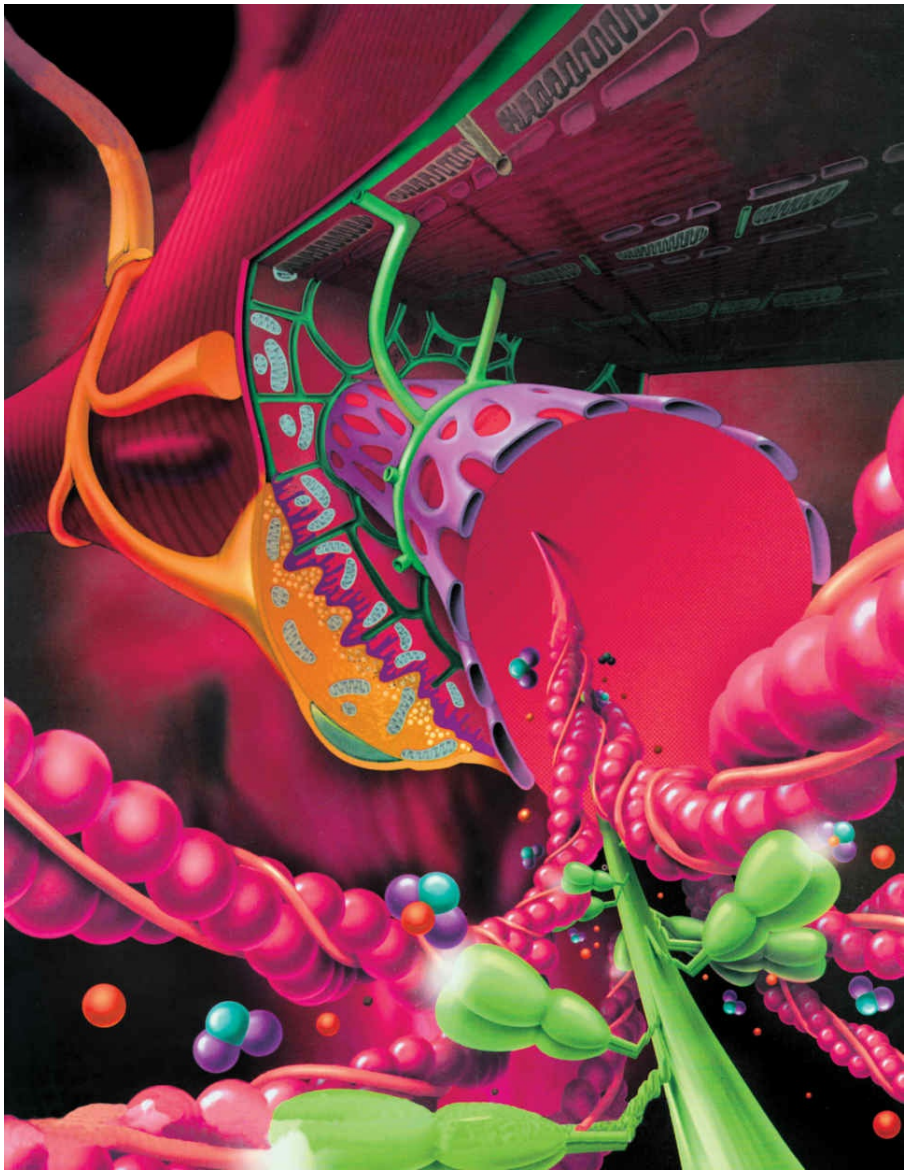
Collins JF, Prohaska JR, Knutson MD (2010). Metabolic crossroads of iron and copper. *Nutrition Reviews* 68: 133-147.

de Baaij JH, Hoenderop JG, Bindels RJ (2015). Magnesium in man: Implications for health and disease. *Physiological Reviews* 95: 1-46.

Monteverde DR, Gómez-Consarnau L, Suffridge C, Sañudo-Wilhelmy SA (2017). Life's utilization of B vitamins on early Earth. *Geobiology* 15: 3-18.

Williams MH, Rawson E, Branch D (2017). *Nutrition for Health,*

Fitness, and Sport (McGraw-Hill, New York), pp. 277-369.



The introductory figure for part II takes you on an imaginary trip to the inner space of a motor unit. At the top left is the motor neuron, which transmits the signals for muscle activity. Its axis is wrapped in myelin up to a point, after which it splits into terminal branches that stick to the muscle fiber. The cutaway view of a terminal branch and the muscle fiber reveals an undulating interface. On the side of the neuron, you can see a wealth of synaptic vesicles and mitochondria; on the side of the muscle fiber, you can see a wealth of mitochondria and transverse tubules. The transverse tubules embrace the myofibrils, one of which projects. It is wrapped in sarcoplasmic reticulum and is full of thick and thin filaments, which dominate the lower part of the picture. One thick filament is surrounded by six thin filaments. Double myosin heads project from the thick filament and split ATP, releasing the energy (flashes) required for motion. The thin filaments consist mainly of actin (the double winding necklace); tropomyosin (the double lace) also appears. The elongated bulge between two terminal branches of the neuron is a satellite cell. Come back to the figure after you have read all of part II and identify

its components without reading the caption.

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PART II

Biochemistry of the Neural and Muscular Processes of Movement

The inertia of objects is deceptive. The inanimate world appears static, "dead," to humans only because of our neuromuscular chauvinism. We are so enamored of our own activity range that we blind ourselves to the fact that most of the action in the universe is unfolding outside our range, occurring at speeds so much slower or faster than our own that it is hidden from us as if by a … veil.

—Tom Robbins, *Skinny Legs and All*

Movement is an integral trait of matter, from its largest to its smallest scale. Galaxies spin in the universe, and electrons move around the nuclei of atoms. Movement is particularly evident in living matter—both plants and animals. Plants move as they grow, as they open and close the petals of their flowers, or—among the carnivorous ones—as they entrap insects. However, the movement of plants doesn't measure up to that found in the animal kingdom. There, movement dominates as a way of life, endowing animals with a vital advantage: the ability to go from one place to another, where the living

conditions (such as climate, environment, abundance of food, and absence of enemies or competitors) are more favorable, thus increasing their chances for survival.

Muscle was the tool that granted animals fast movement. Its “invention” through evolution more than half a billion years ago must have brought about an unimaginable revolution in life on Earth. By the power of their muscles, animals—in an incredible variety of forms, sizes, and capabilities—conquered the oceans and lands of our planet. The climax of this course was the appearance of *H. sapiens*. Based mainly on the abilities of their hands and guided by another astonishing tool, the brain, humans managed to become the sovereigns of Earth in the blink of a (geological) eye.

The nervous and muscular systems star in this part of the book. We will examine, in as much detail as is fitting to a textbook, the chain of biochemical processes that convert the signal for muscle activity into a visible effect.

CHAPTER 7

Neural Control of Movement

Learning Objectives

After reading this chapter, you should be able to do the following:

- Distinguish the two ways of transmitting nerve signals.
- Compare the various body fluids in terms of electrolyte concentrations.
- Describe resting potential and the protein that is responsible for its generation.
- Describe action potential and the proteins that are responsible for its generation.
- Define and compare continuous conduction and saltatory conduction of nerve impulses.
- Explain how a nerve impulse is transmitted from one neuron to another and from nerve to muscle.
- Discuss the generation of a visual signal in response to light hitting the retina in the eye.
- Distinguish voltage-gated from ligand-gated ion channels.
- Discuss the changes in motor neuron activity during exercise and define fatigue.
- List the weapons of some animals that target the electrical or

chemical transmission of nerve impulses and explain how they work.

Movement resulting from muscle activity is impossible without the transmission of signals from the nervous system to the muscles. Most of our movements are involuntary and reflexive: They constitute immediate responses to information provided to the spinal cord by the sensory organs or the muscles themselves through appropriate nerves. In turn, the spinal cord, without "consulting" the brain, transmits signals for the muscles to contract. Even signals coming from the brain down the spinal cord act largely by affecting reflex routes.

Instantly retracting the hand from a hot surface is an example of a reflex.

The area of the brain mainly involved in controlling voluntary movement is the **motor cortex**, a strip across the middle of the cerebral cortex, roughly where the frame of a headset rests when we listen to music in private (figure 7.1). The motor cortex sends signals to the spinal cord through the **brain stem** (or **brainstem**), which, in addition, "reports" to the **cerebellum**. This structure, lying at the back of the brain, integrates the reports from the brain stem with sensory information to coordinate the activity of the muscles involved in a movement. As a result, the movement is smooth, and the body maintains balance while moving. A final structure participating in the control of voluntary movement is the **basal ganglia** (singular: *ganglion*), located deep inside the brain. The basal ganglia suppress unwanted movements and prepare the motor cortex and brain stem (with both of which they are tightly connected) to trigger movement.

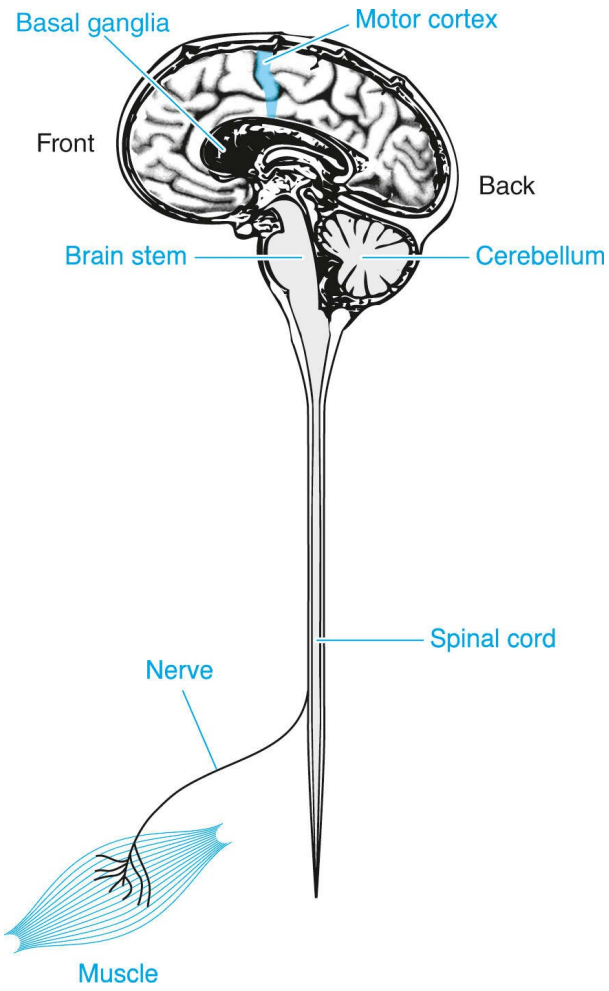

Figure 7.1 **Nervous motorways.** Skeletal muscles receive signals to contract from nerves originating in the spinal cord and brain. The spinal cord generates signals in response to sensory input from all over the body. It also receives signals for motor activity from the brain, notably the motor cortex and brain stem. The cerebellum helps to coordinate these signals and produce smooth motion, whereas the basal ganglia "prime" the motor cortex and brain stem for the initiation of movement.

The brain and spinal cord constitute the **central nervous system**. Its commands reach the muscles (and other organs) through the **peripheral nervous system**, which consists of the nerves that emerge from the central nervous system. The biochemical events that permit the transmission of nerve signals to the muscles are the topic of the present chapter.

7.1 Two Ways of Transmitting Nerve Signals

To travel from its origin to its destination, a nerve signal, like the signal

directing a muscle to contract, needs to go through **neurons**, that is, cells of the nervous tissue. A neuron is highly asymmetric (figure 7.2). It consists of the **cell body**, where the nucleus lies, and two kinds of processes: the **dendrites**, which bring signals to the cell body, and a very long **axon**, which takes the signals away from the cell body. The axon, in turn, ends in numerous thin branches, the **terminal branches**, which can contact other neurons or even other parts of the same neuron.

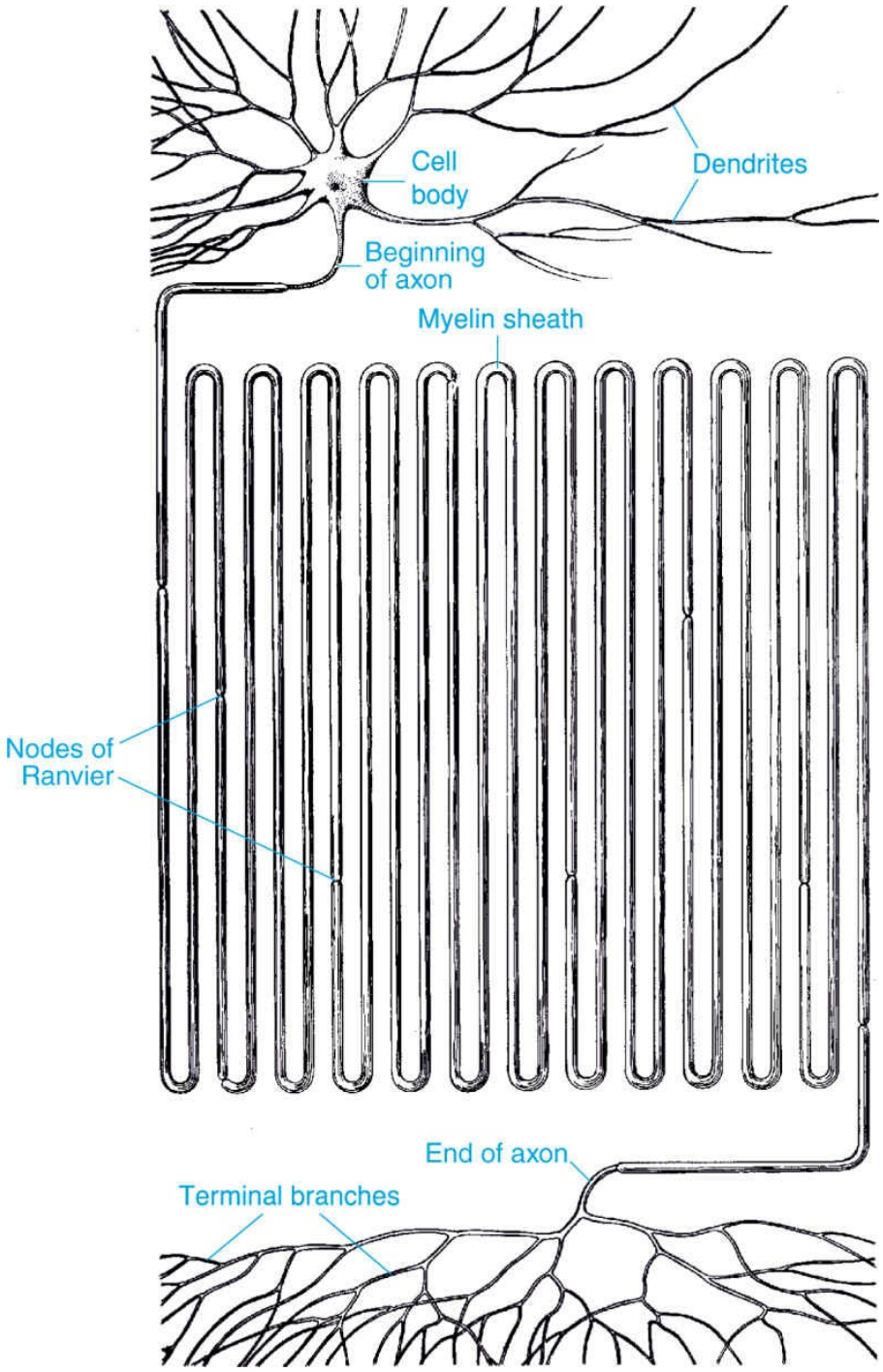

Figure 7.2 **Neuron.** Nerve signals are transmitted through neurons like the one depicted here, enlarged 200 times, with its parts drawn to scale. The actual length of the axon, which is folded to fit in the page, is 1 cm. However, there are axons over 1 m long. The dendrites and the axon's terminal branches may number in the hundreds or thousands. The axon is covered by myelin, which serves as an electrical insulator, speeds up signal transmission, and saves energy. The myelin sheath is interrupted at the nodes of Ranvier, only two of which are pointed out. (Try to locate the rest.) The direction of the nerve signals is from top to bottom,

that is, from the dendrites to the cell body to the terminal branches.
Courtesy of Carol Donner.

A relatively inert substance, **myelin**, surrounds most of the axon's length in many neurons of vertebrates. Myelin is formed by two types of specialized cells: **oligodendroglial cells**, or **oligodendrocytes**, in the central nervous system and **Schwann cells** in the peripheral nervous system. These cells roll their plasma membranes repeatedly around the axon, as shown in the electron micrograph in figure 7.3, thus forming something like a "pig in a blanket" (a sausage wrapped in dough).

An electron micrograph is taken through an electron microscope, which affords a higher resolution than the light microscope. It can discern not only intracellular organelles but also large complexes of biological molecules (such as ribosomes) and even isolated large molecules (such as DNA). Electron micrographs are dark where there is a high density of atoms and light where there is a low density of atoms. Watch out for more electron micrographs throughout the rest of the book!

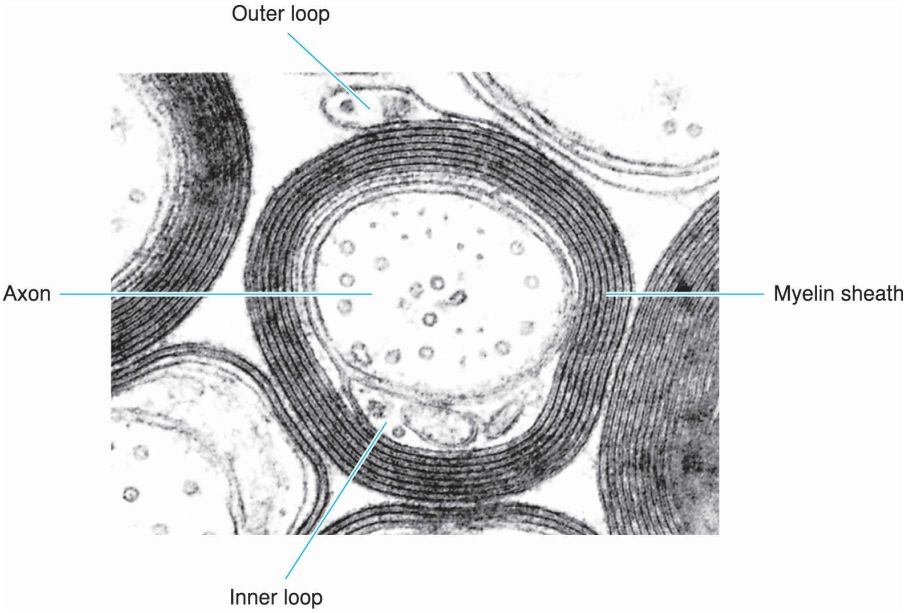

Figure 7.3 **Myelin.** An oligodendrocyte, the maker of myelin in the central nervous system, wraps its plasma membrane tightly around the axon of a neuron in this cross-sectional view. The myelin sheath's beginning (outer loop) and end (inner loop) are marked.
Photograph courtesy of Dr. Cedric S. Raine.

A single oligodendrocyte or a single Schwann cell can provide myelin for

only a short length along an axon. Thus, many myelin-producing cells need to lay their rolls of plasma membrane one next to another in order to cover the entire axon. The cover is not complete, though, because small areas of the axon's plasma membrane between adjacent rolls remain naked. These areas are called **nodes of Ranvier** (figure 7.2) and are spaced every few millimeters along the axon. As we will see in section 7.4, the myelin sheath and the nodes of Ranvier are crucial for the rapid propagation of nerve signals along the axon.

Nerve signals are transmitted differently inside neurons than they are from one neuron to another:

- Inside each neuron, signals are transmitted electrically (as electric current).
- From one neuron to another, signals are usually transmitted chemically.
- Signals are also transmitted chemically from neurons to muscle.

The neurons that innervate muscle are termed **motor neurons**. Let's now examine the two ways of transmitting nerve signals.

7.2 The Resting Potential

The electrical transmission of nerve signals is due to the movements of sodium and potassium cations across the plasma membrane of neurons. (By contrast, the current in an electric wire is due to the flow of electrons through its metal lattice.) These movements are made possible by existing **chemical gradients**, or **concentration gradients** (that is, unequal concentrations), of these cations between the cytosol and the interstitial fluid. Concentration gradients characterize not only neurons but other cells as well and are vital to their operation. Table 7.1 presents the concentrations of Na^+ and K^+ in some body fluids, along with the concentrations of some other important ions, so that you can get a more complete picture of ion concentration gradients in the body. You can see that Na^+ and K^+ are indeed the main electrolytes in the extracellular fluids (interstitial fluid and plasma) and in cytosol, respectively, as mentioned in section 6.3.

Table 7.1 Ion Concentrations in Human Body Fluids (in mmol · L^{-1})

Ion	Cytosol	Interstitial fluid	Plasma	Sweat
Na^+	3-10	133-144	136-145	40-80
K^+	148-156	3.8-4.4	3.5-5.1	4-8
Ca^{2+}	0.0001-0.01	1.4-1.7	1-1.3	1.5-2
Mg^{2+}	0.25-1	0.5-1.7	0.7-1	0.5-2
Cl^-	2-3	112-115	98-107	30-70
HCO_3^-	7-10	21-28	21-29	0-36

Na^+ has a higher concentration in the interstitial fluid (about 140 mmol · L^{-1}) than in the cytosol (10 mmol · L^{-1}). In contrast, K^+ is less concentrated in the interstitial fluid (4 mmol · L^{-1}) than in the cytosol (150 mmol · L^{-1}). The two fluids are separated by the plasma membrane, which prevents Na^+ and K^+—as well as many other substances—from moving spontaneously down their concentration gradients, that is, from the compartment of high concentration to the compartment of low concentration. Because the ions are charged and hydrated—surrounded by a shell of water molecules (figure 7.4)—they cannot penetrate the hydrophobic core of the plasma membrane.

To remember where each of the [Na^+] and [K^+] is higher, you may compare the extracellular fluids to seawater, which was probably the environment of the first cells that appeared on Earth and which has a high salt (Na^+Cl^-) concentration. Thus, Na^+ is more concentrated in the extracellular fluids than in the cytosol, and the opposite is true for K^+.

How are the Na^+ and K^+ concentration gradients formed in the first place? They are formed by a "pump," which throws Na^+ out of the neuron (thus accumulating it in the interstitial fluid) and brings K^+ into the neuron (thus accumulating it in the cytosol). The pump transports Na^+ and K^+ "uphill," that is, each from a compartment of low concentration to a compartment of high concentration. This transport requires an input of energy and is therefore called **active transport**. Energy comes from the hydrolysis of ATP according to reaction 2.5.

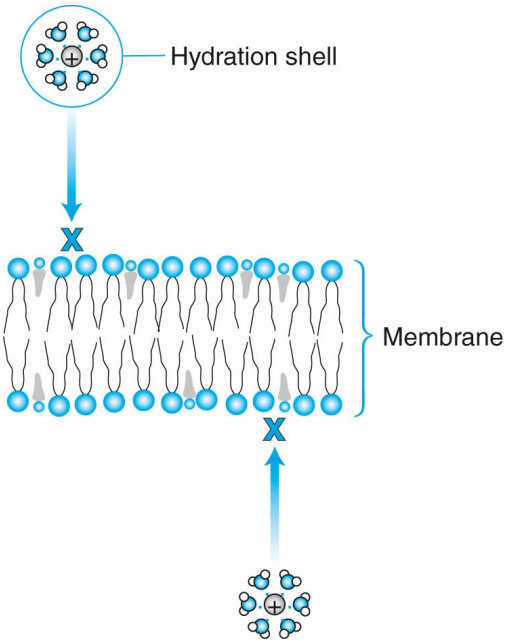

Figure 7.4 **Membrane barrier at work.** Sodium and potassium cations in aqueous solutions attract water molecules (six in this representation) through coordinate bonding (colored dots) with oxygen atoms. The resulting hydration shell cannot pass through the hydrophobic core of a membrane such as the plasma membrane. This barrier contributes to the maintenance of ion concentration gradients across cell membranes. The hydration shell has been magnified relative to the membrane for clarity.

What is the nature of the pump that moves Na^+ and K^+ in opposite directions? It is an integral protein of the plasma membrane (figure 7.5) named the **sodium–potassium pump (Na^+–K^+ pump)**. The same protein is endowed with the ability to hydrolyze ATP; thus, it exhibits enzyme activity. That is why it is also referred to as *sodium–potassium adenosine triphosphatase*, or **Na^+–K^+ ATPase**. For every ATP hydrolyzed, three Na^+ exit and two K^+ enter the cytosol within 10 ms (thousandths of a second).

The pump is composed of two subunits designated α and β (110 and 55 kDa, respectively). Both span the membrane bilayer, but the bulk of each lies on one side, α in the cytosol and β in the extracellular space. Although the α subunit carries out both tasks of the protein (ion transport and ATP hydrolysis), the β subunit is required for proper function.

The plasma membrane is studded with molecules of the pump. Cells spend high amounts of energy to maintain the Na^+ and K^+ concentration gradients. This is particularly true for nerve and muscle cells, because, as we will see,

their excitation results in the disturbance of these gradients.

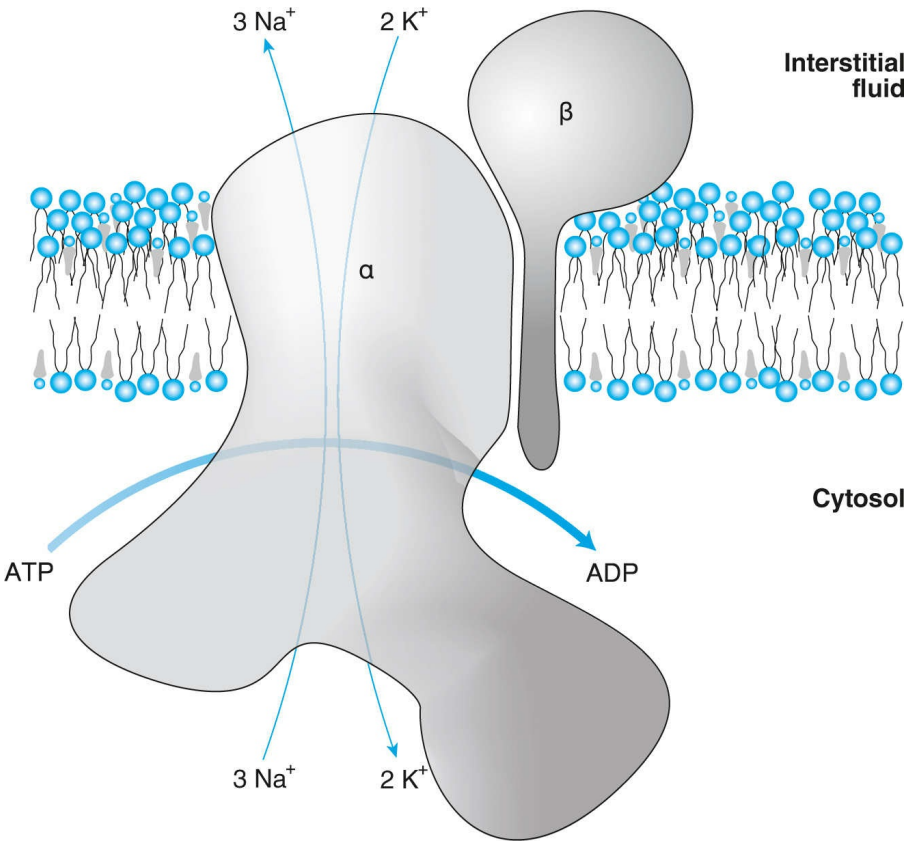

Figure 7.5 **Na^+–K^+ pump.** The Na^+–K^+ pump spans the plasma membrane and maintains an unequal distribution of Na^+ and K^+ between the inside and outside of cells at the expense of energy from the hydrolysis of ATP. The protein is an αβ dimer (complex of two subunits). The protein and the membrane have been drawn to scale.

Because the concentration gradients across the plasma membrane involve charged solutes, they result in the generation of an electric potential difference, or voltage, symbolized as ΔV. This is called the **resting potential**, because it characterizes the resting, not the excited, state. The resting potential is 60 to 70 mV (thousandths of a volt) negative inside relative to outside of the neuron. Thus, there is an **electrical gradient**, as well as a chemical gradient, across the plasma membrane. These two factors combine to produce an **electrochemical gradient**.

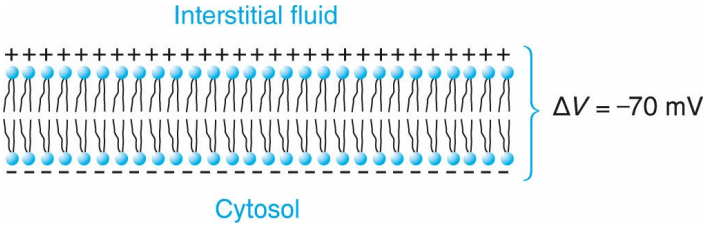

Figure 7.6 Polarized plasma membrane. The unequal distribution of ions between the cytosol and the interstitial fluid polarizes the plasma membrane (presented here and in subsequent figures as consisting only of phospholipids for the sake of simplicity) and gives rise to a resting potential of about –70 mV.

The resting potential forms the basis for the electrical transmission of a nerve signal, which I describe in the next section. We say that the plasma membrane is **polarized** because we can depict it as an electric dipole with its negative pole in the cytosol and its positive pole in the extracellular space (figure 7.6).

7.3 The Action Potential

An electric nerve signal is an instantaneous **depolarization** (reversal of the polarity) of the plasma membrane of a neuron (figure 7.7) in the area where the cell body meets the axon. The depolarization is due to the influx of Na^+ from the extracellular space to the cytosol through pores in the otherwise impermeable plasma membrane. The pores are formed by molecules of a transmembrane protein, the **sodium channel (Na^+ channel)**.

The Na^+ channel is closed most of the time, which hinders the entry of Na^+ into the cytosol and contributes to the maintenance of the resting potential. However, for reasons that we will discuss in sections 7.4 and 7.6, the membrane voltage around the channel can increase. If the increase is large enough, exceeding what is termed the **excitation threshold**, then the shape of the Na^+ channel changes and a narrow opening

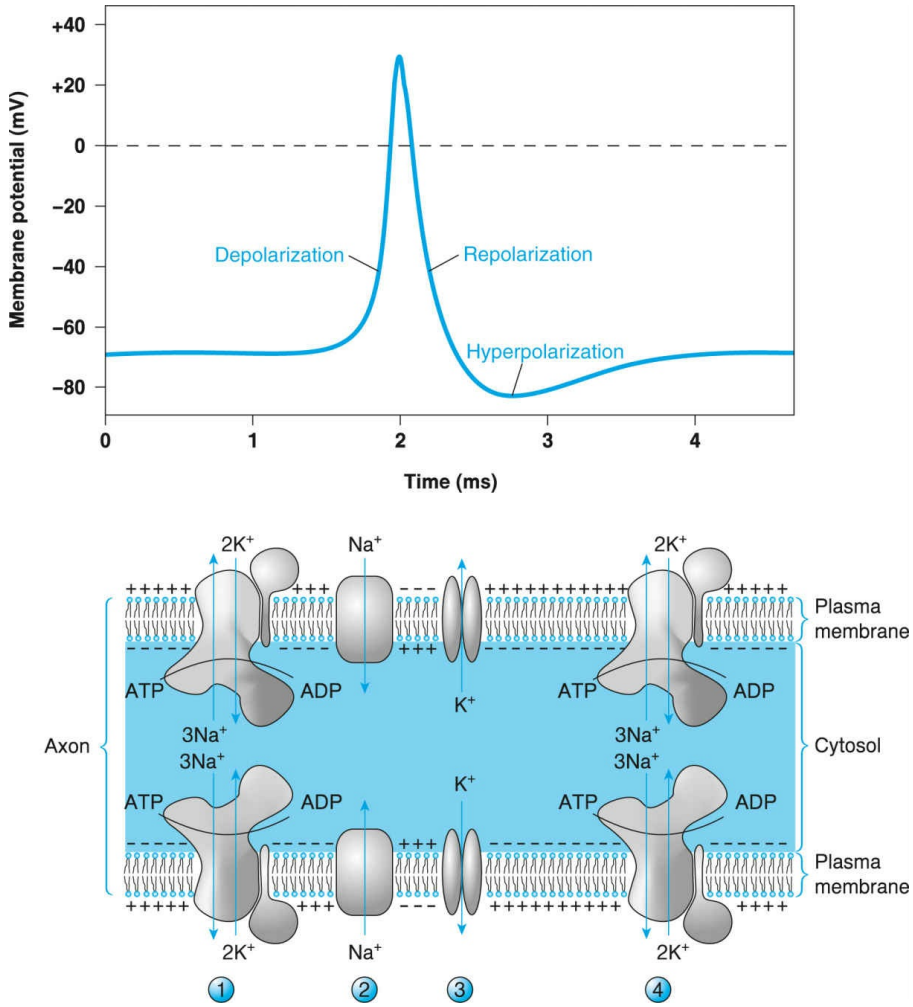

Figure 7.7 **Electrical transmission of a nerve signal.** The nerve signal is transmitted electrically in a neuron thanks to a momentary leap of the plasma membrane potential from a negative to a positive value (upper panel). The negative value (resting potential) is maintained by the sodium–potassium pump (lower panel, *1)*, whereas the positive value (action potential) appears when the sodium channel opens *(2)* and Na^+ ions rush into the cytosol. The potassium channel *(3)* lets K^+ ions out and reinstates the resting potential. The Na^+–K^+ pump restores the disturbance in the Na^+ and K^+ concentrations *(4)*. The position of the proteins in the lower panel corresponds to the different phases of the membrane potential in the upper panel. In reality, however, there is no orderly arrangement of the three proteins; rather, they are scattered all over the membrane.

forms in its interior. Because it opens when the voltage changes, the Na^+ channel is called a **voltage-gated channel**. The excitation threshold is at least 15 mV (meaning that a resting potential of, say, –65 mV will have to increase to at least –50 mV for the Na^+ channel to respond), and its precise value differs from one neuron to another. The higher the threshold, the more

difficult it is for a neuron to get excited.

When the Na^+ channel opens, Na^+ ions pass in a direction dictated by their electrochemical gradient: They move from where the concentration is high and the charge positive (that is, the interstitial fluid) to where the concentration is low and the charge negative (the cytosol). This form of "downhill" movement is termed **passive transport**.

Three ways by which molecules or ions cross membranes are as follows (in order of increasing complexity):

- Simple diffusion, as in the case of O_2 crossing cell membranes down its concentration gradient (sections 3.11 and 3.12): O_2 needs no transporter because it is nonpolar like the core of the lipid bilayer.
- Passive transport (also called *facilitated diffusion*), as in the case of Na^+ entering a cell down its electrochemical gradient with the help of the Na^+ channel
- Active transport, as in the case of Na^+ and K^+ crossing the plasma membrane against their electrochemical gradients through the action of the Na^+–K^+ pump, powered by ATP hydrolysis

Now let's return to the Na^+ channel. Its opening in the plasma membrane of a neuron and the subsequent influx of Na^+ to the cytosol result in a reversal of the membrane potential to values that are positive inside relative to outside (up to 30 mV). The new voltage is called the **action potential**. The depolarization is extremely short: In less than 1 ms the Na^+ channel closes spontaneously and cannot reopen for several milliseconds. At the same time, the change in membrane potential triggers the opening of yet another integral protein of the plasma membrane, which is also a voltage-gated channel. This one is a **potassium channel (K^+ channel)** that lets K^+ exit the cytosol toward the extracellular space, down its electrochemical gradient. In effect, the membrane potential returns to its resting value, which results in **repolarization** of the plasma membrane (restoration of its original polarity), and briefly goes below the resting value, which results in **hyperpolarization**. The K^+ channel too closes spontaneously in a few milliseconds, and the membrane is ready to witness a new action potential.

Although the outward movement of K^+ is down its chemical gradient (from high to low concentration), it is against the electrical gradient (from negative to positive). However, the large concentration difference outweighs the electrical difference, thus resulting in a favorable electrochemical gradient overall.

The generation of action potentials tends to change the Na^+ and K^+ concentrations inside and around a neuron in the direction of diminishing their gradients. However, the Na^+–K^+ pump is there to rid the cytosol of the Na^+ ions that intrude and recall the K^+ ions that escape, at the expense of ATP.

7.4 Propagation of an Action Potential

An action potential is meant not to remain stagnant but to travel along the neuron. The instantaneous depolarization at the beginning of the axon causes neighboring molecules of the Na^+ channel to open, which results in depolarization of the adjacent area. This effect, in turn, causes the membrane to depolarize a bit further, and so on. Because a molecule of the Na^+ channel cannot reopen immediately, an action potential can move only forward (toward molecules that have not yet opened), not backward (toward molecules that have already opened). Thus, an action potential propagates along the axon toward the terminal branches like a wave caused by a pebble falling into a pond. For this reason, a nerve signal is often referred to as a **nerve impulse**.

The propagation of an action potential along axons encased in myelin is somewhat different. Because myelin bars the axon from the interstitial fluid, an action potential cannot run along the axon's myelinated segments. However, it can jump from one node of Ranvier to another like a stone skipping across water. Remember that the plasma membrane is exposed to the interstitial fluid at the nodes. Both the Na^+ and K^+ channels are concentrated there—much more densely, in fact, than in the plasma membrane of unmyelinated axons. The propagation of an action potential from one node of Ranvier to another is termed **saltatory conduction** (figure 7.8), as opposed to the **continuous conduction** that characterizes unmyelinated axons.

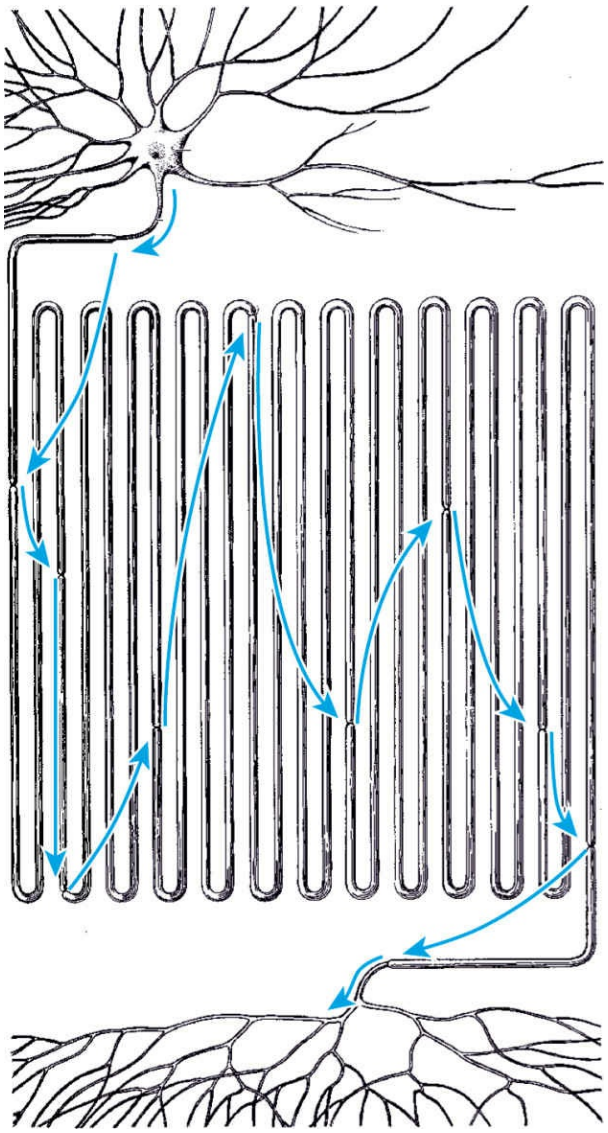

Figure 7.8 **Saltatory conduction.** A nerve impulse jumps from one node of Ranvier to another along a myelinated axon, thus saving time and energy.

Courtesy of Carol Donner.

We are now ready to discuss the role of myelin, which is that of an electrical insulator like the insulators of electric wires. By confining the electrical activity that develops along an axon to certain points (the nodes of Ranvier), myelin minimizes the effect on neighboring axons. Another utility of myelin is that it speeds up the transmission of nerve impulses. Indeed, saltatory conduction is much faster than continuous conduction. Finally, myelin saves energy. Because the movement of Na^+ and K^+ ions through their

respective channels is confined to the nodes of Ranvier, the Na^+–K^+ pump has much less work to do in order to bring the concentrations of these ions back to the resting levels. Thus, the neuron spends less ATP. In summary, myelin

- electrically insulates the axons of neurons,
- speeds up the propagation of nerve signals, and
- saves the neuron ATP.

The great importance of myelin for the proper function of the nervous system becomes evident when it is destroyed from pathological causes. Destruction of myelin leads to the development of degenerative diseases, such as **multiple sclerosis**, which is characterized by gradual degradation of myelin in certain regions of the central nervous system. This degradation slows down the conduction of nerve impulses, thus causing loss of motor coordination and partial paralysis.

7.5 Transmission of a Nerve Impulse From One Neuron to Another

When, having traveled down the axon, an action potential reaches the terminal branches, it needs to jump a small but important hurdle to convey the signal to another neuron: It must cross the space separating the two neurons. This is where the second means of transmission of nerve signals, the chemical one, comes into play.

The point of contact between neurons is called a **synapse** (figure 7.9). At the synapse, the plasma membrane of one neuron comes very close to the plasma membrane of another. The membrane of the neuron transmitting the impulse is termed **presynaptic**, whereas that of the neuron receiving the impulse is termed **postsynaptic**. The space separating them is the **synaptic cleft**, which varies from 20 to 200 nm in width and is filled with interstitial fluid.

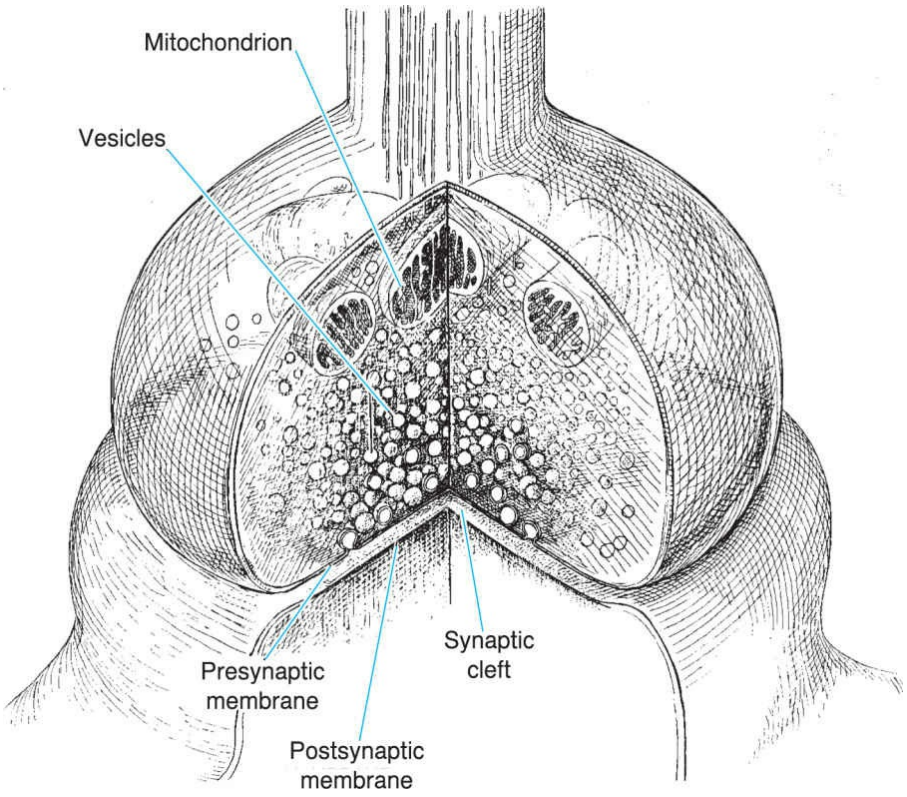

Figure 7.9 **Synapse.** A synapse permits the propagation of an impulse from one neuron to another. The bulged ending of the neuron transmitting the signal (top) and the surface of the neuron receiving the signal (bottom) come close to each other. The former is filled with vesicles containing neurotransmitter molecules.

Courtesy of Carol Donner

The transmission of a nerve impulse across the synaptic cleft is accomplished by compounds referred to collectively as **neurotransmitters**. Several have been identified, some of which were introduced in section 6.1 and figure 6.1. Each neurotransmitter has a specific distribution in the nerve tissue and specific ways of acting, thus contributing to the inconceivable variety of messages handled by the nervous system.

Despite their variety, neurotransmitters share several features. For one, following their synthesis in the neurons, they are stored in **synaptic vesicles** inside the terminal branches, very close to or even in contact with the presynaptic membrane. Like all intracellular organelles, synaptic vesicles are bound by a lipid bilayer. Another common feature of neurotransmitters is the way in which they are liberated: The arrival of an action potential at the presynaptic membrane causes the instantaneous (within hundredths of a

millisecond) fusion of the vesicles with the membrane and the discharge of their contents into the synaptic cleft in a process termed **exocytosis**.

How does the action potential accomplish this feat? The key to neurotransmitter release lies in the influx of calcium cations to the cytosol. Ca^{2+} is another ion with unequal distribution across the plasma membrane; its concentration is higher in the extracellular fluids than in the cytosol (see table 7.1). The plasma membrane contains many molecules of a voltage-gated **calcium channel (Ca^{2+} channel)**, which open when the membrane is depolarized. Through the channel, Ca^{2+} rushes to the cytosol and causes the membrane of the synaptic vesicles to fuse with the plasma membrane (figure 7.10). Researchers believe that **synaptotagmin 1**, an integral protein of the vesicle membrane, senses the rise in the cytosolic [Ca^{2+}] and undergoes a crucial change in its interaction with membrane phospholipids and with a tight complex of three other proteins (one in the vesicle membrane and two in the plasma membrane), called the **SNARE complex**. This change triggers the fusion of the two membranes, as reviewed in detail by Josep Rizo and Junjie Xu.

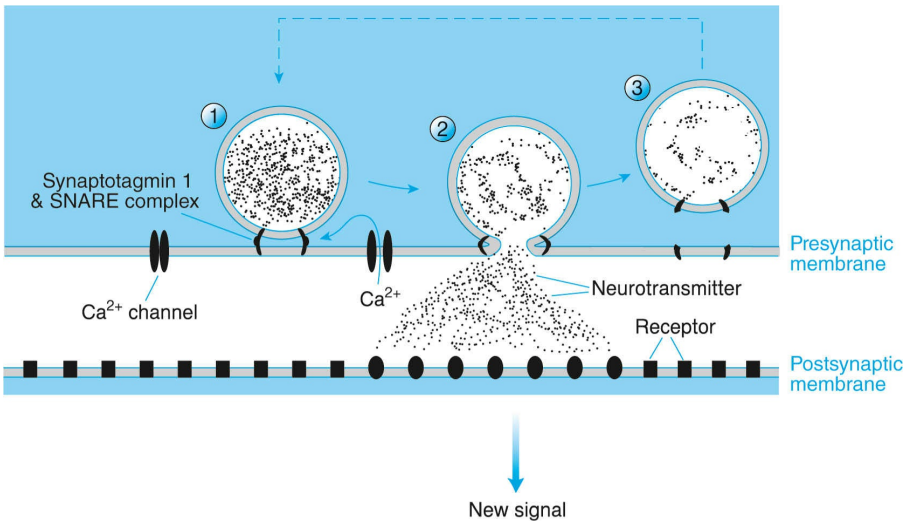

Figure 7.10 **Chemical transmission of a nerve impulse.** *(1)* A synaptic vesicle, full of neurotransmitter molecules (black dots) and ready to release its contents, is tethered to the presynaptic membrane when a neuron is not excited. *(1 → 2)* When an action potential arrives at the presynaptic membrane, a voltage-gated calcium channel opens. The incoming Ca^{2+} binds to synaptotagmin 1, which, in association with the SNARE complex of proteins, causes the vesicle membrane to fuse with the plasma membrane. *(2)* Neurotransmitter molecules are emptied into the synaptic cleft and bind to receptor molecules in the postsynaptic membrane,

causing a change in their shape (schematically, from square to oval) and the generation of a new signal. *(2 → 3)* The SNARE complex is disassembled by other proteins, and the vesicle dissociates from the plasma membrane. *(3)* The vesicle is reconstituted with a small amount of neurotransmitter that was not discharged. *(3 → 1)* The vesicle is refilled by the biosynthetic machinery of the neuron and gets ready to release its contents again.

The neurotransmitter molecules diffuse to the postsynaptic membrane and bind to receptors in it. Remember, receptors are proteins that receive molecular messengers (section 3.8). A neurotransmitter receptor is highly specialized in recognizing and binding a neurotransmitter. Binding modifies the conformation of the receptor, causing a change in its biological activity. As for the synaptic vesicles, they reassemble when cellular proteins dissolve the SNARE complex, leading to detachment of the vesicle membrane from the plasma membrane. The reconstituted vesicles may contain few neurotransmitter molecules initially (those that did not make it to the synaptic cleft), but the neuron synthesizes new ones and fills the vesicles, thus preparing them for a new round of fusion with the plasma membrane, neurotransmitter release, and reassembly.

This is where the common features of neurotransmitters end, as their receptors play a multitude of different roles. Some, for example, serve as ion channels, whereas others regulate the rate of reactions taking place in the cytosol. Thus, one cannot provide a general scheme describing the outcome of the binding of a neurotransmitter to a receptor other than the coarse distinction between **excitatory** and **inhibitory neurotransmitters**. The former facilitate the propagation of a signal from one neuron to another, whereas the latter inhibit it.

When a nervous stimulus ceases, the action of a neurotransmitter needs to stop in order to avoid any further unnecessary or even harmful stimulation. How is this stopping achieved? When action potentials cease to arrive at the presynaptic membrane (or arrive at a slower rate), synaptic vesicles stop fusing with it (or fuse at a slower rate). The neurotransmitter molecules that are present in the synaptic cleft are degraded by enzymes or are taken back up through specific transport proteins in the presynaptic neuron. Thus, the neurotransmitter concentration in the synaptic cleft drops. This drop encourages the dissociation of the neurotransmitter from the receptor and the

subsequent degradation or reuptake of the neurotransmitter. The receptor then reverts to its initial (neurotransmitter-free) state.

The binding of a neurotransmitter N to a receptor R is reversible and concentration dependent. $N + R \rightleftharpoons NR$.

7.6 Birth of a Nerve Impulse

In sections 7.3 and 7.4, we saw how an action potential forms and propagates along an axon as a result of the opening of Na^+ channel molecules in its membrane, which is triggered by depolarization of the membrane in the vicinity of the channel molecules. The obvious question arises: What triggers the very beginning of electrical activity in a neuron? The answer could be the chemical signal from a preceding neuron along the path of transmission of a command (in our context, the command for muscle activity). But this answer merely shifts the question one neuron back, and then another neuron back, and so on until we reach the neuron(s) where the signal directing a muscle to contract originated. Then we are cornered.

The cause of a signal for muscle activity could be a decision born in the mind without a particular external stimulus. For example, while standing, you might decide to start walking. Alternatively, it could be a stimulus from one or more of the sensory organs. For example, you might watch a tennis ball coming your way and decide to hit it with a racket. Thanks to research findings of recent years, the answer to what triggers the beginning of nervous activity leading to an effect, such as muscle activity, has begun to take shape in increasingly satisfactory molecular detail. Because complete coverage of the matter lies beyond the scope of this book, I will describe an example only —that of the birth of a visual stimulus.

A visual stimulus begins when a photon (the elementary quantity of light) causes a change in the structure of retinal, a derivative of vitamin A (section 6.2) that serves as the prosthetic group of **rhodopsin** and other **photoreceptor** (light-receiving) proteins located in the retina. The retina of vertebrates features two kinds of photoreceptor cells: **rods** and **cones**. Rods contain rhodopsin and convey the perception of light, whereas cones contain

three other photoreceptor proteins and convey the perception of red, green, and blue. The biochemistry of vision has been studied especially well in rods, which greatly outnumber cones in the retina and which are more sensitive to light than are cones, as reviewed by Norianne Ingram and coworkers.

A rod (figure 7.11) is a highly specialized cell with an **outer segment** pointing toward the brain, an **inner segment**, a cell body, and a **synaptic body**, which is full of neurotransmitter-laden

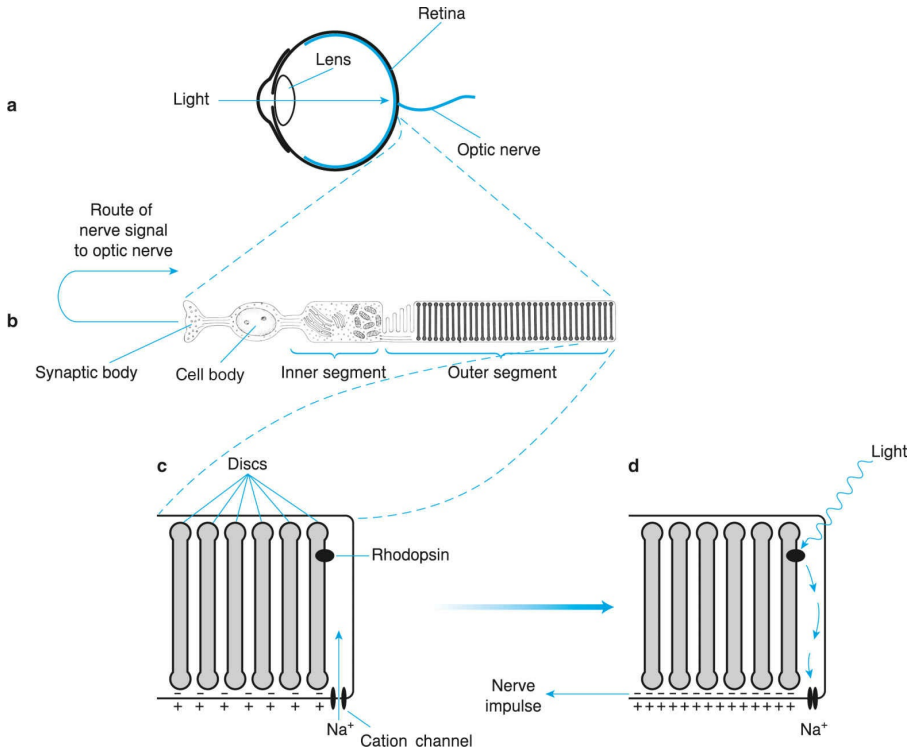

Figure 7.11 **Generation of a visual signal.** *(a)* A visual signal begins when light hits the retina in the eye. *(b)* The retina contains rod cells consisting of an outer segment, an inner segment, a cell body, and a synaptic body. The outer segment, which is the light-sensing part, contains a pile of membrane-bound discs. *(c)* The disc membrane is crowded with molecules of rhodopsin. In the dark, a cation channel in the plasma membrane remains open, permitting an influx of Na^+. *(d)* Excitation of rhodopsin by light triggers a series of reactions that cause the cation channel to close. As a result, the membrane is hyperpolarized (depicted by more plus and minus signs), which generates a nerve signal.

vesicles. The amino acid glutamate serves as neurotransmitter in this case. The synaptic body is in touch with nerve cells in the retina, which in turn communicate with the optic nerve. The outer segment contains a stack of flattened membrane-bound **discs**; rhodopsin is an integral protein of the disc

membrane, being present by the thousands of molecules per disc.

The light-induced change in retinal structure just mentioned causes a conformational change in rhodopsin that activates a series of enzyme reactions leading to the closure of a cation channel in the surrounding plasma membrane. The channel is open in the dark, permitting the influx of Na^+ from the interstitial fluid. This influx, along with the operation of the Na^+–K^+ pump (located in the part of the plasma membrane surrounding the inner segment), maintains the membrane voltage at about –45 mV (inside relative to outside). However, the closure of the channel when rhodopsin is lit hinders Na^+ entry, causing the membrane voltage to become even more negative. This hyperpolarization of the plasma membrane is transmitted to the synaptic body, which reduces the rate of neurotransmitter release to the contiguous nerve cells. Because the neurotransmitter is inhibitory, the lowering of its concentration in the synaptic cleft excites the postsynaptic membrane. This excitation, in turn, is transmitted to the optic nerve and finally to the brain for processing as a visual stimulus.

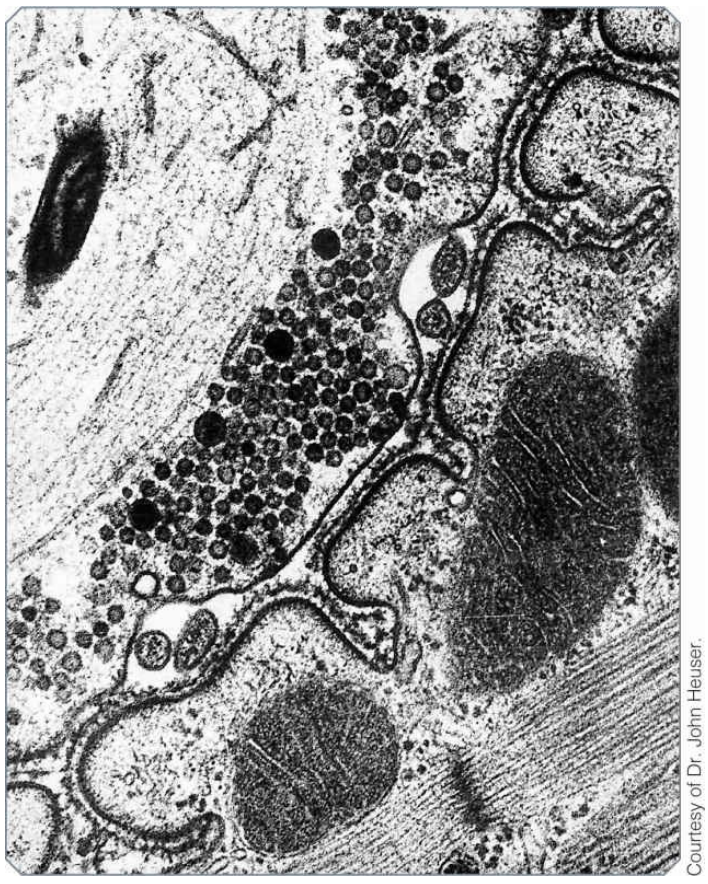

Figure 7.12 **Synapse at the neuromuscular junction.** A narrow synaptic cleft, running diagonally like a stream through this electron micrograph, separates a neuron (upper left) from a muscle cell (lower right). Tens of synaptic vesicles, filled with acetylcholine, crowd the ending of the neuron, very close to or in contact with the presynaptic membrane. On the opposite bank, the plasma membrane of the muscle cell forms invaginations called *transverse tubules* (see section 8.11). Further inside the muscle cell, you can make out two mitochondria (the dark, round structures) and a myofibril with a Z line (the bundle with the dark ribbon), which we will examine in section 8.1.

7.7 The Neuromuscular Junction

As a motor neuron approaches a muscle, it splits into hundreds or thousands of branches, each of which contacts a muscle cell. The motor neuron and the muscle cells it innervates form a **motor unit**. A nerve signal for muscle activity ends in the **neuromuscular junction**—the interface between a branch of a motor neuron and a muscle cell. Each neuromuscular junction contains many synapses like the one shown in figure 7.12. These synapses are similar to those found between neurons. The synapse at the neuromuscular junction has been studied in more detail and is the source of most of our knowledge about synapses in general.

A specific neurotransmitter, **acetylcholine**, operates at the neuromuscular junction. Acetylcholine is a relatively small compound consisting of an acetyl group and a choline unit (figure 7.13). You have already met the acetyl group, as the product of stage 2 of catabolism (section 2.6), and choline, as a component of phospholipids (section 5.9). Choline contains a nitrogen atom connected to four carbons, and this assembly of atoms, known as a **quaternary amino group,** carries a positive charge; thus, acetylcholine is a cation.

Acetylcholine is formed in neurons from acetyl coenzyme A (also introduced in section 2.6) and choline by the catalytic action of **choline acetyltransferase**.

$$\text{Acetyl coenzyme A} + \text{choline} \rightarrow \text{acetylcholine} + \text{coenzyme A}$$
$$\Delta G^{\circ\prime} = -2.3 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 7.1)}$$

After being synthesized, acetylcholine molecules gather in the synaptic vesicles (each vesicle is about 18 nm in diameter and contains about 10,000 molecules) and are discharged when action potentials arrive at the

presynaptic membrane. Some 300 vesicles per synapse empty their contents into the synaptic cleft, raising the acetylcholine concentration by 50,000 times (from 0.01 to 500 μmol · L^{-1}) in less than 1 ms.

Acetylcholine carries the signal for contraction from a nerve to a muscle thanks to the **acetylcholine receptor**, which is an integral protein of the plasma membrane of muscle cells. We call this type of acetylcholine receptor **nicotinic** to distinguish it from the **muscarinic** receptor, which dominates in the autonomic, rather than the somatic, nervous system. Acetylcholine serves as a neurotransmitter there too, but the muscarinic receptor functions differently from the nicotinic receptor. The names stem from **nicotine** (the infamous component of tobacco smoke) and **muscarine** (a substance found in some mushrooms). Investigators have used these compounds as ligands (a term defined in section 3.8) for distinguishing the two receptors.

In the nervous system, we can distinguish between the **somatic** part (serving the skeletal muscles) and the **autonomic** part (serving the internal organs).

Let's focus on the nicotinic acetylcholine receptor, as reviewed by Edson Albuquerque and collaborators. This receptor is a relatively large protein with a molecular mass of about 280 kDa and five similar subunits, two of which are identical (figure 7.14). Hence, its structure is symbolized as $\alpha_2\beta\gamma\delta$ or $\alpha_2\beta\delta\varepsilon$ (ε indicating *epsilon*). The five subunits surround a pore at the center.

The receptor has two binding sites for acetylcholine located on its extracellular surface, one in each α subunit at or near the α-γ (or α-ε) and α-δ subunit interfaces. When two molecules of acetylcholine bind to these sites, the conformation of the receptor changes, which results in a widening of the pore within about 20 μs (figure 7.15). Na^+ and K^+ ions then move through the pore, down their electrochemical gradients: Na^+ moves from the extracellular space to the cytosol, while K^+ moves in the opposite direction.

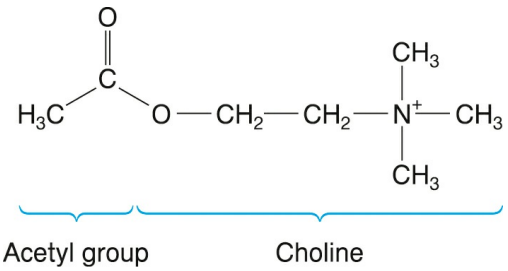

Figure 7.13 **Acetylcholine.** The neurotransmitter at the neuromuscular junction derives from the attachment of an acetyl group to choline.

The pore is almost equally permeable to Na^+ and K^+. However, many more Na^+ ions enter than K^+ ions exit because the existing electrical gradient (negative inside) favors the influx and inhibits the efflux of cations. In other words, the electrochemical gradient of Na^+ is higher than that of K^+. Thus, the plasma membrane of the muscle cell is depolarized, and a new action potential, analogous to the ones dashing through neurons, appears. This potential is often termed **postsynaptic potential**. Its development is aided by a voltage-gated Na^+ channel, which is also located in the plasma membrane and opens in response to the depolarization caused by the opening of the acetylcholine receptor. Then, just as happens in the neurons, a voltage-gated K^+ channel opens, lets K^+ out of the cytosol, and brings the membrane potential back to its resting value.

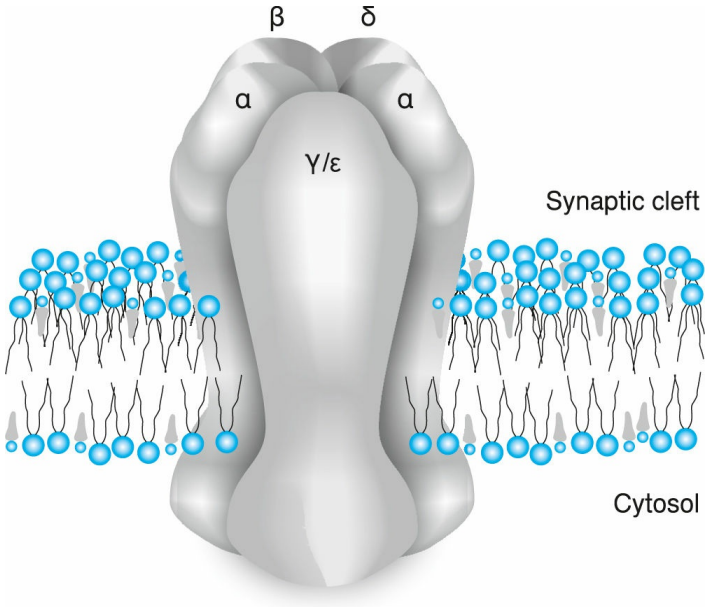

Figure 7.14 **Nicotinic acetylcholine receptor.** The receptor spans the plasma membrane of muscle cells. It is formed by five cylindrically arranged subunits, which protrude markedly toward the extracellular space and leave a narrow pore in the center (visible only at the top). The protein and membrane are drawn to scale.

In contrast to the voltage-gated Na^+, K^+, and Ca^{2+} channels that we encountered in sections 7.3 and 7.5, the acetylcholine receptor is a **ligand-gated channel**. Its shape, position in the membrane, and function are reminiscent of the magnificent gates that led in and out of cities at a time when cities were surrounded by walls.

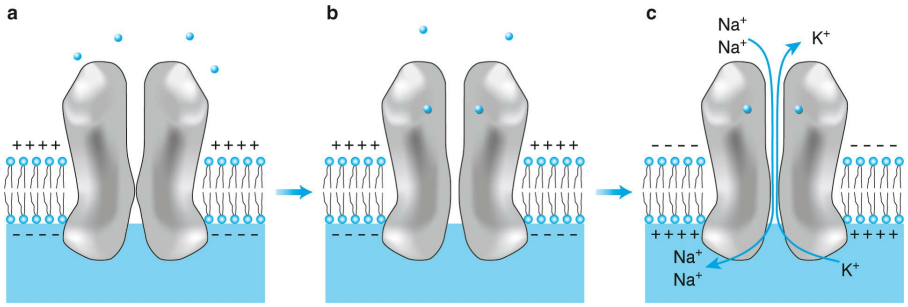

Figure 7.15 **Signal transmission across the neuromuscular junction.** Acetylcholine causes the generation of an action potential at the plasma membrane of a muscle cell by interacting with the acetylcholine receptor, of which I present just two of the five subunits to make the pore visible. *(a)* Acetylcholine molecules (colored orbs) are released into the synaptic cleft. *(b)* Two acetylcholine molecules bind to the receptor and dilate the pore. *(c)* Many Na^+ ions flow into the cytosol, and fewer K^+ ions flow out to the extracellular space through the pore, which results in depolarization of the membrane (note the reversal of charges from *b* to *c*).

The change in the receptor's structure caused by acetylcholine is reversible. After about 1 ms, the pore shrinks. Free (unbound) acetylcholine in the synaptic cleft is hydrolyzed by the enzyme **acetylcholinesterase**. The hydrolysis reaction is

$$\text{Acetylcholine} + H_2O \rightarrow \text{acetate} + \text{choline} + H^+ \qquad \Delta G^{\circ\prime} = -4.9 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 7.2)}$$

The elimination of free acetylcholine from the synaptic cleft leads to the dissociation of bound acetylcholine from the receptor according to the description in section 7.5. Thus, the receptor returns to its initial state, ready to receive a new swarm of acetylcholine molecules when the synapse is excited again. In the meantime, the Na^+–K^+ pump (which is present in the

plasma membrane of muscle cells as well as neurons) restores the resting Na^+ and K^+ concentrations, along with the resting potential, so that a new postsynaptic potential can be generated.

Malfunctioning of the acetylcholine receptor is related to **myasthenia gravis** (the term, which blends Greek and Latin, means "severe muscle disease"). In most cases of this disease, the body produces antibodies against the acetylcholine receptor. Less often, mutations prevent the receptor from operating properly. Problems like these result in inability of the muscles to receive nerve signals, leading to muscle weakness and atrophy.

We have reached the end of the nervous transmission of the signal for muscle activity. Postsynaptic potentials excite the plasma membrane of muscle cells and trigger a series of delicate biochemical processes culminating in the contraction of a muscle. These processes are the subject of the next chapter.

7.8 Changes in Motor Neuron Activity During Exercise

How does the central nervous system instruct a muscle to go from the resting state to the exercising state? Basically, it does so by generating action potentials along the motor neurons that innervate the muscle. And how does the nervous system instruct a muscle to contract more strongly or less strongly? One might think that the amplitude of action potentials rises to deliver a more forceful signal, but this is not the case: The amplitude remains at about 100 mV (from –70 to +30 mV). The way the nervous system controls contractile force is, first, through the number of motor units that are excited, or *recruited*, as we say.

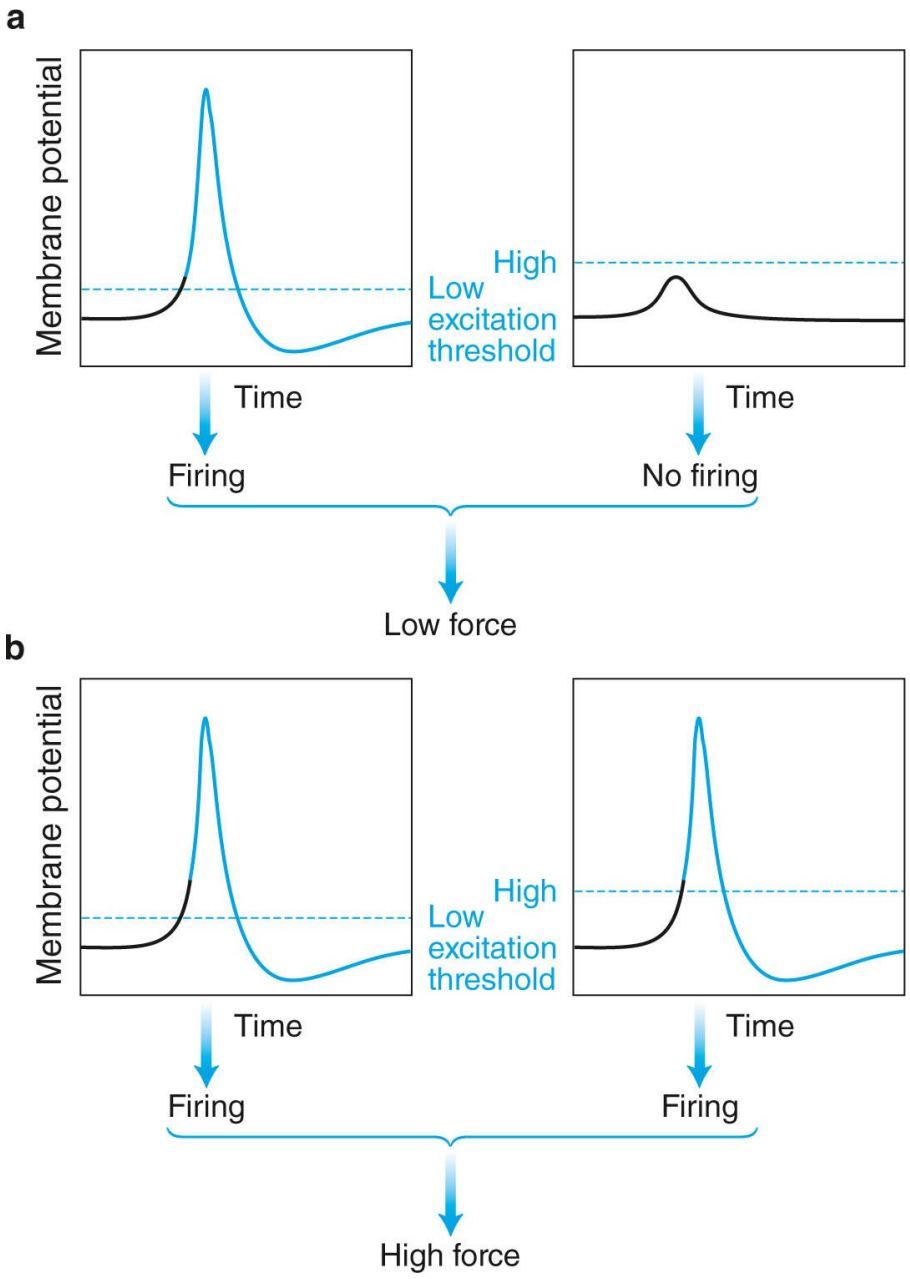

Figure 7.16 **Control of muscle contractility by motor unit recruitment.** The amount of force exerted by a muscle depends on how many of its motor units fire. *(a)* A slight rise in the resting potential (black) makes a neuron with a low excitation threshold fire (color) but is insufficient to cause a neuron with a high excitation threshold to fire; thus it results in low force development. *(b)* A higher rise in the resting potential makes both neurons fire, thus resulting in high force development.

As mentioned in section 7.3, there is a threshold of membrane potential, above the resting potential, that must be exceeded for an action potential to develop. As it turns out, the excitation threshold is not the same for all

neurons. Thus, a slight increase in membrane potential will excite only neurons with a low excitation threshold (figure 7.16). As the central nervous system commands more forceful contractions, the membrane potential gradually rises above the excitation threshold of more and more neurons, thus causing their additive recruitment. As more motor units are recruited, more force develops. The magnitude of the excitation threshold generally hinges on motor neuron size; specifically, on axon thickness: Motor neurons with thin axons have low excitation thresholds, whereas motor neurons with thick axons have high excitation thresholds.

A second, and finer, means of controlling contractile force is through the frequency of action potentials fired by motor neurons. This frequency, termed the **motor unit firing rate**, may go from about 5 Hz in light exercise to 65 Hz in maximal exercise. (Hz is the symbol of the hertz, a unit of frequency equal to one event per second.) An increase in motor unit firing rate results in a higher rate of acetylcholine release at the neuromuscular junction and a stronger stimulus for the muscle to contract (figure 7.17). I will return to this way of controlling contractile force to provide a full explanation in section 8.11.

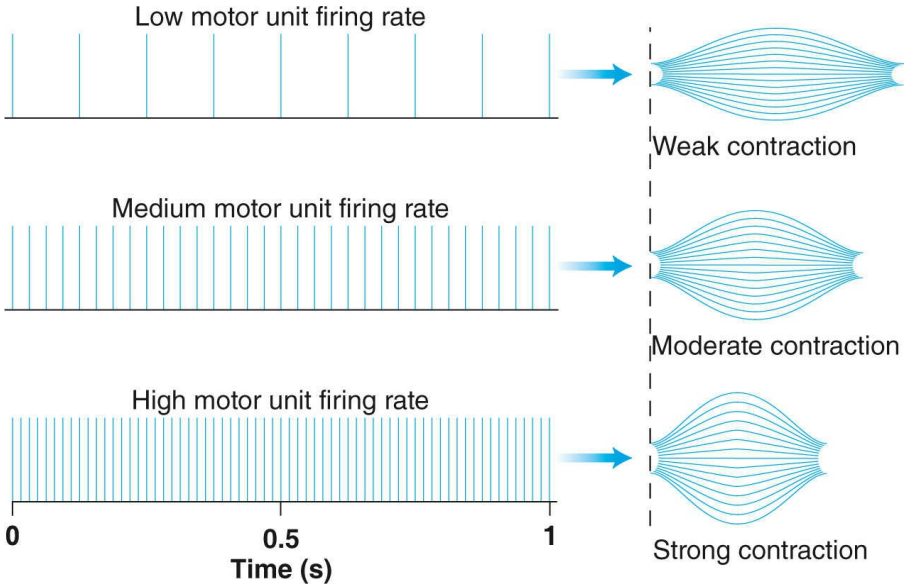

Figure 7.17 Control of muscle contractility by the motor unit firing rate. As the frequency at which motor neurons fire action potentials (colored spikes) increases (from top to bottom), so does the force of muscle contraction.

An inevitable corollary of exercise is **muscle fatigue**, or plain **fatigue** in the context of this book. Fatigue has traditionally been defined as *failure to maintain required or expected force* and divided into central fatigue (emanating from the central nervous system) and peripheral fatigue (resulting from inability of the motor unit to perform). Over the years, however, researchers have realized that this definition missed an important component —namely, the sensation of fatigue in the exerciser. Thus, Roger Enoka and Jacques Duchateau have proposed that we define fatigue as *a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability.* I will return to this definition in section 14.25.

Although fatigue has been studied extensively for more than a century, its etiology remains largely unclear. Moreover, the etiology of fatigue in one exercise may be quite different from that in another. Thus, what we usually describe are *possible* causes of fatigue in various exercises. These causes involve processes described in the present chapter (that is, the electrical and chemical transmission of nerve signals, as well as muscle excitation) and processes described in subsequent chapters. For this reason, we will consider the matter of fatigue several times throughout the rest of the book.

7.9 A Lethal Arsenal at the Service of Research

An unusual danger lurks in the swamps and streams of the vast Amazon basin. In places where other fish barely survive because of the low oxygen content of water, a fish answering to the telltale name *Electrophorus electricus* thrives. *E. electricus,* commonly known as “electric eel” (although technically not an eel), is a living battery, with the positive pole at its head and the negative one at its tail. While immobile, it produces no electricity. Once it starts moving, however, it generates discharges of as much as 800 V. Small animals in its vicinity (or, worse, in contact with it) are killed, and larger mammals get dizzy and may drown. We humans can withstand a single shock.

An equally unpleasant surprise awaits the fish that swim in temperate and

tropical seas. This one is an electric ray of the *Torpedo* genus, which uses discharges reaching 200 V to capture prey or fend off prospective predators. In antiquity, Roman physicians used the electric ray as a therapeutic agent.

The fugu, or puffer fish, is a culinary delicacy in Japan. However, if the person preparing the food does not meticulously remove the skin and liver, the fish is going to be someone's last meal. Fugu is armed with **tetrodotoxin**, an extremely potent poison, of which a mere 1 to 2 mg is estimated to be lethal to an adult human.

Equally horrible is **saxitoxin**, a poison produced by marine microorganisms called *dinoflagellates*. Humans are not usually affected directly but can be poisoned after consuming filter-feeding mollusks (shellfish), such as mussels and clams, which concentrate the toxin in their flesh. Less than 1 mg of saxitoxin is believed to be fatal to a human. Dinoflagellates can grow at amazingly rapid rates under certain environmental conditions, giving rise to the spectacular phenomenon known as *red tides*.

What do all of these lethal weapons have to do with the subject of this chapter? The biological warfare that I just described relies on either the electrical transmission of nerve impulses or its inhibition. The electric eel and electric ray, to begin with, are endowed with electric organs containing **electroplaxes**, or **electroplaques—**that is, stacks of flattened cells called **electrocytes**, which derive from muscle cells. Electrocytes have lost their contractile apparatus but have maintained the electrically excitable plasma membrane.

An electrocyte has two distinct faces (figure 7.18). One receives nerve endings and can be excited electrically from a resting potential of –90 mV to an action potential of +60 mV. The other is not excitable and remains at –90 mV. Thus, when the brain sends a nerve impulse to the innervated face, a voltage of +60 – (–90) = 150 mV develops between the two faces. Electrocytes are placed one on top of another, so that the voltages are additive. As a result, an electroplax of 5,000 electrocytes can cause a discharge of 750 V.

Thanks to their high content of excitable membranes, the electric organs are rich sources of the proteins responsible for the generation of action

potentials. Thus, researchers have used them as starting materials for isolating the Na^+ channel and the acetylcholine receptor. In fact, a large portion of our knowledge about these proteins comes from the study of purified Na^+ channel from the electric eel and purified acetylcholine receptor from the electric ray.

Tetrodotoxin and saxitoxin, on the other hand, owe their toxicity to their blocking of the electrical transmission of impulses along the axons of neurons, which leads to inability of the muscles to contract. Death is caused by asphyxiation, when the diaphragm and other muscles that control respiration become paralyzed. Both neurotoxins bind very strongly and selectively to the Na^+ channel, thus preventing Na^+ ions from flowing into the cytosol and triggering action potentials. Both also contain positively charged **guanidinium** groups in their structures (figure 7.19), which form electrostatic bonds with (negatively charged) carboxyl group at the extracellular opening of the Na^+ channel. In this way, the toxin molecule obstructs the opening and does not allow Na^+ to pass. Thanks to these two toxins' high affinity and selectivity for the Na^+ channel, they have proven to be valuable tags for measuring the content of membranes in the Na^+ channel and monitoring the purification of the Na^+ channel in the laboratory.

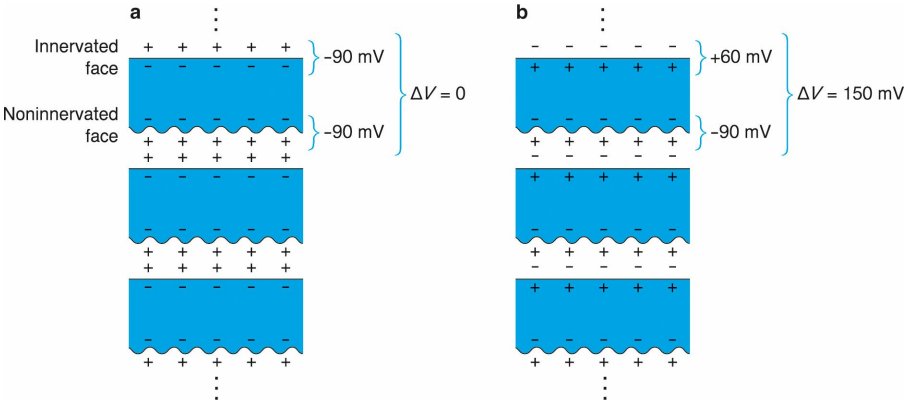

Figure 7.18 **Living battery.** The electric organs of certain fish contain piles of electrocytes with two different faces. One is relatively flat, innervated, and, thus, excitable; the other is intensely folded, not innervated, and, thus, not excitable. When the cells are not excited *(a)*, there is no potential difference between the two faces. However, when a nerve impulse elicits an action potential on the innervated face *(b)*, a large ΔV develops. The stacking of thousands of electrocytes in an electric organ results in the generation of stunning electric shocks.

The poisonous ingredients in the venoms of many snakes are also

neurotoxins. **Dendrotoxin**, present in the venom of the African black mamba, occludes the K^+ channel in neurons, thus preventing K^+ ions from flowing out of the cytosol and restoring the resting potential after an action potential has developed. This effect prolongs the action potential, which results in excessive release of acetylcholine at the neuromuscular junction, uncontrolled muscle contraction, and asphyxiation. Dendrotoxin is a small protein (around 7 kDa) bearing a high percentage of amino acid residues with positively charged side chains (Lys and Arg), which are believed to bind to carboxyl groups in the K^+ channel in a way that prevents K^+ from passing through. By analogy to tetrodotoxin and saxitoxin, the high affinity and selectivity of dendrotoxin for the K^+ channel has rendered it a valuable tool in studying the structure and function of the protein.

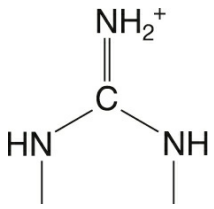

Figure 7.19 **Guanidinium.**

Other snake neurotoxins exhibit high affinity and selectivity for the acetylcholine receptor. Several species of cobra and bungarus (a South Asian snake) kill their prey thanks to **α-cobrotoxin**, **α-cobratoxin** (which has a somewhat larger molecule than that of α-cobrotoxin), and **α-bungarotoxin**. These toxins are small proteins (7–8 kDa) too, with high percentages of positively charged amino acids. They bind to the acetylcholine receptor in the plasma membrane of muscle cells, thus blocking acetylcholine binding and, hence, neuromuscular communication. This effect leads to paralysis and death by asphyxiation. The toxins have been used to locate the acetylcholine receptor in membranes and track its isolation.

Finally, a poison of plant origin, curare, has also proven useful in biochemical research. Curare is a complex plant extract, in which the Amazon natives used to dip their arrow tips before hunting or fighting. Curare's most active ingredient, **tubocurarine**, is produced by the *Chondodendron tomentosum* plant and was isolated from a form of curare

that was transported from South America to Europe in bamboo tubes (hence the name). Tubocurarine, like the snake toxins mentioned earlier, competes with acetylcholine for binding to the acetylcholine receptor. The tubocurarine molecule contains two quaternary amino groups like the ones in acetylcholine, which explains why the former competes successfully with the latter. This competition provides another proof of the fundamental principle of modern biochemistry that *structure determines function,* or, in the words of one of the most eminent figures in the history of art, technology, and science, “Shape is the plastic representation of function” (Leonardo da Vinci).

Summary

The nervous system controls movement through intricate processes that take place in neurons of its central and peripheral sections. The signals that direct the muscles to move are of alternating electrical and chemical nature. They originate in the brain or spinal cord as action potentials in response to sensory input from all over the body. Action potentials are made possible by the presence of a resting potential across the plasma membrane of neurons. The resting potential is negative inside as compared with outside the neuron and is due to the action of the Na^+–K^+ pump, or Na^+–K^+ ATPase, which maintains the cytosolic Na^+ and K^+ concentrations lower and higher, respectively, than the extracellular ones at the expense of ATP. An action potential, then, is an instantaneous depolarization of the plasma membrane due to the opening of the Na^+ channel, which permits Na^+ to enter the cytosol, followed by the opening of the K^+ channel, which permits K^+ to exit the cytosol. Action potentials run along unmyelinated (naked) neurons or jump from one node of Ranvier to another in neurons that are myelinated (that is, surrounded by myelin sheath) until they reach the terminal branches, which form synapses with other neurons.

At the synapses, action potentials cause the opening of a Ca^{2+}

channel in the plasma membrane, through which Ca^{2+} enters the cytosol and forces synaptic vesicles to empty the neurotransmitter they contain into the synaptic cleft. Synaptotagmin 1 in the vesicle membrane serves as the Ca^{2+} sensor for this process and works with membrane phospholipids and the SNARE complex of proteins to achieve fusion of the vesicle and plasma membrane. The released neurotransmitter binds to receptor molecules in the postsynaptic membrane and alters their biological activity.

By employing electrical transmission (action potentials) and chemical transmission (neurotransmitter release), the command directing a muscle to contract reaches the neuromuscular junction, at which a motor neuron contacts the muscle cells that it controls in a motor unit. There, a specific neurotransmitter, acetylcholine, is released. Acetylcholine diffuses to the surface of muscle cells and binds to the acetylcholine receptor. The receptor opens, permitting mainly Na^+ ions to enter. In effect, the plasma membrane is depolarized, which triggers muscle activity. During exercise, we control contractile force through the number of motor neurons that fire and the motor unit firing rate. Fatigue appears as a decline in physical and cognitive function due to interactions between performance fatigability and perceived fatigability. The crucial roles played by the Na^+ channel, the K^+ channel, and the acetylcholine receptor in nervous transmission and neuromuscular communication are responsible for the evolution of a variety of very effective, and deadly, electrical and chemical weapons. These weapons, used by marine and land animals to capture prey and defend themselves, have also proven to be invaluable tools in biochemical research.

Problems and Critical Thinking Questions

1. Which protein is responsible for the resting potential in nerve and muscle cells?

2. Which proteins are responsible for action potentials in nerve and muscle cells?
3. Is the resting potential due to passive or active transport of ions? What about the action potential?
4. Distinguish voltage-gated from ligand-gated ion channels and give examples of each kind.
5. Although multiple sclerosis and myasthenia gravis share muscle weakness as a symptom, they have different causes. Which tissue (nervous or muscle) and which way of transmitting nerve signals (electrical or chemical) does each of the two diseases affect?
6. Neostigmine is a synthetic compound that acts as an acetylcholinesterase inhibitor (that is, it blocks the activity of the enzyme). Which of the two diseases—multiple sclerosis or myasthenia gravis—do you think neostigmine fights? How does it do so?
7. Acetylcholinesterase inhibitors are a double-edged sword: Although neostigmine is a therapeutic agent, a class of acetylcholinesterase inhibitors known as *organophosphates* (and including sarin) act as nerve agents and are used as weapons of mass destruction. Why are these agents lethal?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

motor cortex
brain stem, brainstem
cerebellum
basal ganglion
central nervous system
peripheral nervous system

neuron
cell body
dendrite
axon
terminal branch
myelin
oligodendroglial cell, oligodendrocyte
Schwann cell
node of Ranvier
motor neuron
chemical gradient, concentration gradient
active transport
sodium–potassium pump, Na^+–K^+ pump
Na^+–K^+ ATPase
resting potential
electrical gradient
electrochemical gradient
polarized plasma membrane
depolarization
sodium channel, Na^+ channel
excitation threshold
voltage-gated channel
passive transport
action potential
potassium channel, K^+ channel
repolarization
hyperpolarization
nerve impulse
saltatory conduction
continuous conduction

multiple sclerosis
synapse
presynaptic
postsynaptic
synaptic cleft
neurotransmitter
synaptic vesicle
exocytosis
calcium channel, Ca^{2+} channel
synaptotagmin 1
SNARE complex
excitatory neurotransmitter
inhibitory neurotransmitter
rhodopsin
photoreceptor
rod
cone
rod outer segment
rod inner segment
synaptic body
disc
motor unit
neuromuscular junction
acetylcholine
quaternary amino group
choline acetyltransferase
acetylcholine receptor
nicotinic acetylcholine receptor
muscarinic acetylcholine receptor
nicotine

muscarine
somatic nervous system
autonomic nervous system
postsynaptic potential
ligand-gated channel
acetylcholinesterase
myasthenia gravis
motor unit firing rate
muscle fatigue, fatigue
tetrodotoxin
saxitoxin
electroplax, electroplaque
electrocyte
guanidinium
dendrotoxin
α-cobrotoxin
α-cobratoxin
α-bungarotoxin
tubocurarine

References and Suggested Readings

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009). Mammalian nicotinic acetylcholine receptors: From structure to function. *Physiological Reviews* 89: 73-120.

Chakraborti S, Dhalla NS, eds. (2016). *Regulation of Membrane Na^+–K^+ ATPase* (Springer, Berlin).

Dolphin AC (2016). Voltage-gated calcium channels and their auxiliary subunits: Physiology and pathophysiology and pharmacology. *Journal of Physiology* 594: 5369-5390.

Enoka RM, Duchateau J (2016). Translating fatigue to human

performance. *Medicine and Science in Sports and Exercise* 48: 2228-2238.

Ingram NT, Sampath AP, Fain GL (2016). Why are rods more sensitive than cones? *Journal of Physiology* 594: 5415-5426.

Rizo J, Xu J (2015). The synaptic vesicle release machinery. *Annual Review of Biophysics* 44: 339-367.

Zheng J, Trudeau MC, eds. (2015). *Handbook of Ion Channels* (CRC Press, Boca Raton).

CHAPTER 8

Muscle Activity

Learning Objectives

After reading this chapter, you should be able to do the following:

- List the unique or unusual features of a skeletal muscle cell, including the proteins that constitute the myofibrils.
- Discuss the sliding filament theory.
- Describe the properties and structure of myosin and assign each property to a segment of myosin.
- Describe the two forms of actin and explain its interaction with myosin and ATP.
- Draw a sketch of the sarcomere showing the positions of its major proteins.
- Discuss the mechanism of force generation in a sarcomere.
- Contrast the three types of muscle activity and explain how myosin and actin interact in each case.
- List and differentiate the main types of human muscle fiber.
- Describe how muscle activity is controlled at the level of the thin filaments.
- Explain how excitation of a muscle fiber is coupled with contraction.

In this chapter, we will examine how skeletal muscle activity, the most impressive form of movement in living organisms, is carried out and controlled. Your appreciation of the processes involved will grow as we explore the exceptional organization of the contractile elements of muscle cells and the multitude of delicate molecular interactions among these elements. Proteins play the leading part in these processes, thus justifying their name once more. The most abundant of muscle proteins, myosin, possesses the rare (though not unique) ability to convert the free-energy change of a reaction into kinetic energy. For this reason, we often call myosin a **molecular motor**.

8.1 Structure of a Muscle Cell

A muscle cell is extremely specialized. Although it obeys the basic principles of structure and function of all animal cells, it exhibits some features that are uncommon or even unique. To begin with, it is **multinucleated**; that is, it contains many nuclei lying just underneath the plasma membrane. This is the case because each muscle cell is formed by the fusion of many mononucleated **myoblasts** (precursors of muscle cells) during development. In addition, a muscle cell is **postmitotic**, meaning that it cannot divide by mitosis. Because muscle cells look like long tubes, they are better known as **muscle fibers**. Biochemists and physiologists refer to two components of a muscle cell by means of special terms: **sarcolemma** (“wrapping of the flesh” in Greek) for the plasma membrane and **sarcoplasm** for the cytoplasm.

When observed under a light microscope, a skeletal muscle fiber presents, perpendicular to its longitudinal axis, densely packed striations of alternating dark and light color (figure 8.1). Dark stripes are known as **A bands** and light stripes as **I bands**. The presence of bands is the reason that skeletal and cardiac muscles are called **striated muscles**. In contrast, striations are not as evident in smooth muscle, because it is less well organized.

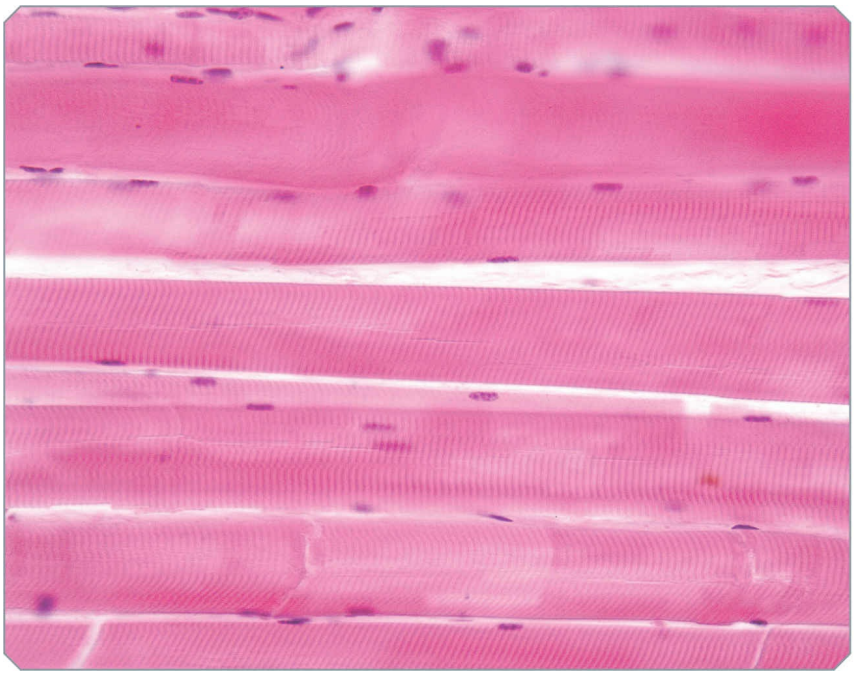

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Figure 8.1 **Muscle fibers.** Skeletal muscle fibers present intense transverse stripes under a light microscope. We call the dark stripes *A bands* and the light stripes *I bands*. The micrograph also shows many dark, elongated dots, which are nuclei belonging to the muscle fibers themselves, to satellite cells (see section 13.5), to connective tissue cells, and to blood vessel cells.

A muscle fiber is filled with about a thousand parallel rods of contractile material (think of a pack of spaghetti) embedded in cytosol. Each rod, or **myofibril**, measures 1 to 2 μm in diameter. The alternation of A and I bands, which, as we saw, characterizes the muscle fiber as a whole, persists in every myofibril. In fact, a muscle fiber looks striated because of the alignment of A bands in adjacent myofibrils.

At higher resolution—under an electron microscope—one can see that the A and I bands are not uniform (figure 8.2*a*). Instead, the A band has a stripe, the **H zone**, in the middle, which is less dense than the rest of the band. In the middle of the H zone lies a dense line called the **M line**. The I band also bears a dense line in the middle; it is called the **Z line**, or **Z disc**.

The Z line is actually a disc if you think of it as a transverse section of a myofibril. Of course, one could argue the same about the M line. However, authors do not use the term *M disc.*

This symmetrical pattern is repeated along the entire myofibril and permits the definition of a minimal complete functional unit, within which we can witness and examine muscle activity. This unit, called the **sarcomere**, is defined as *the segment of a myofibril between two Z lines.* The sarcomere has a filamentous appearance and is approximately 2.3 μm long in a muscle at rest.

What lies behind this mosaic of bands, zones, and lines of different densities? Electron micrographs of cross-sections of a myofibril (figure 8.2*b*) reveal that it contains two kinds of filaments: **thick filaments**, with a diameter of about 15 nm, and **thin filaments**, with a diameter of about 7 nm. The I band consists of thin filaments, while the A band has a mixed makeup. Its H zone consists of thick filaments, whereas the rest of the band contains both kinds of filaments in a symmetrical arrangement: Every thick filament is surrounded by six thin filaments, and every thin filament is surrounded by three thick filaments. Filaments are composed of **contractile proteins**, which provide an example of the motile proteins mentioned in section 3.8. The thick filaments contain mainly **myosin**, whereas the thin filaments contain mainly **actin**, **tropomyosin**, and **troponin**. In addition, the M line contains **myomesin** and **M-protein**, whereas the Z line contains **α-actinin**.

The information just presented shows that thick and thin filaments interdigitate at the two ends of the A band (figure 8.3). A wealth of other information convinces one that, in addition, the two kinds of filaments interact through **cross-bridges** that are part of myosin. Approximately 500 cross-bridges protrude from the surface of each thick filament and point toward the surrounding thin filaments. The gap between thick and thin filaments covered by the cross-bridges is 13 nm. As we will see, the interaction of the myosin cross-bridges with actin generates the force of contraction.

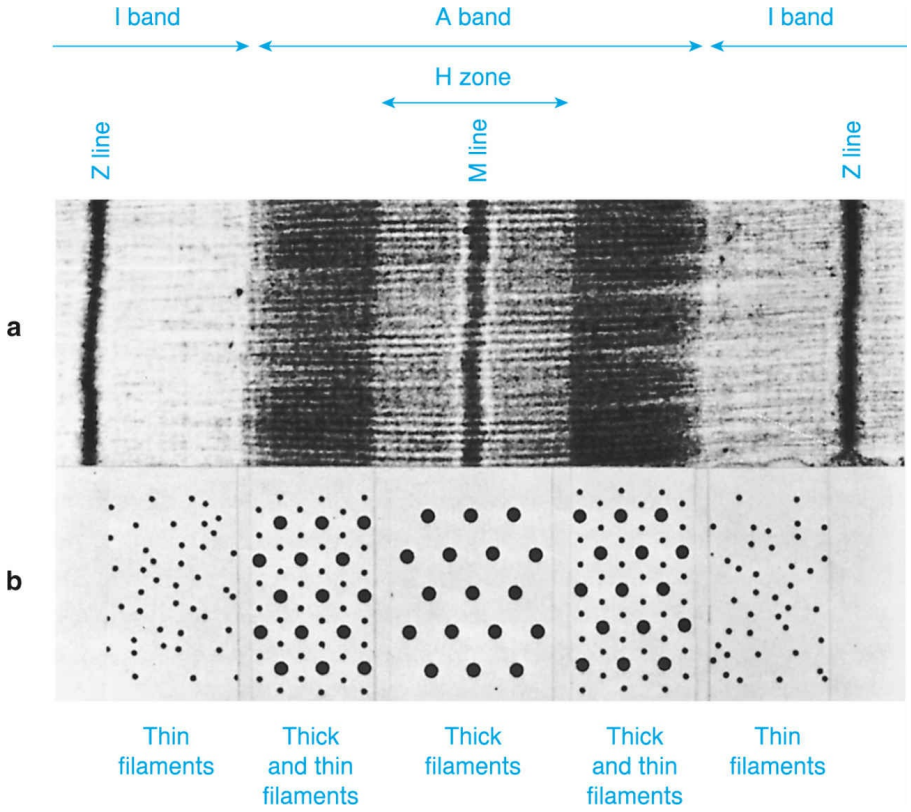


Figure 8.2 **Electron micrograph of a sarcomere.** *(a)* A longitudinal section of a myofibril presents a repetitive symmetrical pattern. The part between two Z lines shown here is a sarcomere. *(b)* Cross-sections at different points along the sarcomere reveal its structural details.

Courtesy of Dr. H.E. Huxley.

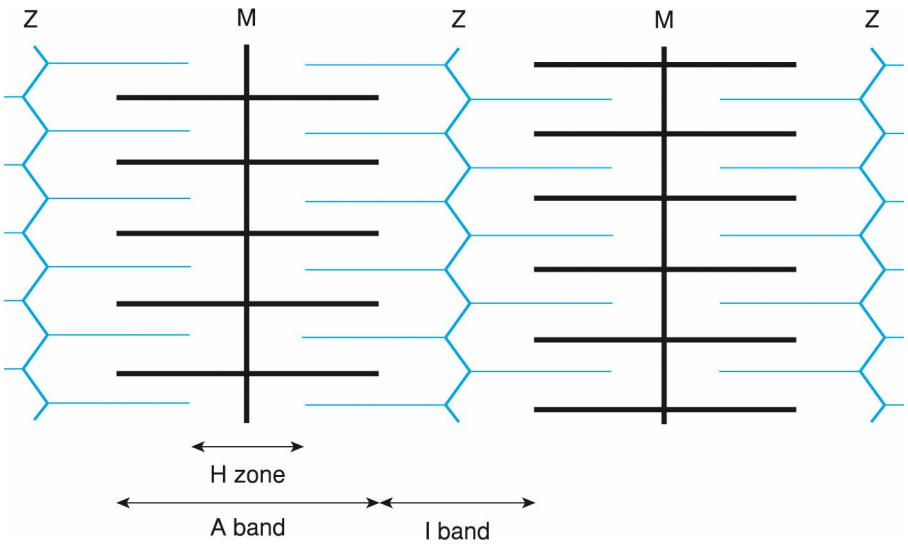


Figure 8.3 **Filament arrangement.** Deduced from the electron micrographs in figure 8.2, this diagram depicts two sarcomeres consisting of overlapping thick (black) and thin (colored)

filaments.

8.2 The Sliding Filament Theory

When a muscle fiber is activated and meets no insurmountable resistance, it contracts (shortens). This shortening is reflected in the fiber's elementary functional unit, the sarcomere; indeed, each sarcomere shortens, roughly in proportion to the whole fiber. However, the parts of the sarcomere do not shorten proportionally. Instead, as shown by examination of sarcomeres via electron microscopy and X-ray crystallography, the A band does not change in length, whereas the I band and the H zone shorten during contraction (figure 8.4). In the early 1950s, these observations led two independent groups of British investigators—one consisting of physiologists Andrew Huxley and Ralph Niedergerke and the other consisting of molecular biologist Hugh Huxley and biophysicist Jean Hanson—to propose the **sliding filament model**.

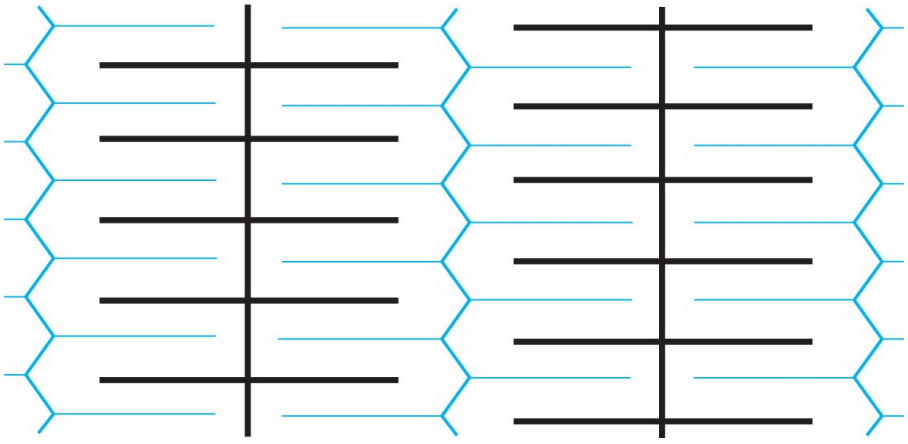


Figure 8.4 Sarcomere shortening. The length of each sarcomere in a muscle decreases when the muscle contracts. The decrease is not due to a shortening of the filaments but to an increase in their overlap. (Compare the length of the filaments and sarcomeres in this figure to those in figure 8.3.)

The sliding filament model maintains that *when the sarcomere contracts, the lengths of the thick and thin filaments do not change, but their overlap increases.* Therefore, *contraction is caused by the active sliding of thick and thin filaments past each other*. The sliding filament model has been verified

repeatedly by experimental data and is now accepted as a theory. But what draws thick and thin filaments past each other? To answer this question, we need to explore the major players in force generation—myosin and actin—along with their interplay.

> The difference between a model and a theory is that the former is a proposal, or hypothesis, which researchers put forward, based on empirical evidence, to explain a phenomenon. When evidence accumulates to such a degree that the model is indisputable (or accepted by the vast majority of experts in the field), it is "promoted" to the status of theory.

8.3 The Wondrous Properties of Myosin

Myosin is the leader of the protein pack during muscle contraction. It is the most abundant protein of muscle tissue, accounting for half of its protein mass, hence the name (after *mys*, the Greek word for muscle). Thanks to this abundance, myosin can be purified and studied with relative ease. Decades of research have ascribed three important biological properties to this protein.

- **Self-assembly.** Myosin is soluble in aqueous solutions of high ionic strength. If we start with such a solution of myosin at physiological pH and gradually add water until the ionic strength drops to the physiological level (0.3 mol · L^{-1}), the solution becomes turbid because the myosin molecules form filaments spontaneously. These filaments resemble the thick filaments of intact myofibrils. Thus we say that *myosin is capable of self-assembling.*
- **Enzyme activity.** Apart from constituting the main structural protein of muscle, myosin is an enzyme. As the Soviet biochemists Vladimir Engelhardt and Militsa Lyubimova discovered in 1939, myosin hydrolyzes ATP to ADP and P_i. This action renders myosin an ATPase. *The ΔG of ATP hydrolysis is exactly what drives muscle contraction.*
- **Binding to actin.** Myosin binds to the polymerized form of actin, the main component of the thin filaments (see section 8.5). This binding is necessary for the movement of thick and thin filaments past each other during contraction.

8.4 Myosin Structure

The structure of myosin is as interesting as its properties. The main features of the myosin molecule are as follows:

- It is very large, having a molecular mass of 520 kDa.
- It consists of six subunits (figure 8.5*a*): two large ones (220 kDa each) named **heavy chains** and four small ones (15-22 kDa each) named **light chains**.
- It is asymmetric, consisting of a double-headed region linked to a very long (170 nm) tail. The tail contains part of each heavy chain in α-helical conformation, coiled around the same part of the other heavy chain. Each of the two heads is formed by the rest of the heavy chain and two light chains. Each head is 16.5 nm long.

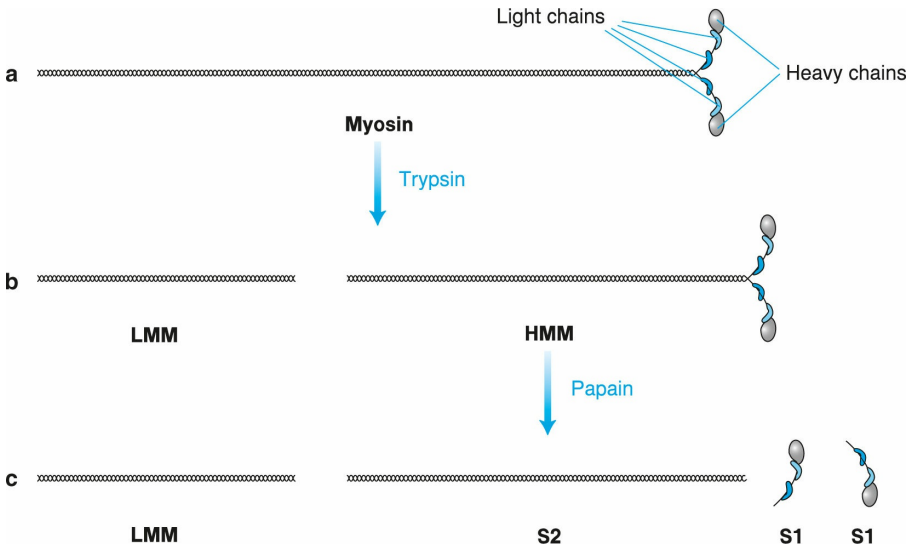


Figure 8.5 Myosin. *(a)* Myosin looks like two intertwined snakes. It consists of six polypeptide chains: two heavy chains, accounting for the tail and most of the two heads, and four light chains, shared by the heads. *(b)* Myosin can be cut by limited proteolysis. Treatment with trypsin (a protease) splits it into light meromyosin (LMM) and heavy meromyosin (HMM). *(c)* Further treatment of HMM with papain (another protease) divides it into two subfragments S1 and one subfragment S2.

The three properties described in the previous section may be attributed to specific regions of the myosin molecule. This specificity can be shown experimentally by **proteolysis**, that is, protein breakdown through hydrolysis

of their peptide bonds. Proteolysis is catalyzed by enzymes known collectively as **proteases**. Under mild conditions, proteolysis may yield information about functional **domains** (that is, parts that fold into compact shapes and have distinct functions) of a protein because such domains are usually separated by stretches of the polypeptide chain exposed to the environment and, therefore, vulnerable to mild hydrolysis.

Although myosin consists of six molecules (its six polypeptide chains), we often refer to it as a single molecule, as we do with double-stranded DNA.

Treatment with **trypsin,** a protease secreted by the pancreas and further discussed in section 12.1, cleaves myosin into two segments, named **light meromyosin** and **heavy meromyosin** (figure 8.5*b*). Heavy meromyosin contains the double-headed region and part of the tail, whereas light meromyosin corresponds to the remaining length of the tail. Another protease, **papain**, deriving from the papaya fruit, cleaves heavy meromyosin further into three **subfragments**: two identical heads denoted by S1 and one rod denoted by S2 (figure 8.5*c*).

How then are the three properties of myosin distributed among its segments? Light meromyosin has the property of self-assembly (figure 8.6*a*); that is, it forms filaments, just as intact myosin does. However, whereas cross-bridges protrude from the filaments of intact myosin, the filaments of light meromyosin are smooth. This difference shows that light meromyosin constitutes the trunk of the thick filaments, whereas heavy meromyosin corresponds to the cross-bridges. Light meromyosin lacks ATPase activity and does not bind to actin.

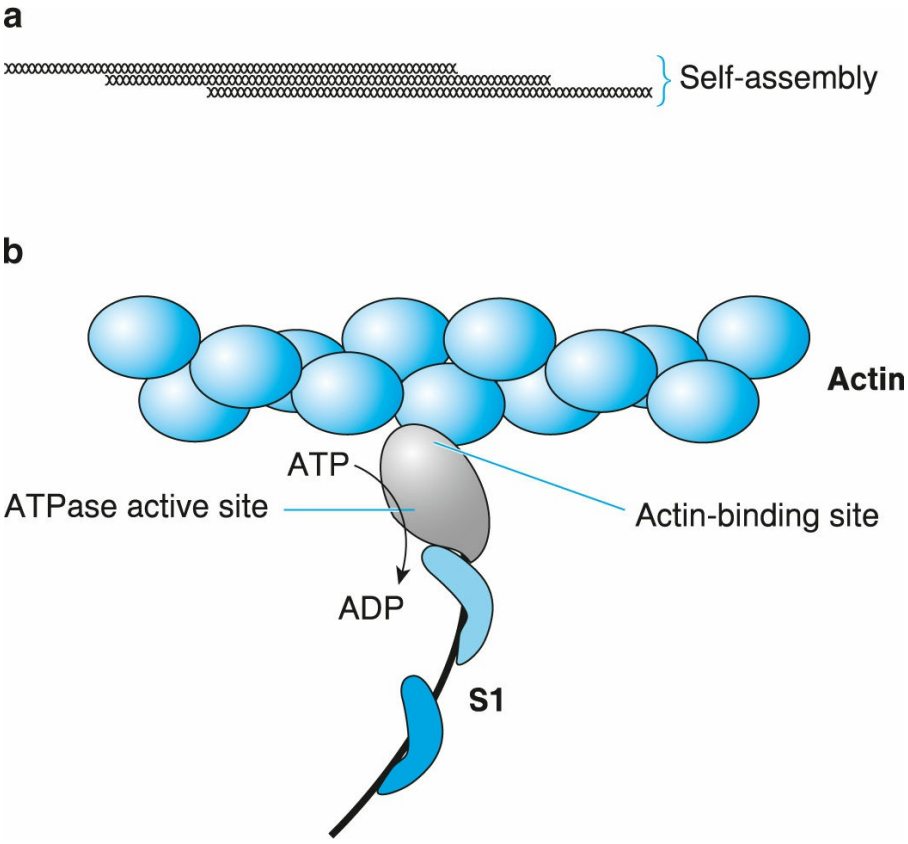


Figure 8.6 **Assigning properties to myosin's segments.** *(a)* LMM is capable of self-assembling to form smooth filaments corresponding to the trunk of the thick filaments in intact muscle. *(b)* S1 is capable of hydrolyzing ATP and binding to actin. The ATPase active site and actin-binding site are close to each other but distinct.

In contrast to light meromyosin, each S1 hydrolyzes ATP and binds to actin (figure 8.6*b*) but does not form filaments. Finally, S2 possesses none of myosin's properties; it just serves to link the other segments.

8.5 Actin

Actin is the main component of the thin filaments. It consists of a single 42 kDa polypeptide chain and exists in two forms. In solutions of low ionic strength (a term introduced in section 3.16), actin molecules are present as monomers. This form is designated **G-actin** (*G* stands for *globular*, as the form is almost that). If we increase the ionic strength toward the physiological value, monomers polymerize to form fibers of **F-actin** (*F* stands for *fibrous*). In F-actin, monomers are arranged in such a way that they

give the impression of a necklace with two intertwined strands of beads (figure 8.7). F-actin forms the trunk of the thin filaments, to which tropomyosin and troponin attach, as we will see in section 8.10.

Actin polymerization is accompanied by the hydrolysis of ATP to ADP. In the 1940s and 1950s, this fact misled several investigators to propose that the mechanism of muscle contraction was based on actin.

F-actin greatly increases the ATPase activity of myosin. To be exact, it does not increase the efficiency of ATP hydrolysis (since myosin alone is as good at that), but it does increase the rate at which the products (i.e., ADP and P_i) are released from the ATPase active site (this rate is low in the absence of F-actin). Thus, the active site is emptied of the products sooner and can host more ATP molecules per unit of time. To use a term introduced in section 3.16, F-actin increases the *turnover number* of myosin ATPase. Actin owes its very name to this activation of myosin ATPase.

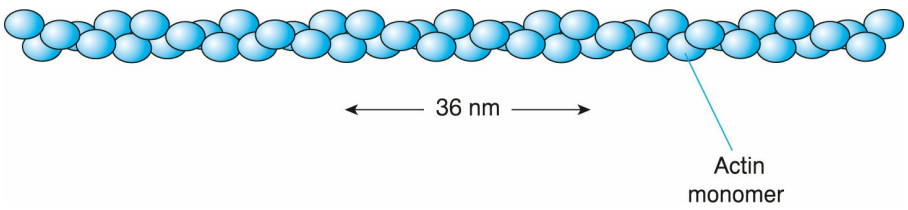


Figure 8.7 **Actin.** F-actin is formed by the welding of actin monomers that turn gradually as they go, giving the impression of two intertwined fibers. A full turn of F-actin encompasses 13 monomers and is 36 nm long.

When F-actin is present in solution with myosin, a complex called **actomyosin** forms, and the mixture becomes viscous (it thickens). The mixture can become thin by the addition of ATP, which means that *ATP dissociates actin and myosin.* However, because of the presence of F-actin, myosin hydrolyzes ATP quickly. When ATP is depleted, actomyosin reforms, but it can be dissolved by another addition of ATP. The description of these interactions by the Hungarian physiologist Albert Szent-Györgyi in the 1940s shed the first light on the mechanism of muscle contraction.

For ATP to dissolve actomyosin, magnesium ions must be present. The reason is that the actual substrate of myosin ATPase—and all ATPases—is

the complex of Mg^{2+} with the ATP^{4-} ion shown in figure 2.4.

8.6 Sarcomere Architecture

Now that we have examined the structure and interaction of myosin and actin in detail, we are able to explore the organization of the fundamental contractile unit of muscle. As already mentioned, the sarcomere is delimited by two Z lines composed mainly of α-actinin. Thin filaments, approximately 0.9 μm long, sprout from the Z lines (figure 8.8). The assembly of filaments may be aided by **nebulin**, an extremely large (about 600 kDa) and elongated protein that runs along every thin filament. It is believed to serve as a template on which the filament grows during myofibril formation or to stabilize its structure, as reviewed by Christine Henderson and associates.

The thick filaments, 1.5 μm long, lie in the center of the sarcomere, holding onto the M line. Cross-bridges protrude from the surface of the thick filaments in a helical arrangement every 14.3 nm along the filament axis, except for a 150 nm bare zone (that is, a zone without cross-bridges) in the middle.

The sarcomere owes part of its exquisite architecture to yet another giant protein, **titin**. This extraordinary, elastic protein is the largest polypeptide known (3,000-3,700 kDa). Twelve titin molecules, six in each half of the sarcomere, extend through each thick filament from the M line all the way to the Z lines. Between the end of the thick filament and the Z line, titin forms flexible connections, which endow muscle fibers

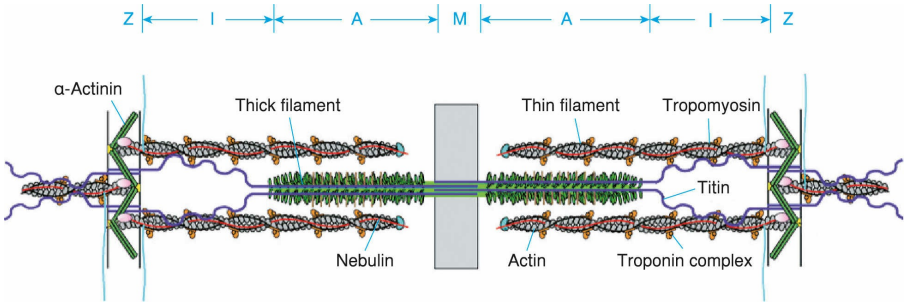


Figure 8.8 **Sarcomere organization.** A sarcomere contains precisely aligned thick and thin filaments anchored to the M and Z lines, respectively. The direction of the myosin molecules is reversed in the middle of the sarcomere. This reversal is also the case for the actin, tropomyosin, and troponin molecules in the thin filaments. These opposite directions ensure

the coordinated movement of the Z lines toward the M line during contraction. Thick and thin filaments are held in place by two giant elongated proteins, titin and nebulin. Note that the diagram depicts only a longitudinal section of the sarcomere. To get the whole picture, imagine a cylinder filled with thin filaments arranged in hexagonal symmetry around the thick filaments.

Reprinted from *Trends in Cardiovascular Medicine,* vol. 13(5), A.S. McElhinny et al., Nebulin: The Nebulous, Multifunctional Giant of Striated Muscle, pg. 15, Copyright 2003, with permission from Elsevier.

with passive elasticity. These connections center thick filaments in the sarcomere, so that myosin develops equal forces in both halves of the sarcomere.

Although titin is credited with the elasticity of muscle fibers, the elasticity of the whole muscle derives primarily from the connective tissue (mainly in the form of collagen) that surrounds muscle fibers (the endomysium), bundles (or fascicles) of muscle fibers (the perimysium), and the entire muscle (the epimysium).

The direction of the myosin molecules is opposite in the two halves of the thick filament: The tails point to the middle of the sarcomere (hence the bare zone), whereas the heads point to the Z lines. The thin filaments are directional too. The direction is the same for all thin filaments in one half of the sarcomere but is reversed in the other half. Thus, we can generalize by saying that *both thick and thin filaments have opposite directions in the two halves of the sarcomere.* This reversal of directions enables the thick filaments to pull the thin filaments toward the M line during contraction, thus decreasing the distance between the Z lines.

8.7 Mechanism of Force Generation

We have now all that is needed to answer the question posed at the end of section 8.2: What draws thick and thin filaments past each other? Although we do not know the mechanism of this process in every molecular detail, we do have a fairly good picture of it.

Contractile force is generated by repeated cycles of attachment, pulling, and detachment between the myosin heads in the thick filaments and F-actin in the thin filaments. Each head in a myosin molecule operates independently

of the other. At rest (figure 8.9*a*), the heads protrude from the thick filaments but are unable to bind to the thin filaments because, as we will see in section 8.10, tropomyosin covers the binding sites on actin. ATP is hydrolyzed on the heads at a slow rate because the binding of actin (which, as we saw in section 8.5, activates myosin ATPase) is hindered.

When the muscle is excited, tropomyosin moves to the side (for a reason that we will see in section 8.10), allowing the myosin heads to stick to actin monomers on the thin filaments (figure 8.9*b*). This action is followed by an important conformational change in S1, that is, a swinging of the stalk to which the light chains are bound. The stalk is known as the **lever arm** and swings in the direction of the M line. Since S1 is hooked to the thin filament, the swinging of the lever arm results in a movement of the thin filament relative to the thick filament (figure 8.9*c*), bringing the Z line a bit closer to the M line. The movement of the myosin head is called the **power stroke** because it generates muscle contraction.

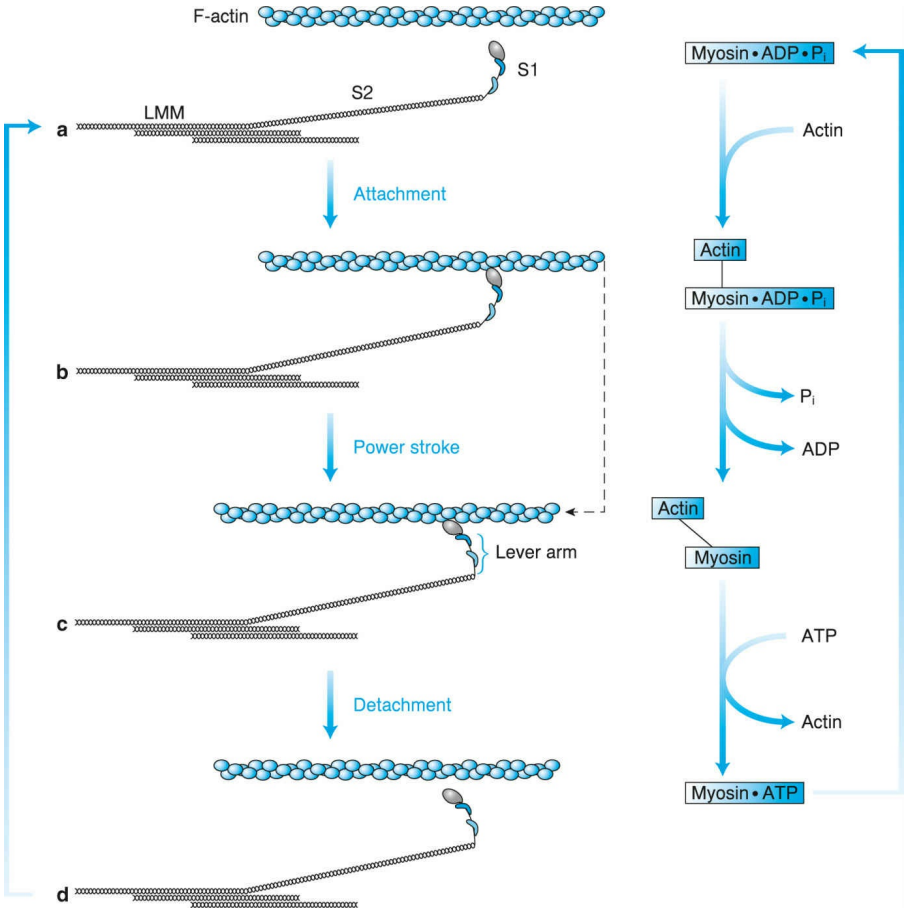


Figure 8.9 **Mechanism of muscle contraction.** The most probable mechanism of muscle contraction, depicted schematically at the left and in terms of molecular interactions at the right, is as follows. *(a)* At rest, S1 of myosin hydrolyzes ATP to ADP and P_i but does not liberate the products readily and does not bind to F-actin. (For clarity, only one of the two S1 in a myosin molecule is shown.) *(b)* Excitation of the muscle fiber leads to the attachment of S1 to F-actin. *(c)* The lever arm swings, moving the thin filament relative to the thick filament. (Note the black broken line and arrow.) At the same time, P_i and ADP leave the ATPase active site. *(d)* ATP enters the active site, forcing S1 to detach from actin. *(d → a)* S1 hydrolyzes ATP, resumes its initial orientation, and is ready to start all over.

The power stroke is accompanied by the departure of P_i and then ADP from the ATPase active site. Once the active site is emptied of the ATP hydrolysis products, ATP moves in, breaking actomyosin (figure 8.9*d*). S1 hydrolyzes ATP, returns to its original conformation, and is ready for another cycle of attachment, pulling, and detachment. The cycle is repeated for as long as the binding of actin and myosin is allowed. This mechanism of force generation explains what I mentioned in the introduction of this chapter, that is, the fact that myosin converts the free-energy change of a reaction (ATP hydrolysis) into kinetic energy, thus acting as a molecular motor.

8.8 Types of Muscle Activity

The swinging of myosin's lever arm is unidirectional: It moves only toward the M line, not toward the Z line, within a sarcomere. As a result, *a muscle can be shortened but not stretched by its own power*. Because such shortening activity moves the Z lines toward the center of the sarcomere, it is termed **concentric**. This is the kind of activity that I described in the previous section.

Although myosin can only shorten a muscle, concentric activity is not the only type of muscle activity. When the muscle meets a resistance it cannot overcome, such as when we push against a firm wall, the muscle's length does not change. This type of activity is **isometric** (meaning "measuring equal" in Greek). Finally, it is even possible for a muscle to lengthen despite developing contractile force, if a higher opposing force is applied to it. In that case, it performs **eccentric** activity, as does the biceps brachii of a person losing a *bras de fer*.

What does the contractile machinery do in the case of isometric or eccentric activity? In both cases, the myosin heads attach to and detach from the thin filaments, as described in the previous section. Here, however, myosin is unable to move the thin filaments toward the M line because of the high opposing force. In the case of isometric activity, the myosin heads attach to roughly the same points on the thin filaments over and over again (figure 8.10), just as a car's wheels spin in the mud. In the case of eccentric activity, the myosin heads attach to points on the thin filaments that are farther away from the Z line each time, just as a car can be pulled downhill by gravity although the wheels are spinning in an uphill direction.

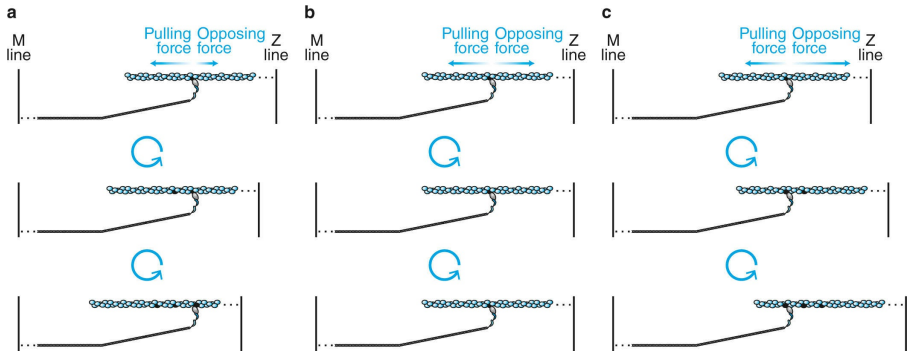


Figure 8.10 **Three types of muscle activity.** *(a)* In concentric activity, the myosin heads are able to pull the thin filaments toward the center of the sarcomere because the force they develop is higher than the force that opposes the movement. As a result, the muscle shortens and, in each cycle of attachment, pulling, and detachment, a myosin head grasps actin monomers (marked in black) that are closer to the Z line. *(b)* In isometric activity, the myosin heads are unable to pull the thin filaments toward the center of the sarcomere because the force they develop is merely equal to the force that opposes the movement. As a result, the muscle's length does not change, and in each cycle a myosin head grasps the same actin monomer. *(c)* In eccentric activity, the myosin heads are again unable to pull the thin filaments toward the center of the sarcomere, this time because the force they develop is lower than the force that opposes the movement. As a result, the muscle lengthens, and in each cycle a myosin head grasps actin monomers that are farther away from the Z line.

Regarding eccentric activity, Walter Herzog and coworkers have proposed that titin plays a particular role in protecting muscle from excessive and potentially damaging stretching by increasing its stiffness and decreasing its free length. The increase in titin's stiffness is due to its binding of Ca^{2+} (the cytosolic concentration of which increases during muscle activity, as we will see in sections 8.10 and 8.11), whereas the decrease in titin's free length is

due to the attachment of a longer part of it to the thin filaments near the Z line (see figure 8.8).

Although a muscle produces no work during isometric or eccentric activity, it nevertheless spends energy, since ATP is hydrolyzed to fuel the futile cycles of myosin head attachment to and detachment from the thin filaments. Our everyday life involves a variety of concentric, isometric, and eccentric muscle activities that you can easily think of. The same, of course, is true of sporting events and training practice.

8.9 Myosin Isoforms and Muscle Fiber Types

The genome of an organism often contains genes, differing little in base sequence, that encode proteins differing little in amino acid sequence and serving essentially the same function. Slightly different forms of a protein can also arise from a single gene by alternative splicing of exons or by RNA editing, two processes that produce two or more similar mRNA, as we have seen in section 4.13. Such different forms of a protein are called **isoforms** and usually differ quantitatively in biological activity; that is, they possess more or less of it. Quantitative differences among isoforms often serve the specific needs of the cells in which they are synthesized.

> If a protein is an enzyme, its different forms are referred to as *isoenzymes* or *isozymes.*

Myosin is a protein displaying isoforms. Animals, including we humans, have multiple forms of both the heavy and the light chains of this protein, although the ones primarily affecting myosin action are the heavy chain isoforms. Adult humans have three such isoforms, denoted as I, IIa, and IIx (formerly IIb). Investigators separate and measure myosin heavy chain (MHC) isoforms by gel electrophoresis, a technique that I will explain in section III.7. A muscle fiber usually contains one MHC isoform, although some fibers contain two: I and IIa or IIa and IIx.

The MHC isoform classification forms the basis for the classification of muscle fibers as type I, IIA, or IIX (we use capital *A* and *X* when it comes to muscle fibers). To assign muscle fiber types, researchers take advantage of

the fact that fibers containing different MHC isoforms are stained differently when thin sections of muscles are soaked in specific solutions and can thus be discerned under a light microscope.

How do the three MHC isoforms differ? As reviewed by Stefano Schiaffino and Carlo Reggiani, myosin molecules with MHCI have the lowest ATPase activity, whereas those with MHC IIx have the highest. Consequently, type I muscle fibers have the lowest maximal shortening velocity, whereas type IIX have the highest. Type I fibers are often characterized as **slow twitch** and types IIA and IIX (collectively, type II) as **fast twitch**. Fast-twitch fibers are less economical (just as fast cars are), because they spend more ATP to maintain a given force. Finally, resistance to fatigue (assessed experimentally by measuring how slowly force drops when a fiber is repeatedly stimulated) decreases as we go from type I to type IIA to type IIX fibers. Table 8.1 summarizes the differences between fiber types regarding contractile activity.

Table 8.1 Properties of the Main Muscle Fiber Types in Humans Regarding Contractile Activity

	Muscle fiber type		
Property	**I**	**IIA**	**IIX**
Myosin ATPase activity	Lower	Intermediate	Higher
Maximal shortening velocity	Lower	Intermediate	Higher
Economy	Higher	Intermediate	Lower
Resistance to fatigue	Higher	Intermediate	Lower

A motor unit comprises muscle fibers of the same type, which are all excited when the motor neuron fires action potentials. The electrical activity in a muscle can be studied through **electromyography** by attaching electrodes to the skin over the muscle or, better, inserting needle electrodes into the muscle. It is believed that motor units with low excitation thresholds (section 7.8) control few muscle fibers of type I, whereas motor units with high excitation thresholds control many muscle fibers of type II. Thus, type I fibers are recruited first, followed by type II, as force develops.

Differences between muscle fiber types are not confined to contractile behavior but are extended to metabolism. However, before we discuss the

metabolic differences of muscle fiber types, we need to explore muscle metabolism both at rest and during exercise. Therefore, I will keep returning to the issue of muscle fiber types throughout part III, which addresses exercise metabolism.

8.10 Control of Muscle Activity by Ca_{2+}

For more than 130 years (since 1883), it has been known that muscles cannot contract in the absence of calcium cations. However, 80 years had to pass before the exact role of Ca^{2+} was discovered. Today we know that *Ca^{2+}controls muscle activity* and that it does so by permitting the binding of myosin to F-actin. This action of Ca^{2+} is not direct but indirect. As the Japanese physiologist Setsuro Ebashi discovered in the 1960s, control is exerted through tropomyosin and troponin, the two proteins that coexist with actin in the thin filaments, constituting about one third of the thin-filament mass. Let's meet them.

Tropomyosin has a molecular mass of 70 kDa and consists of two similar stringlike subunits in α-helical conformation. The two subunits wind around each other just as the myosin heavy chains do in the myosin tail. The resulting extremely long tropomyosin molecules join in a row to form fibers. Two such fibers run along each thin filament while following the twisting of the actin monomers (figure 8.11).

Tropomyosin owes its very name to the similarity of its structure and some of its physical properties to myosin; *trópos* means "mode" in Greek.

Troponin, symbolized as Tn, is a complex of three different subunits: TnC (18 kDa), TnI (24 kDa), and TnT (37 kDa). TnC binds Ca^{2+}, TnI binds to actin, and TnT binds to tropomyosin. Two troponin complexes appear on the two sides of a thin filament every 39 nm, which is approximately the length of a tropomyosin molecule. One troponin complex attached to one tropomyosin molecule controls approximately seven actin monomers.

When a muscle is at rest (relaxed), the cytosol has a very low [Ca^{2+}], approximately 10^{-7} mol · L^{-1}. Researchers believe that, in this case,

interactions among F-actin, TnI, TnT, and tropomyosin hold the latter close to the sites on the actin monomers where the myosin heads bind. Thus, tropomyosin hinders the interaction of thin and thick filaments. As we will see in the next section, muscle excitation by the nervous system results in the release of Ca^{2+} from an intracellular reservoir called the *sarcoplasmic reticulum*. This release causes a 100-fold surge in the cytosolic [Ca^{2+}], from 10^{-7} to 10^{-5} mol · L^{-1}.

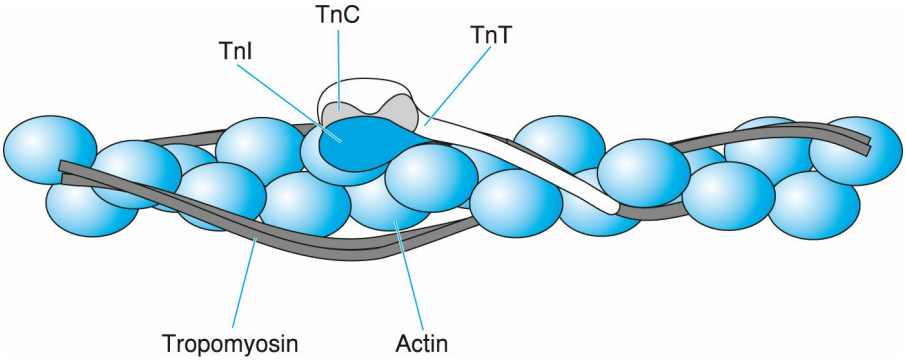


Figure 8.11 **Thin-filament proteins.** A thin filament consists of an F-actin fiber (the double necklace of figure 8.7), two series of tropomyosin molecules, and troponin (Tn) complexes placed at regular intervals. Each troponin complex consists of TnC, which is the Ca^{2+} acceptor; TnI, which binds to actin; and TnT, which extends by way of a long tail along tropomyosin.

Adapted from L. Smillie and S. Ebashi, *Essays in Biochemistry*, vol. 10, edited by P.N. Campbell and F. Dickens (Orlando, FL: Academic, 1974), 1–35; C. Cohen *Scientific America* 233 (1975): 36 - 45. Courtesy of L. Smillie.

The increased Ca^{2+} ions encounter TnC, bind to it, and elicit a change in its conformation. As a result, TnC detaches TnI from F-actin. This detachment lets tropomyosin move over the surface of the thin filament, away from the binding sites of myosin on F-actin. Myosin then binds to F-actin, and the muscle contracts. In fact, there is evidence that myosin binding to F-actin is cooperative; that is, the binding of some myosin heads facilitates the binding of additional ones by promoting the displacement of tropomyosin. This effect is reminiscent of the cooperative binding of O_2 to hemoglobin (section 3.12).

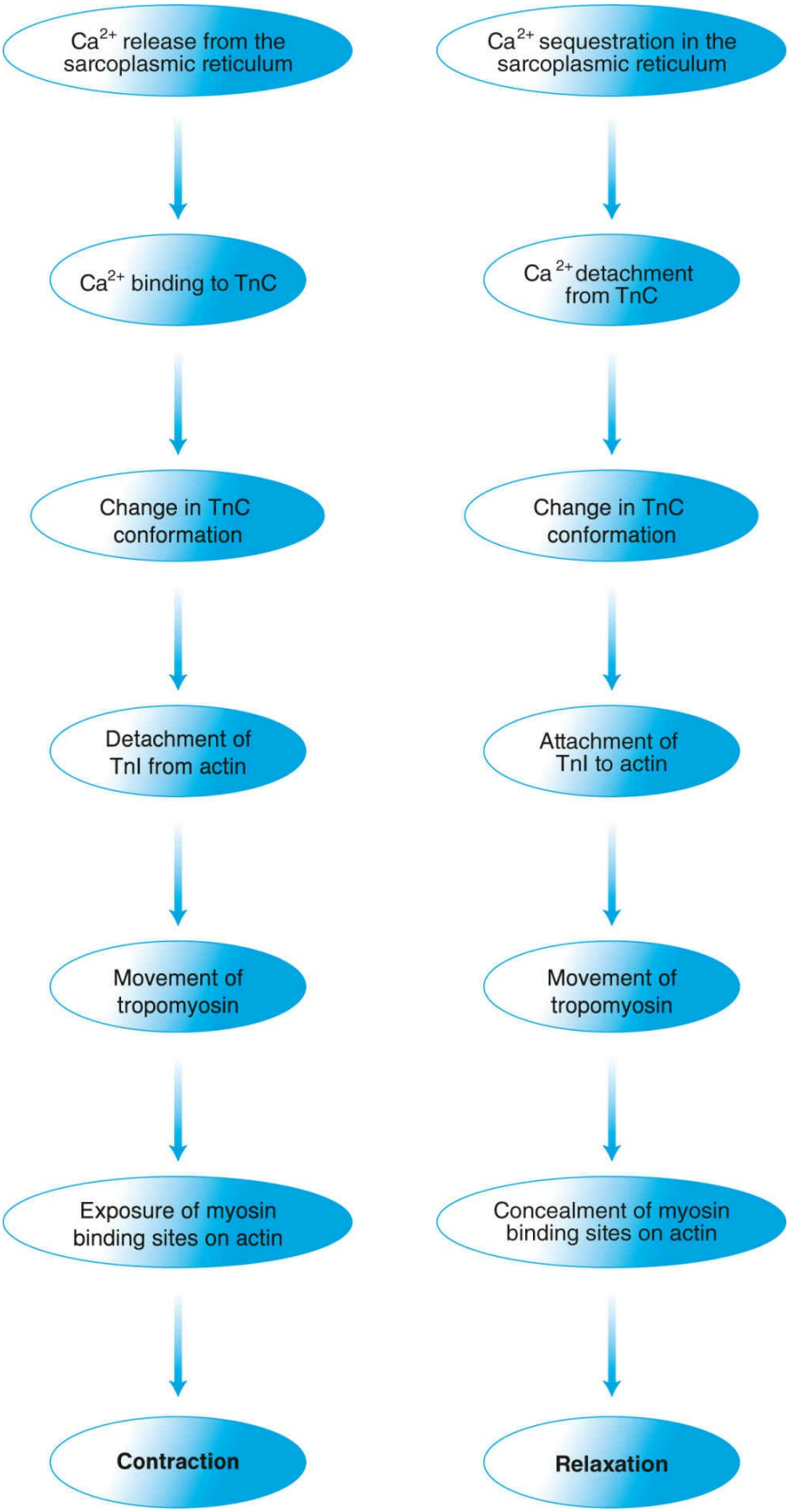

Ca^{2+} release from the sarcoplasmic reticulum
Ca^{2+} binding to TnC
Change in TnC conformation
Detachment of TnI from actin
Movement of tropomyosin
Exposure of myosin binding sites on actin
Contraction
Ca^{2+} sequestration in the sarcoplasmic reticulum
Ca^{2+} detachment from TnC
Change in TnC conformation
Attachment of TnI to actin
Movement of tropomyosin
Concealment of myosin binding sites on actin
Relaxation

Figure 8.12 Control of muscle contraction and relaxation by Ca^{2+}. Ca^{2+} controls muscle contraction (left) and relaxation (right) through a series of protein interactions triggered by, respectively, Ca^{2+} release from and sequestration in the sarcoplasmic reticulum and involving troponin, tropomyosin, actin, and myosin.

Muscle activity continues until Ca^{2+} is sequestered in the sarcoplasmic reticulum (in a manner that we will examine shortly). The actin–TnI–TnT–tropomyosin interaction is then restored. Tropomyosin returns to a position hindering cross-bridge formation, and the muscle relaxes. The chain of events through which Ca^{2+} controls muscle contraction is summarized in figure 8.12.

8.11 Excitation–Contraction Coupling

In chapter 7, we examined how a nerve impulse travels from its birthplace to the surface of a muscle fiber. In the present chapter, we have considered how the interior of a muscle fiber is constructed, how the fiber contracts, and how contraction is controlled by Ca^{2+}. Now we can complete the sequence of events leading from nervous excitation to muscle activity by providing the missing link: the transmission of the signal from the surface to the interior of a muscle fiber.

We saw that the release of acetylcholine at the neuromuscular junction and the subsequent opening of the acetylcholine receptor in the plasma membrane of a muscle fiber (the sarcolemma) results in its depolarization and in generation of action potentials (section 7.7). Action potentials are then transmitted to the interior of the fiber, traveling along a system of **transverse tubules**, also known as the **T system** (refer to the introductory figure of part II). The transverse tubules form an extension of the sarcolemma and are closely apposed to a complex membranous system of delicate sacs surrounding the myofibrils and covering the entire length of the sarcomere. This membranous system is called the **sarcoplasmic reticulum** and constitutes the Ca^{2+} reservoir that responds to nervous excitation. The sarcoplasmic reticulum is tethered to the sarcomere at the M line by what is believed to be **obscurin**, another giant sarcomeric protein (about 720 kDa), as discussed by Davide Randazzo and colleagues.

Ca^{2+} accumulates in the sarcoplasmic reticulum by the action of an integral protein of its membrane, the **Ca^{2+} pump** (figure 8.13). This protein consists of a single 110 kDa polypeptide chain, which is similar to the α subunit of the Na^+–K^+ pump. The Ca^{2+} pump constitutes 80% of the protein mass of the sarcoplasmic reticulum membrane and occupies nearly half of its surface. The pump sequesters Ca^{2+} from the cytosol and raises the [Ca^{2+}] inside the sarcoplasmic reticulum to above 10^{-3} mol · L^{-1}. As with the Na^+–K^+ pump, the energy for the operation of the Ca^{2+} pump comes from the hydrolysis of ATP. Hence, the pump is also referred to as **Ca^{2+} ATPase**. For every molecule of ATP it hydrolyzes, the ATPase transports two Ca^{2+} ions.

The similarity between the Ca^{2+} pump and Na^+–K^+ pump illustrates the existence of families of related proteins in living organisms. These proteins usually have a common ancestor, that is, a protein initially encoded by a single gene. This gene gives rise to many similar genes through repeated duplications and mutations in the course of evolution.

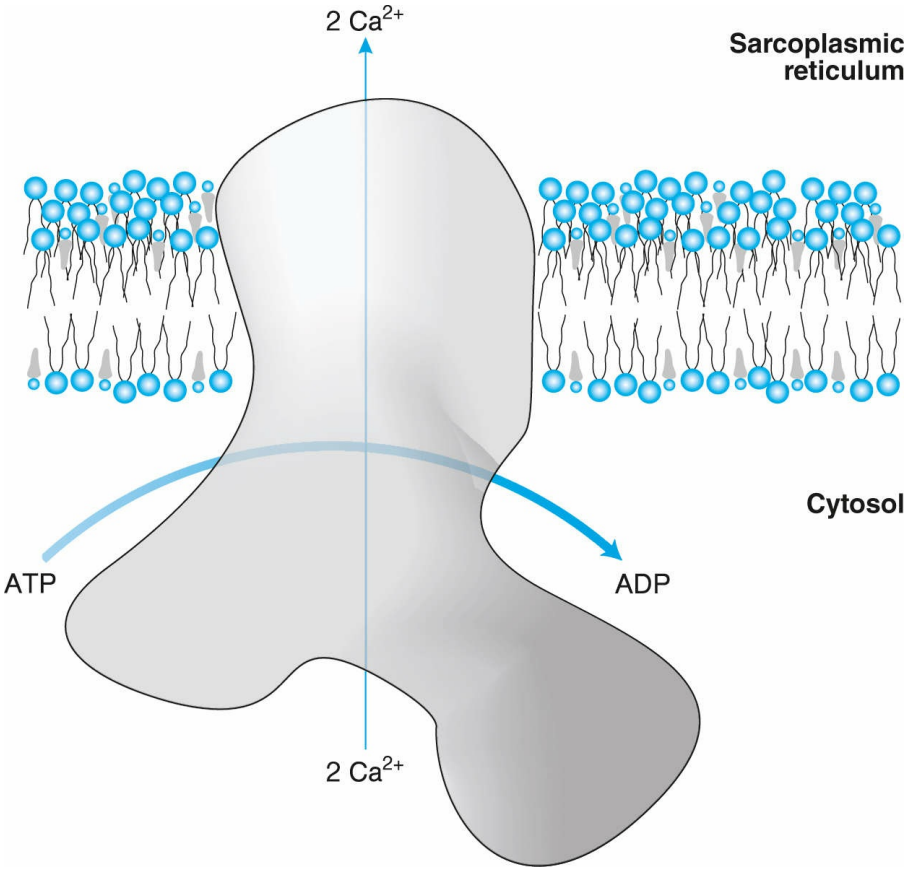


Figure 8.13 Calcium pump. The sarcoplasmic reticulum Ca^{2+} pump resembles the α

subunit of the plasma membrane Na^+–K^+ pump (cf. figure 7.5). The Ca^{2+} pump concentrates Ca^{2+} inside the sarcoplasmic reticulum at the expense of ATP. The protein and membrane are drawn to scale.

The maintenance of such a steep concentration gradient across the sarcoplasmic reticulum membrane ($10^{-3}/10^{-7}$ = 10,000-fold at rest) is facilitated by **calsequestrin**. This protein is located inside the sarcoplasmic reticulum and features approximately 40 low-affinity binding sites for Ca^{2+}, thus acting as a molecular sponge: By binding calcium cations loosely, it lowers their tendency to leak out of the sarcoplasmic reticulum without totally preventing them from doing so.

The transmission of an action potential from the sarcolemma to the transverse tubules causes the sudden opening of another integral protein of the sarcoplasmic reticulum membrane, one acting as a Ca^{2+} channel (figure 8.14). This channel is known as the **ryanodine receptor**. Exactly how the action potential opens the ryanodine receptor is not known with certainty. Possibly, it causes a conformational change in a protein of the transverse tubule membrane called the **dihydropyridine receptor**. It is believed that the dihydropyridine receptor is in touch with the ryanodine receptor in the adjacent sarcoplasmic reticulum membrane, and it may transmit the conformational change to it, thus resulting in its opening. You can find details about the structure and function of these remarkable proteins, which lie at the heart of excitation–contraction coupling, in the studies by Amédée des Georges and collaborators and by Montserrat Samsó.

Ryanodine and dihydropyridine are ligands that have been used in the laboratory to study the Ca^{2+} channel of the sarcoplasmic reticulum and the voltage-sensing protein of the transverse tubules, respectively. However, these substances are not physiological ligands for these proteins.

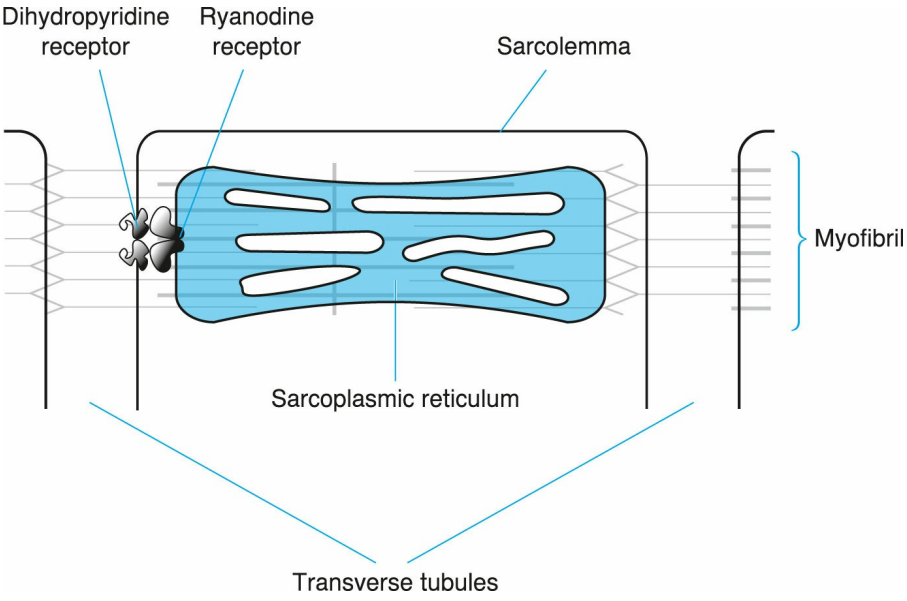


Figure 8.14 **From nervous excitation to muscle contraction.** The transverse tubules and the sarcoplasmic reticulum, two delicate membranous systems inside muscle fibers, permit the coupling of excitation to contraction. The transverse tubules carry action potentials from the sarcolemma to the depths of a muscle fiber. A voltage-sensitive protein of the transverse tubule membrane, the dihydropyridine receptor, undergoes a change in shape that opens an adjacent Ca^{2+} channel, the ryanodine receptor, in the sarcoplasmic reticulum membrane. The ryanodine receptor consists of four identical subunits, two of which are shown in this cutaway view. (Imagine two more lying behind.) Each subunit is thought to be in contact with a molecule of the dihydropyridine receptor. When the dihydropyridine receptor causes the ryanodine receptor to open, Ca^{2+} ions flow from the sarcoplasmic reticulum to the cytosol, where they bind to TnC and permit the interaction of actin with myosin. The sizes of the dihydropyridine receptor and the ryanodine receptor have been exaggerated for clarity.

The $[Ca^{2+}]$ gradient between the sarcoplasmic reticulum and the cytosol is so steep in the resting state that the rate of efflux through the ryanodine receptor upon excitation of the muscle fiber exceeds the rate of influx through the Ca^{2+} pump. Thus, the $[Ca^{2+}]$ in the cytosol rises 100 fold, although it remains well below the $[Ca^{2+}]$ inside the sarcoplasmic reticulum (figure 8.15). By analogy, think of a rich person possessing 10,000 gold coins and a poor person possessing one gold coin. If the rich person gives the poor person 99 coins, the poor person's money will increase 100 fold, but the rich person will still be wealthier and will have lost just 1% of his or her money.

When the cytosolic $[Ca^{2+}]$ rises to 10^{-5} mol $\cdot$ L^{-1}, the binding of Ca^{2+} to TnC activates muscle contraction as described in the previous section. When the action potential passes, the ryanodine receptor closes and prevents the

efflux of Ca^{2+}. Ca^{2+} ATPase then gets the upper hand: It brings Ca^{2+} back to the sarcoplasmic reticulum, thus decreasing the cytosolic [Ca^{2+}] from 10^{-5} to 10^{-7} mol · L^{-1} and causing muscle relaxation. This process is thought to require one third of the ATP hydrolyzed in an active muscle (the rest being hydrolyzed primarily at the myosin heads). Thus, energy is needed not just for contraction but also for relaxation. The two processes take place within a fraction of a second each. This kind of instantaneous change in contractile force is referred to as a **twitch** (hence the terms *slow-twitch* and *fast-twitch* in relation to fibers).

Myosin ATPase and Ca^{2+} ATPase are the major energy consumers in an active muscle. The kinetic energy produced is generally less than half of the energy spent; the precise value depends on the mechanical characteristics of muscle activity (such as type and load). The rest of the energy spent is released as heat, which is why body temperature rises during exercise. This effect may be undesirable in some cases (for example, during prolonged exercise or during exercise in the heat) and desirable in others (for example, during warm-up before a main exercise or during exercise in the cold). An additional instance of useful heat production with exertion is shivering (the uncontrolled shaking activity of muscle) in response to cold exposure. The resulting **shivering thermogenesis** helps to raise body temperature and protects us from dangerous hypothermia.

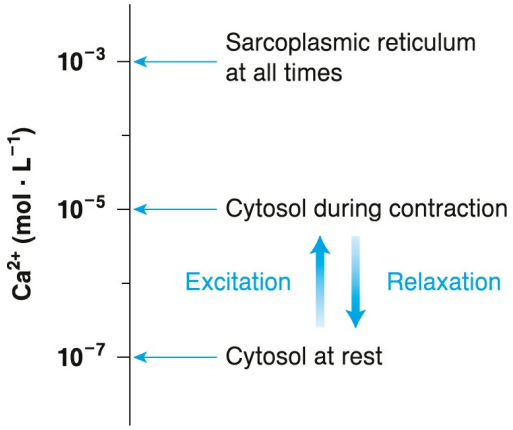


Figure 8.15 **Ca^{2+} concentration gradients in muscle fibers.** A large Ca^{2+} reservoir, the sarcoplasmic reticulum, causes an instantaneous 100-fold increase in the cytosolic [Ca^{2+}] when the muscle is excited by a nerve impulse. The cytosolic [Ca^{2+}] returns to its resting value during relaxation. The scale is logarithmic (as in figure 4.11).

In section 7.8 we saw that one way of controlling contractile force is through the motor unit firing rate. At that point we did not have all that was needed to fully explain this process, but now we do. When the motor unit firing rate is low, there is sufficient time for the cytosolic [Ca^{2+}] to return to baseline after each action potential and for the muscle fibers of the motor unit to relax. However, as the motor unit firing frequency rises, the cytosolic [Ca^{2+}] cannot make it back to baseline between action potentials, and the muscle fibers do not relax. Instead, the twitches begin to overlap, and force begins to rise, initially with oscillations, until it reaches a plateau called **tetanus** (not to be confused with the namesake disease), at which point the motor unit develops maximal force (figure 8.16).

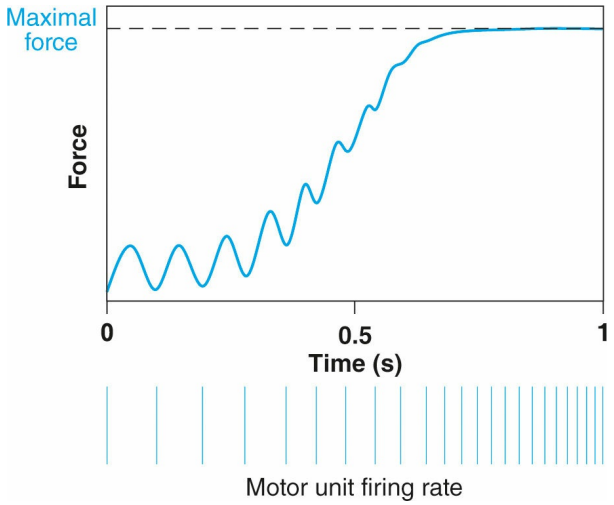


Figure 8.16 Controlling the contractile force through the motor unit firing rate. Increasing the motor unit firing rate results in the fusion of muscle twitches until a tetanic contraction appears, corresponding to maximal force. The colored spikes represent action potentials. This figure helps us better understand figure 7.17.

Summary

Muscle activity is due to the exquisite design of muscle fibers, a design that allows the development of a wide range of forces under control by the nervous system. A unique feature of muscle fibers is the presence of myofibrils in the sarcoplasm, consisting of thick and thin filaments arranged parallel to each other within

the sarcomeres, which are the elementary contractile units of muscle. Thick and thin filaments interact through cross-bridges formed by the heads of myosin, the main protein of the thick filaments. The myosin heads, which possess ATPase activity, bind to F-actin, the main protein of the thin filaments. F-actin then drives P_i and ADP, the products of ATP hydrolysis, away from the heads. This change causes a swinging of the lever arm, which is a flexible stalk in the heads. In effect, the thick filaments pull the thin filaments, which slide past the thick filaments toward the center of the sarcomere. This sliding results in shortening of the sarcomere, the muscle fiber, and the whole muscle in what is known as concentric activity.

A muscle does not change its length if it meets a resistance that it cannot overcome, in which case it performs isometric activity. Likewise, a muscle may lengthen despite developing force if a higher opposing force is applied to it, in which case it performs eccentric activity. All types of activity require expenditure of energy. Adult human muscle contains three myosin heavy chain (MHC) isoforms (I, IIa, and IIx), usually in different muscle fibers (types I, IIA, and IIX). MHC isoforms largely determine how fast a muscle fiber contracts.

Muscle activity is triggered by the release of acetylcholine at the neuromuscular junction, opening of the acetylcholine receptor in the sarcolemma, and generation of action potentials in the muscle fibers that are controlled by a firing motor neuron. Action potentials are transmitted deep inside the muscle fibers thanks to an extensive network of transverse tubules. A voltage-sensitive protein in the tubule membrane, the dihydropyridine receptor, causes the release of Ca^{2+} from the adjacent sarcoplasmic reticulum through the ryanodine receptor, which is a calcium channel. Ca^{2+} rushes to the cytosol and binds to troponin C (TnC), which lies in the thin filaments along with two other troponin subunits (TnI and TnT) and tropomyosin. The binding of Ca^{2+} to TnC detaches TnI from F-actin and lets

tropomyosin slide away from the myosin-binding sites on actin, thus permitting the development of contractile force. Ca^{2+} ATPase, or the Ca^{2+} pump, sequesters Ca^{2+} in the sarcoplasmic reticulum when there are no action potentials, thus relaxing the muscle fibers. When action potentials arrive at high rate, muscle twitches fuse, tetanus ensues, and maximal force develops.

Problems and Critical Thinking Questions

1. Place the following terms in a logical order and explain that order: thick and thin filaments, muscle, myofibril, sarcomere, muscle fiber.
2. Fill the following table with the proteins present in each part of a sarcomere.

Thick filaments	Thin filaments	M line	Z line	Interfilament space

3. Consider a muscle having average sarcomere, A band, and H zone lengths at rest as indicated in the following table (all in μm). Suppose that the muscle shortens by 20% during concentric activity and lengthens by 20% during eccentric activity. Fill in the missing lengths of the parts of the sarcomere.

	Rest	Concentric activity	Eccentric activity
Sarcomere	2.5		
A band	1.5		
H zone	0.7		
I band			
Thick filaments			
Thin filaments			
Overlap of thick and thin filaments			

4. What is the direct energy source for muscle contraction and muscle relaxation? Which parts of the muscle machinery use it?

5. What is the cause of rigor mortis, that is, the stiffening of a body after death?
6. Is muscle contraction feasible if we remove tropomyosin and troponin from the thin filaments by means of an experimental treatment?
7. Addition of a caffeine solution to a muscle with severed sarcolemma (to permit the entrance of caffeine to the sarcoplasm) causes contraction. Suggest a mode of action for caffeine.
8. When studying living organisms and the phenomenon of life, it is important to remember that most biological processes are reversible and that the reversal of a process may be as important as the process itself. One case in point is that of muscle activity: Unless a muscle relaxes after it has contracted, it is unable to contract again. In this regard, it is quite fortunate that what many consider the “golden age” of muscle research (the 1950s and 1960s) was marked by great discoveries not only about how muscle contracts but also about how it relaxes. One of those was the discovery of a factor that causes relaxation of fragmented muscle fibers. The factor has the following characteristics: It is natural (that is, present in muscle), contains lipids, exhibits ATPase activity, and concentrates Ca^{2+} when ATP is present. Can you guess what it is?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

molecular motor
multinucleated cell
myoblast
postmitotic cell

muscle fiber
sarcolemma
sarcoplasm
A band
I band
striated muscle
myofibril
H zone
M line
Z line, Z disc
sarcomere
thick filament
thin filament
contractile protein
myosin
actin
tropomyosin
troponin
myomesin
M-protein
α-actinin
cross-bridge
sliding filament model
heavy chain
light chain
proteolysis
protease
domain
trypsin
light meromyosin

heavy meromyosin
papain
subfragment
G-actin
F-actin
actomyosin
nebulin
titin
lever arm
power stroke
concentric
isometric
eccentric
isoform
slow-twitch fiber
fast-twitch fiber
electromyography
transverse tubules, T system
sarcoplasmic reticulum
obscurin
Ca^{2+} pump
Ca^{2+} ATPase
calsequestrin
ryanodine receptor
dihydropyridine receptor
twitch
shivering thermogenesis
tetanus

References and Suggested Readings

Caremani M, Melli L, Dolfi M, Lombardi V, Linari M (2015). Force and number of myosin motors during muscle shortening and the coupling with the release of the ATP hydrolysis products. *Journal of Physiology* 593: 3313-3332.

des Georges A, Clarke OB, Zalk R, Yuan Q, Condon KJ, Grassucci RA, Hendrickson WA, Marks AR, Frank J (2016). Structural basis for gating and activation of RyR1. *Cell* 167: 145-157.

Dolphin AC (2016). Voltage-gated calcium channels and their auxiliary subunits: Physiology and pathophysiology and pharmacology. *Journal of Physiology* 594: 5369-5390.

Heckman CJ, Enoka RM (2012). Motor unit. *Comprehensive Physiology* 2: 2629-2682.

Henderson CA, Gomez CG, Novak SM, Mi-Mi L, Gregorio CC (2017). Overview of the muscle cytoskeleton. *Comprehensive Physiology* 7: 891-944.

Herzog W, Schappacher G, DuVall M, Leonard TR, Herzog JA (2016). Residual force enhancement following eccentric contractions: A new mechanism involving titin. *Physiology* 31: 300-312.

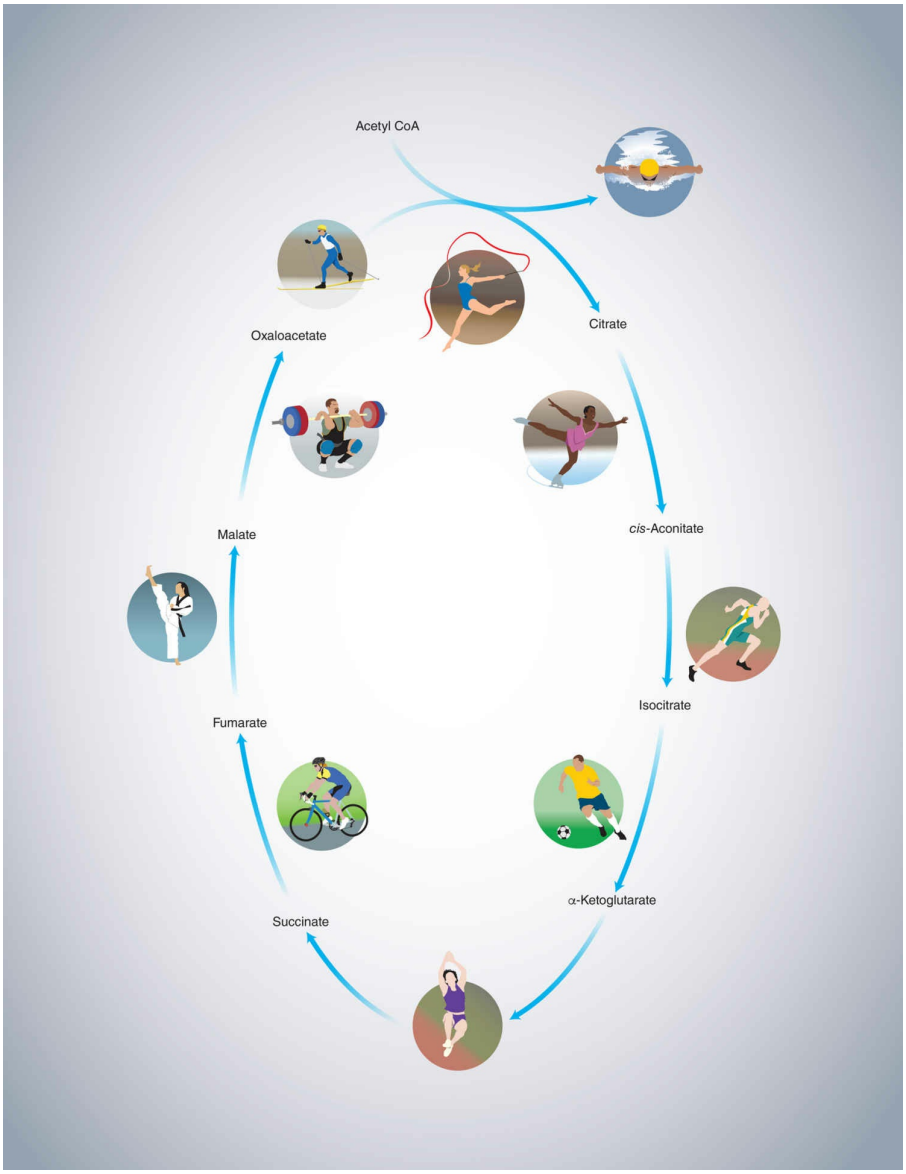
Rall JA (2014). *Mechanism of Muscular Contraction* (Springer, Berlin).

Randazzo D, Blaauw B, Paolini C, Pierantozzi E, Spinozzi S, Lange S, Chen J, Protasi F, Reggiani C, Sorrentino V (2017). Exercise-induced alterations and loss of sarcomeric M-line organization in the diaphragm muscle of obscurin knockout mice. *American Journal of Physiology Cell Physiology* 312: C16-C28.

Samsó M (2015). 3D structure of the dihydropyridine receptor of skeletal muscle. *European Journal of Translational Myology* 25: 27-33.

Schiaffino S, Reggiani C (2011). Fiber types in mammalian skeletal muscles. *Physiological Reviews* 91: 1447-1531.

Zheng J, Trudeau MC, eds. (2015). *Handbook of Ion Channels* (CRC Press, Boca Raton).


Acetyl CoA
Citrate
cis-Aconitate
Isocitrate
α-Ketoglutarate
Succinate
Fumarate
Malate
Oxaloacetate

PART III

Exercise Metabolism

Our study [of muscle], however, needs light also from another aspect, it requires the skilled labours of the organic and biochemists…. To take another analogy, the completion of the drawing will rest with the chemists: we physicists can only provide a sketch; we can indicate the type of machine and its properties, the chemists must describe it in detail.

–Archibald Vivian Hill (Nobel Prize in Physiology or Medicine 1922), *Nobel Lecture*

In chapter 2, we considered metabolism as the sum of the chemical reactions occurring in a living organism or part of it. In that context, I introduced some basic knowledge, including the energy changes that accompany metabolic reactions, the compounds that play central roles in energy transactions and redox reactions, and the fundamental processes that yield energy from foodstuffs.

The present part of the book deals with metabolism during and after physical exercise. Let me clarify right from the beginning that the metabolic reactions taking place in the body are the same whether we are resting or exercising. What changes spectacularly upon shifting from one state to the other is the rate at which reactions take place: Some reactions speed up, while others slow down, thus enabling the body to respond to the demands of each

state. This process constitutes **metabolic control** and is manifest not only in exercise but also in any state of an organism (be it growth, food consumption, starvation, stress, or disease). Each state is characterized by the predominance of certain reactions. The purpose of part III is to explore the *metabolic processes that characterize exercise.*

III.1 Defining Exercise and Physical Activity

Although it is obvious to most of us what exercise is, it would be useful, for the sake of accuracy, to define it (just as we defined another obvious term, *life*, in the part I opener). Based on several existing definitions, I will define **exercise** here as *planned and structured bodily movement, which, when repeated (in exercise training), results in learning and improving one or more physical skills or in maintaining and improving one or more physical abilities.* This definition distinguishes exercise (performed once) from exercise training (performed repeatedly), a distinction that we will need in the rest of the book. It also highlights the two main outcomes, or purposes, of exercise training: learning and improving skills (such as dribbling in soccer) and maintaining and improving abilities (such as aerobic endurance).

A related, and more general, term is **physical activity**, which can be defined as *any bodily movement produced by muscle activity that results in energy expenditure above the resting level* based on the American College of Sports Medicine position stand (Garber et al., 2011). Physical activity is broader than exercise because it includes movement that is not planned or structured. However, deciding whether or not an activity is planned and structured may be quite subjective. For example, some might argue that hiking is neither planned nor structured (and, hence, does not qualify as exercise), whereas others might argue that they put a lot of planning and structure into a hiking activity. Thus, it may not always be possible (or necessary, for that matter) to tell exercise from physical activity.

III.2 Principles of Exercise Metabolism

Metabolic control (both in exercise and in general) is exerted through

biochemical mechanisms that we have only begun to comprehend in recent decades. Although most metabolic pathways have been elucidated, our knowledge about their regulation is incomplete. (In the context of metabolism, I will use the terms *control* and *regulation* interchangeably.) From what we know, however, it seems that metabolic control in a healthy individual is both efficient and flexible.

Exercise is the most powerful healthy modulator of metabolism. Indeed, only severe diseases can rival exercise in magnitude of metabolic changes. The metabolic changes caused by exercise are the subject of the chapters to come, through which the following basic principles will emerge:

1. Exercise metabolism obeys the need to supply energy for muscle activity.
2. Exercise is almost always fueled by a combination of energy sources (not by a single source), the major ones being carbohydrates and lipids.
3. Exercise changes metabolism not only in the active muscles but also in other organs and tissues (such as the liver, adipose tissue, and heart).
4. Changes in metabolism with exercise depend on exercise parameters, characteristics of the exercising individual, and environmental factors.
5. Metabolism does not return to the characteristics of the resting state immediately after the end of exercise. Many changes persist for hours or days, and others are manifest during recovery from exercise rather than during exercise.
6. Frequent repetition of exercise (that is, exercise training) can make the metabolism of an individual different from that of an individual who does not train, both at rest and during exercise.
7. Many of the metabolic changes elicited by exercise training increase performance and improve health or protect it against disease.

III.3 Exercise Parameters

Exercises do not all exert the same effects on metabolism. Although a thorough examination of all possible kinds of exercise goes beyond the scope of this book, we need to be clear and specific as to which kinds the metabolic

changes that we discuss apply to. Four main identifying parameters of exercise are **type**, **intensity**, **duration**, and **frequency**.

Exercise Type

Three widely recognized (though not the only) types of exercise are **endurance**, **resistance**, and **sprint**. Endurance exercise is characterized by prolonged periods of muscle activity against low resistance that are either continuous or intermittent (that is, interspersed with rest periods). One example of continuous endurance exercise is jogging. An example of intermittent (or interval) endurance exercise is the biathlon, the winter Olympic event that encompasses cross-country skiing interspersed with rifle shooting from a standing or prone position.

In contrast to endurance exercise, resistance exercise involves short periods of muscle activity against high resistance; one example is weightlifting. Finally, sprint exercise consists of short periods of maximal muscle activity against low resistance—for instance, a competitive 50 m swim.

An alternative way of describing exercise type is to use the terms **aerobic** and **anaerobic**, which refer to the predominant means of energy production during exercise. Aerobic exercise draws energy mainly from metabolic processes requiring oxygen, either directly or indirectly, whereas anaerobic exercise draws energy mainly from processes not requiring oxygen in any way. (We will explore all of these processes in the coming chapters.) Endurance exercise is aerobic, whereas resistance and sprint exercises are usually anaerobic.

Characterizing exercise as either aerobic or anaerobic comes up against two problems. First, the little word *mainly* is often ignored, leading one to believe that an exercise can be totally aerobic or totally anaerobic. However, as we will see on multiple occasions, any type of exercise relies on a mixture of aerobic and anaerobic processes in some proportion. Second, what is considered (mainly) anaerobic exercise may in fact be (mainly) aerobic. Two cases in point are repeated sprints with short intervals, which start as mainly anaerobic and end up as mainly aerobic (see section 14.8), and exercise above the so-called anaerobic threshold, which is mainly aerobic in reality

(see section 14.7). For this reason, I recommend avoiding the use of the ambiguous terms *aerobic* and *anaerobic* when describing exercise or training.

Exercise Intensity

Exercise intensity can be expressed in a variety of ways. Some ways are exercise specific (for example, running speed and weight lifted), whereas others apply generally (for example, heart rate and oxygen uptake). Another general measure of exercise intensity is the **metabolic equivalent of task**, or **MET**. One MET is defined as the oxygen uptake at rest and, by convention, is given a value of 3.5 mL · kg $^{-1}$ · min^{-1} (although, naturally, oxygen uptake at rest varies among individuals). Thus, an exercise eliciting an oxygen uptake of 14 mL · kg $^{-1}$ · min^{-1} has an intensity of 4 MET.

Intensity can also be expressed as a percentage of maximal intensity. This approach is used in the case of percentage of $\dot{V}O_2$max, a favorite measure of the relative intensity of exercise, particularly endurance exercise. The favorite measure of the relative intensity of resistance exercise, on the other hand, is the percentage of one-repetition maximum (1RM)—the maximal force that can be generated in a single effort for a particular exercise (for example, bench press).

Another useful measure of exercise intensity is the **rating of perceived exertion (RPE)**. This rating consists of a dimensionless number that one gives when asked to rate how hard an exercise felt on a scale of 6 to 20. The numbers correspond to a usual resting heart rate of 60 beats · min^{-1} and a usual maximal heart rate (HRmax) of 200 beats · min^{-1} for young individuals. This range constitutes the renowned Borg scale, after the Swedish psychologist Gunnar Borg, who proposed it in the 1960s.

HRmax is either determined during an exercise test to exhaustion or assumed to be equal to 220 – age.

A final means of stating exercise intensity is qualitative rather than quantitative—that is, by using adjectives such as *low*, *moderate*, and *high*. This approach allows for a generalization that is useful when one does not

need to be very precise. In table III.1, I have compiled the various ways to express exercise intensity, based on the position stand of the American College of Sports Medicine cited in section III.1.

Two additional adjectives used to describe exercise intensity are *submaximal* and *supramaximal*. The former usually refers to intensities ranging from moderate to near-maximal, depending on the author. The latter refers to efforts that exceed the power corresponding to $\dot{V}O_2max$ for a short while, such as during sprints.

Table III.1 Classification of Exercise Intensity

Qualitative descriptor	MET	% HRmax	% $\dot{V}O_2$max	% 1RM	RPE
Light exercise	<3	≤63	≤45	<50	6-11
Moderate-intensity exercise	3-5.9	64-76	46-63	50-69	12-13
Hard, or vigorous, exercise	6-8.7	77-95	64-90	70-84	14-17
Near-maximal to maximal exercise	≥8.8	≥96	≥91	≥85	18-20

Exercise Duration

Exercise duration is easier to measure than intensity because there is only one way of expressing it (time). There is no agreed-upon classification of exercise duration; that is, there is no consensus as to which exercise should be called *short* and which *prolonged*. You may wonder, then, what I meant when I used these very terms to describe endurance, resistance, and sprint exercise earlier in this discussion. I would consider an exercise lasting up to one minute as short and an exercise lasting more than ten minutes as prolonged. An exercise lasting between one and ten minutes, then, would be of intermediate duration. However, because this is a subjective classification, I will try to be as specific as possible when referring to duration.

Exercise Frequency

A final exercise parameter that we need to consider relates to whether exercise is executed once or is repeated (on separate days). Exercise executed once is **acute**, whereas repeated exercise constitutes **exercise training**, or plain **training** from now on. Alternative terms for training are **chronic** and **long-term exercise**. Though not formally defined, these terms are usually

reserved for training that lasts more than three months. Shorter periods of training may be described as **short-term exercise**.

If exercise is repeated regularly, then frequency is a parameter that affects the metabolic responses of the body. For instance, a competitive athlete might train twice daily, whereas a person engaging in leisure-type activities might train only three times per week. Again, there is no classification of exercise frequency, so I will be specific when referring to it.

The effects of acute exercise on metabolism usually last from a few minutes to a few days after the end of exercise, whereas the effects of training usually last from several days to several months after one has stopped. The latter effects are also called **adaptations to exercise** or **adaptations to training** in the sense that the body modifies its metabolism to meet the requirements of repeated exercise with a slighter disturbance of its **homeostasis** (section 1.12) and a lower chance for damage as compared to the first time it exercised.

III.4 Means of Metabolic Control in Exercise

Mammals (including humans) have evolved a multitude of mechanisms to modify their metabolism during exercise. Let's try to group these mechanisms.

Allosteric Regulation

Metabolic reactions are catalyzed by enzymes. A usual way of regulating the rate of enzyme reactions is through allostery, as introduced in section 3.12 for a protein that is not an enzyme (hemoglobin). Allosteric regulation applies to exercise metabolism in numerous cases. For example, the binding of a compound whose concentration increases with exercise to a site on an enzyme that is distinct from the active site may modify the enzyme's activity. If the activity increases, the compound is called an **activator**; if the activity decreases, the compound is an **inhibitor**.

Modification of enzyme activity by an **effector**—that is, an activator or inhibitor—is instantaneous; it takes place within milliseconds. Moreover, it is reversible: If the effector concentration decreases, then its dissociation from

the enzyme is favored and the enzyme reverts to its initial activity. We will encounter many cases of enzyme activation or inhibition by compounds whose concentrations change with exercise.

A usual control point in a metabolic pathway is the enzyme catalyzing the first irreversible (or hard-to-reverse) reaction, that is, one with highly positive ΔG, as we discussed in section 2.1. This reaction is termed the **committed step** because it commits the compound entering the pathway to a course of no return. A common inhibitor of the enzyme catalyzing the committed step is the product of the pathway (figure III.1). This effect, called **feedback inhibition**, constitutes a homeostatic mechanism that keeps the concentration of the product within limits: If too much of it is produced, production slows down; if too little is present, the inhibition is lifted and production speeds up.

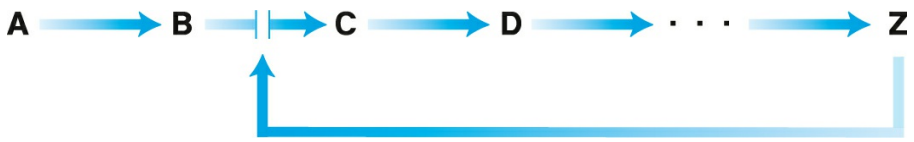


Figure III.1 **Feedback inhibition.** A usual way to control the rate of a metabolic pathway from compound A to compound Z is to inhibit the enzyme catalyzing the first irreversible reaction (which happens to be the second in this example) with the end product Z. This inhibition signals the production of Z to slow down when its concentration becomes exceedingly high.

Covalent Modification

The activity of certain enzymes is modified by the reversible addition of chemical groups to their molecules. This **covalent modification** takes place within seconds. The addition and subsequent removal of groups are catalyzed by specific enzymes, which can be activated or inhibited by biochemical factors affected by exercise. One case in point is the control of glycogen synthesis and breakdown by phosphorylation of the enzymes catalyzing these processes, as discussed in chapter 10.

Changing Substrate Concentration

An increase or decrease in the concentration of a substrate during exercise may cause a similar change in the rate of an enzyme reaction, as explained in

section 3.16. For example, the increase in the amount of glucose entering the exercising muscles from blood raises its cytosolic concentration and speeds up its breakdown for energy supply. Like changes in the concentration of enzyme effectors, changes in substrate concentrations can take place within milliseconds.

Changing Enzyme Concentration

The quantity as well as the activity of enzymes is controlled. Exercise creates biochemical stimuli that modify the concentrations of various enzymes. Cells often achieve this modification by altering the transcription rates of the genes encoding these enzymes. Changing enzyme concentration is the slowest means of regulation—it requires hours. Nevertheless, it is also the most lasting, and it constitutes the basis for adaptations to exercise. We will consider the effect of exercise on gene expression in chapter 13.

Nervous and Hormonal Control

Metabolic regulation in animals goes beyond the boundaries of cells thanks to two large communication systems, the nervous and the hormonal ones. The two systems convey signals, to which cells bearing the appropriate receptors respond by altering their function. The route from a signal to a cellular response can be quite complicated, involving intricate molecular interactions and conversions, as we saw in part II and will also see in subsequent chapters. These routes are termed **signal transduction pathways**. We have already dealt with the nervous system and its signals (action potentials and neurotransmitters) in chapter 7. I have also mentioned hormones in passing in chapters 3 to 6. Therefore, now it is proper to present the hormonal system.

The term *hormone* derives from the Greek *hormón,* meaning “the one who rushes.” It thus implies a mobilization of the body, although some hormones repress bodily functions.

Hormones are compounds that coordinate the functioning of different cells in multicellular organisms. Coordination enables an organism to respond to external or internal stimuli in a concerted manner, thus avoiding

conflicting activities of individual tissues or cells. The many known hormones display a wide variety of structures and functions. Nevertheless, they share three features:

- They are synthesized in minuscule quantities in organs or tissues characterized as **endocrine glands**.
- They are secreted to blood, which disseminates them to the rest of the body.
- They alter in specific manners the function of tissues, organs, or cells called **target tissues**, **target organs**, or **target cells**.

The nervous and hormonal systems intertwine and collaborate to afford the smooth operation of a body in health. Likewise, they collaborate in exercise and dictate the coordinated modification of metabolism in different tissues and organs such as muscle, the liver, and adipose tissue. In this way, for example, there is an increased flow of glucose from the liver to the exercising muscles and an increased breakdown of body fat. Both processes (which we will examine in detail in chapters 10 and 11) result in the supply of energy sources to the exercising muscles from other tissues.

III.5 Energy Sources in Exercise

As we saw in section III.2, exercise metabolism obeys the need to supply energy for muscle activity. The following four classes of biological substances serve as energy sources in exercise and, as such, will be examined in the next four chapters in detail:

- Compounds of high phosphoryl-transfer potential
- Carbohydrates
- Lipids
- Proteins

The breakdown of these substances for energy production is often referred to as **energy metabolism**. As we will see, there is almost no kind of exercise that derives its energy from only one source. On the contrary, two or more

sources contribute energy, depending on the exercise parameters, characteristics of the exerciser, and environmental factors, as I have pointed out in section III.2 (refer to the second and fourth principles).

III.6 How Researchers Study Exercise Metabolism

Researchers have several methods at their disposal when they wish to study how exercise affects metabolism. Depending on the role of the researcher, studies can be categorized as either observational or interventional (figure III.2). In an **observational study**, participants exercise on their own, and researchers try to deduce whether different levels of exercise training or physical activity relate to differences in metabolism. In contrast, in an **interventional** or **experimental study**, researchers dictate the exercise to the participants and compare metabolic characteristics after the intervention either to those before the intervention or to those of participants who did not exercise. The latter are often termed the **control group** and are included in the study to make sure that metabolic changes found by researchers in the **exercise group** are due to exercise itself rather than to other factors, such as the passing of time. The two groups must be comparable as to the characteristics that may affect the outcome of the study (for example, sex, age, and training state). Studies of this type are known as **controlled**.

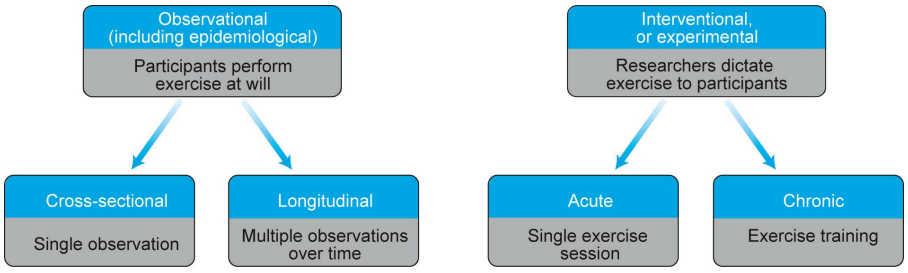


Figure III.2 **Types of studies on exercise metabolism**.

Observational studies are usually conducted on large numbers of individuals (often in the thousands), in which case they are also called **epidemiological studies**. They can be further categorized as either **cross-**

sectional or **longitudinal**. In the former type, investigators examine participants at a single point in time (effectively performing a cross-section in the flow of time), whereas in the latter type participants are examined at least twice over time. Similarly, interventional studies are categorized as either **acute** or **chronic** (including short-term, as defined during the description of exercise frequency in section III.3), depending on whether the participants perform one or multiple exercise sessions.

Each of the types of studies mentioned here has its strengths and weaknesses, the examination of which exceeds the breadth of this book. Investigators weigh the pros and cons of each type and design their studies accordingly. The information presented in the chapters to follow relies on findings from all types of studies, as I will point out in many instances. A finding supported by more than one type of study is usually considered stronger than a finding supported by just one type of study.

A powerful tool for deciphering the effects of exercise on metabolism is the meta-analysis. To perform one, scholars meticulously search the scientific literature for studies on a given subject (for example, the effect of endurance exercise on plasma lipid concentrations). Then they carefully select the studies that meet certain quality criteria and, through specialized statistical analysis, process the outcomes of these studies to arrive at results that reflect a consensus. The conclusions emanating from such results are considered more accurate and objective than those emanating from a narrative review of the literature. Thus, a meta-analysis constitutes one of the best evidence-based approaches for applying scientific outcomes in practice.

Naturally, humans are the preferred subjects of research on exercise metabolism. Nevertheless, a study may be impossible to conduct on humans for ethical reasons. For example, it is too dangerous to perform biopsy on vital organs (such as the liver, heart, and brain) in order to study their metabolism in healthy humans. In such cases, investigators may resort to using experimental animals, such as mice, rats, and dogs, under conditions that meet established ethical standards. Although results from such studies carry a theoretical interest of their own (that is, they show how exercise modulates metabolism in a certain animal species), the main interest usually lies in whether the results are applicable in humans. For this purpose, they

require confirmation by some other means such as imaging, rather than invasive, techniques.

Finally, investigators may resort to experiments conducted on isolated cells **in vitro** (literally meaning "in glass" in Latin) as opposed to **in vivo** (in a live organism). Examples of in vitro experiments include electrical stimulation of muscle fibers to elicit contraction or stretching of muscle fibers to mimic eccentric activity. The advantage of such designs is that they allow almost complete control of the cells' environment and removal of the influence of many known or unknown factors that are altered simultaneously in an exercising organism. In vitro studies are valuable for the discovery of the molecular mechanisms underlying the metabolic effects of exercise.

III.7 Laboratory Techniques in the Study of Exercise Metabolism

A multitude of laboratory techniques are available for the study of exercise metabolism. Most are invasive: Researchers pierce or (mainly in the case of experimental animals) cut open a body and remove a biological sample (such as blood, muscle, or adipose tissue) to measure one or more substances in it. Invasive techniques also include the injection of substances (such as hormones or energy sources) into the body in order to examine how they interact with exercise in modifying metabolism or how they are metabolized during exercise.

Muscle is obviously a particularly popular tissue for studying exercise metabolism. A small sample (around 50 to 200 mg) can by removed from one's muscle by **percutaneous needle biopsy** with minimal damage to the tissue. The sample is then processed in any of various ways, including **lyophilization**, or **freeze-drying** (removing the sample's water under vacuum after freezing it); **homogenization** (mechanically breaking up the cells to produce a uniform mixture of their contents, termed a *homogenate*); **centrifugation** (spinning a homogenate at high speed to separate its components on the basis of differences in density); and **extraction** (removing a particular class of biomolecules by treating the sample with a suitable solvent). These processes are necessary before a sample is analyzed by

sensitive methods, that is, methods enabling the measurement of low amounts of biomolecules.

One of the oldest (though not one of the most sensitive) analytical methods used in biochemical research is **spectrophotometry**. It is based on the principle that *many compounds in solution absorb visible light in proportion to their concentration.* Light absorbance is responsible for colors: An object has color if it absorbs visible light; otherwise, it is colorless. The more light an object absorbs, the darker it appears. Thus, much in the way that you can judge how concentrated or dilute a juice is by looking at it through a transparent container, biochemists can deduce the concentration of a biomolecule of interest (be it a protein, nucleic acid, carbohydrate, lipid, or other metabolite) by accurately measuring its absorbance in a **spectrophotometer** (some preparatory steps are usually needed). Different compounds absorb light of different wavelengths, thus allowing their distinction.

Wavelength is a property of light and, in general, of electromagnetic radiation (one type of which is light). The fact that different compounds absorb light of different wavelengths is responsible for their different colors.

Spectrophotometry is not limited to colored compounds. One can let a colorless compound react in a specific and controlled manner with other substances (termed **reagents**) to produce a colored compound. One then measures the colored product, knowing that its concentration corresponds to that of the colorless **analyte** (the substance being measured). We can even measure colorless compounds in specialized **spectrometers** without converting them into colored ones if they happen to absorb electromagnetic radiation around the visible area, that is, in the ultraviolet or infrared areas (figure III.3).

Another technique that capitalizes on how light interacts with matter is **fluorometry**. Rather than measuring how much light a compound absorbs, it measures how much light a compound emits at a specific wavelength when it returns to its basal energy state, or ground state, after having been excited by light of a different wavelength. This phenomenon is called **fluorescence**,

hence the name of the method. Fluorometry is more sensitive than spectrophotometry.

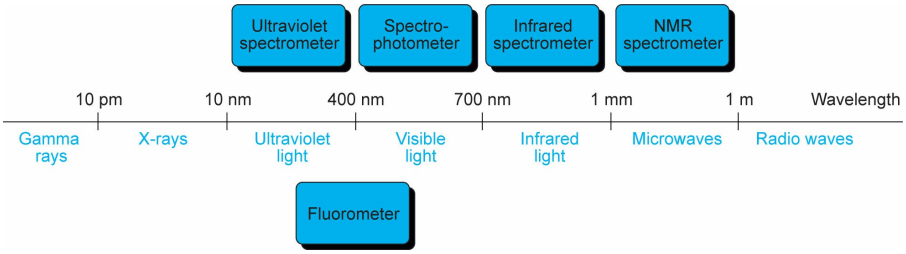


Figure III.3 **Putting electromagnetic radiation into a researcher's service.** A variety of measuring instruments take advantage of the interaction of electromagnetic radiation with matter. Ultraviolet spectrometers, spectrophotometers, and infrared spectrometers send beams of ultraviolet, visible, and infrared light, respectively, through solutions and measure how much of that light is absorbed by solutes, thus calculating their concentration. A fluorometer, on the other hand, emits (usually) ultraviolet light to excite a solute and measures how much (usually) visible light the solute emits back when it returns to the ground state, thus again calculating its concentration. A nuclear magnetic resonance (NMR) spectrometer, working in the microwave area of the electromagnetic spectrum, can both identify and measure solutes in a complex sample, based on the magnetic properties of certain nuclei. The wavelength axis is not to scale.

Another type of electromagnetic radiation (besides ultraviolet, visible, and infrared light) that interacts with matter is that of microwaves. Researchers exploit this interaction in **nuclear magnetic resonance spectroscopy**, or **NMR spectroscopy**, a technique based on the magnetic properties of the nuclei of certain elements such as ^{1}H and ^{31}P. When such nuclei find themselves in a strong magnetic field, they can jump from a low-energy state to a high-energy one by absorbing electromagnetic radiation of an appropriate frequency (in the area of microwaves) emitted by a transmitter. We say then that the nuclei resonate with this frequency. At a given magnetic field strength, the frequency with which a nucleus in a compound resonates depends on the minuscule magnetic field formed by the surrounding atoms in the compound. Thanks to this property, nuclei of the same element resonate with different frequencies if they belong to different compounds or even if they are at different positions within the same compound. Thus, by placing a sample in an **NMR spectrometer**, we can identify the compounds in it based on the frequencies at which radiation is absorbed, and we can measure their

concentrations from the amount of radiation absorbed.

Frequency is another property of electromagnetic radiation; it is inversely proportional to wavelength.

Considerable knowledge about exercise metabolism comes from experiments in which researchers administer compounds with rare isotopes to humans, experimental animals, excised tissues, or cells in culture. The compounds are then given sufficient time to metabolize, so that researchers can trace which biomolecules the isotopes have been passed on to and thus reconstruct the metabolic pathways followed by the compounds (see problem 4.3). This process is much like installing a GPS tracking device in a car to know where it goes. The isotopes can be either radioactive or nonradioactive (that is, stable)—for example, ^{14}C and ^{13}C, respectively (both introduced in section 1.1). Radioactive and stable isotopes are measured with different instruments, called **radioactivity counters** and **mass spectrometers**, respectively.

Mass spectrometry can work with common as well as rare stable isotopes. It is based on the premise that when the molecules of a compound are bombarded by electrons or treated in other extreme ways, they become ionized or break into fragments that are characteristic of the compound. The fragments are identified in the mass spectrometer on the basis of their mass-to-charge ratio, which, in turn, allows identification of the compound on the basis of information stored in libraries of mass spectra. In addition, it is possible to measure the concentration of the compound in a biological sample on the basis of how strong are the signals its fragments produce. Mass spectrometry is one of the most sensitive analytical methods.

Two other analytical methods, chromatography and electrophoresis, allow the separation, isolation, and measurement of biomolecules in complex samples. In **chromatography**, compounds in a liquid or gaseous solution are forced by pressure, gravity, or capillary action to migrate through a solid or liquid medium. Because they differ in chemical structure, the compounds migrate at different speeds and are thus separated from each other in the end. (Think of a swim race, in which athletes who started together finish

separately because they swam at different speeds.) By determining the position or "finishing time" of each compound, we can identify or isolate it; by measuring the intensity of its signal, we can determine its concentration.

In **electrophoresis**, on the other hand, ionic compounds in a liquid solution are driven by electricity through a gel and are separated on the basis of size and charge. As in chromatography, each compound assumes a position that characterizes it, and the intensity of its signal corresponds to its concentration.

You may have noticed that three of the methods presented here—that is, NMR spectroscopy, chromatography, and electrophoresis—have the capacity to identify and measure multiple biomolecules at once (as opposed to, say, spectrophotometry and fluorometry, which usually measure one compound at a time). This capacity renders them ideal for **metabolomics**, especially when chromatography and electrophoresis are each combined with mass spectrometry. By analogy to proteomics, genomics, and transcriptomics (introduced in chapters 3 and 4), metabolomics is the large-scale, comprehensive study of the **metabolome** (that is, the sum of metabolites in an organism, tissue, cell type, or biological fluid) in terms of metabolite identity, quantity, and function. These four "omics" contribute to **systems biology**—the study of complex biological systems, such as cells, tissues, and organisms—through the integrated and combined examination of all of their components in terms of both structure and function.

This discussion is not meant to serve as an exhaustive presentation of the research tools available for the study of exercise metabolism but, rather, as an introduction. More techniques will be presented along the way in part III.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

metabolic control
exercise
physical activity

exercise type
exercise intensity
exercise duration
exercise frequency
endurance exercise
resistance exercise
sprint exercise
aerobic
anaerobic
metabolic equivalent of task, MET
rating of perceived exertion, RPE
acute exercise
exercise training, training
chronic exercise, long-term exercise
short-term exercise
adaptation to exercise, adaptation to training
homeostasis
activator
inhibitor
effector
committed step
feedback inhibition
covalent modification
signal transduction pathway
hormone
endocrine gland
target tissue
target organ
target cell
energy metabolism

observational study
interventional study, experimental study
control group
exercise group
controlled study
epidemiological study
cross-sectional study
longitudinal study
acute study
chronic study
in vitro
in vivo
percutaneous needle biopsy
lyophilization, freeze-drying
homogenization
centrifugation
extraction
spectrophotometry
spectrophotometer
reagent
analyte
spectrometer
fluorometry
fluorescence
nuclear magnetic resonance spectroscopy, NMR spectroscopy
NMR spectrometer
radioactivity counter
mass spectrometer
mass spectrometry
chromatography

electrophoresis
metabolomics
metabolome
systems biology

Reference

Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, Nieman DC, Swain DP (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: Guidance for prescribing exercise. *Medicine and Science in Sports and Exercise* 43: 1334-1359.

CHAPTER 9

Compounds of High Phosphoryl-Transfer Potential

Learning Objectives

After reading this chapter, you should be able to do the following:

- List and define compounds of high phosphoryl-transfer potential.
- Discuss the ATP–ADP cycle and the processes that propel it.
- Distinguish direct from indirect energy sources.
- Explain why the turnover rate of the ATP–ADP cycle rises during exercise.
- Explain how ADP replenishes ATP during exercise through the myokinase reaction.
- Describe the advantages of phosphocreatine over ATP in muscle.
- Write down the interconversion of phosphocreatine and ATP, name the enzyme that catalyzes the reaction, and list the enzyme's isoforms.
- Calculate changes in the muscle concentrations of compounds related to energy production during exercise.
- State the exercises in which phosphocreatine is the major energy source.

- Draw the pathway of degradation of purine-containing compounds during hard exercise.

Compounds of high phosphoryl-transfer potential are the first class of energy sources in exercise that we will consider. These compounds share two features:

- They bear phosphoryl groups.
- Their hydrolysis releases high amounts of energy.

In accordance with what you learned in chapter 2, we can symbolize a compound of high phosphoryl-transfer potential as A~P and write the following equation:

$$A{\sim}P + H_2O \rightarrow A + P_i \qquad \Delta G^{\circ\prime} < 0 \qquad \text{(equation 9.1)}$$

In a variation of this equation, compound A~P can transfer its phosphoryl group to a compound B, thus increasing the energy content of the latter.

$$A{\sim}P + B \rightarrow A + B\text{-}P \qquad \Delta G^{\circ\prime} < 0 \qquad \text{(equation 9.2)}$$

This capacity justifies calling A~P a compound of high phosphoryl-transfer potential. The product B-P is usually not such a compound because part of the energy of ~P is lost to the surroundings. As a result, reaction 9.2 has a negative $\Delta G^{\circ\prime}$ and is favored. Nevertheless, it is possible that A~P has such a high phosphoryl-transfer potential that it endows B-P with a high potential too (thus producing B~P). This is the case with the production of ATP from phosphocreatine, as we will see in section 9.3.

We will consider three compounds of high phosphoryl-transfer potential: ATP, ADP, and phosphocreatine. (AMP is not a compound of high phosphoryl-transfer potential, as it lacks a ~P.)

Compounds of high phosphoryl-transfer potential are also known by the shorter, though less accurate, term **phosphagens**.

9.1 The ATP–ADP Cycle

In chapter 2, we considered ATP as the energy currency of cells. We saw that ATP hydrolysis to ADP and P_i feeds anabolism with energy, whereas the energy released during catabolism feeds ATP synthesis from ADP and P_i. It is now time to complete the picture of this interconversion by inserting four more biological processes: photosynthesis, movement, active transport, and signal amplification (figure 9.1).

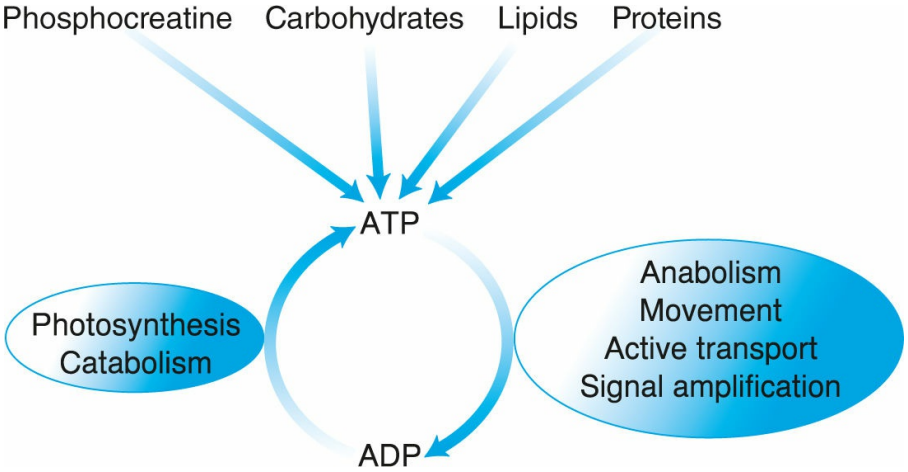


Figure 9.1 Energy exchange in living systems. The ATP–ADP cycle is the main route of energy exchange in biological systems. Shown in the ellipses are the processes driving the conversion of ADP into ATP and vice versa. ATP and, to a lesser degree, its "siblings," GTP, UTP, and CTP (which participate in similar cycles with GDP, UDP, and CDP, respectively, but for clarity are not shown), are direct energy sources for the processes in the right-hand ellipse. All other energy sources (shown above and below ATP) are indirect energy sources: To power the processes in the right-hand ellipse they need to provide the direct energy sources first. This figure extends figure 2.6.

Photosynthesis is a source of ATP for plant cells; it does not operate in animals, which must rely solely on catabolism for ATP synthesis. Let's then turn our attention to the processes that degrade ATP.

- **Anabolism.** I have already presented (in section 2.4) the need to couple the endergonic biosynthetic reactions to the exergonic hydrolysis of ATP for anabolism to be thermodynamically favored.
- **Movement.** We have seen (in chapter 8) that muscle activity is made possible by ATP hydrolysis in the myosin heads. ATP hydrolysis is also

needed for other forms of movement in living organisms, such as the unwinding of the DNA double helix by helicase during replication (section 4.7) and the migration of chromosomes toward opposite ends of a cell during mitosis (figure 4.16).

- **Active transport.** The transport of a substance against a concentration gradient requires ATP hydrolysis by proteins such as the Na^+–K^+ pump (section 7.2) and the Ca^{2+} pump (section 8.11).
- **Signal amplification.** Certain biological signals, such as those initiated by hormones, are despairingly weak, because hormones and other signal biomolecules are synthesized in minuscule amounts. To affect metabolism, the signals need to be amplified. Signal amplification is performed by regulatory mechanisms operating at the expense of ATP. We will encounter such mechanisms when we examine the effect of exercise on carbohydrate metabolism, lipid metabolism, and gene expression.

Signal amplifiers inside us resemble the amplifier in a stereo sound system, which amplifies weak signals such as the one from a laser beam reading a compact disc. This amplifier spends energy too (electric energy, of course, rather than ATP), as evidenced by the warmth you will feel if you touch the device at the vents.

ATP serves as the **direct energy source** in most cases of anabolism, movement, active transport, and signal amplification. Any other energy source, whether ADP, phosphocreatine, carbohydrate, lipid, or protein, is an **indirect energy source**, since it cannot drive any of the energy-demanding processes unless it somehow provides the direct source. Note that I am not referring to *conversion* of the indirect energy sources into direct ones, since no part of the carbohydrate, lipid, or protein molecules ends up in the molecule of ATP. Thus the appropriate expression is that the former provide (or supply, or replenish) the latter. This process will become clearer in chapters 10 through 12, substantial parts of which are devoted to how these indirect energy sources provide ATP.

In some cases, the other three ribonucleoside triphosphates—GTP, UTP, and CTP—are used as direct energy sources. Remember that I have mentioned them in section 4.19.

As ATP is hydrolyzed to ADP and P_i in our bodies to propel energy-demanding processes, it is simultaneously regenerated mainly thanks to the catabolism of carbohydrates and lipids. Thus, ATP and ADP participate in a process known as the **ATP–ADP cycle**. This cycle has a high turnover rate: A human with a low to moderate energy expenditure of 2,000 kcal in a day hydrolyzes and resynthesizes approximately 45 kg of ATP. Nevertheless, the amount of ATP in the body at any moment is a mere 0.1 kg. This amount would suffice for just a few minutes of operation were it not for the catabolic pathways for replenishing it. (By analogy, each of us consumes hundreds of kilograms of food each year, yet we have only a few kilos stored at home at any time.)

9.2 The ATP–ADP Cycle in Exercise

Exercise stimulates three of the four processes that require ATP: movement, active transport, and signal amplification. Stimulation of movement is self-evident. Stimulation of active transport consists mainly of the increased operation of the Na^+–K^+ pump in the plasma membranes of neuronal axons and muscle fibers participating in exercise, as well as the Ca^{2+} pump in the membrane of the sarcoplasmic reticulum in active muscle fibers. The increased operation of the Na^+–K^+ pump is due to the elevated frequency of action potentials, which augments the perturbation of the electrochemical Na^+ and K^+ gradients across the plasma membranes and activates the pump. The increased operation of the Ca^{2+} pump is due to the high [Ca^{2+}] in the cytosol of active muscle fibers. Finally, signal amplification is stimulated because of enhanced secretion of certain hormones, such as epinephrine, as we will see in section 10.6. In contrast, most anabolic processes are inhibited during exercise, as we will see in chapters 10 through 12.

Remember that brackets around the formula or name of a substance denote its concentration (section 1.9).

The net result of these changes is the acceleration of ATP breakdown. An

athlete spending 3,500 kcal daily degrades as many as 80 kg, and the rate of ATP hydrolysis during maximal exercise may rise to 1 kg per minute (!). But what happens on the other side of the ATP–ADP cycle? Naturally, catabolism is activated to meet the increased demand for ATP in accordance with the first principle of exercise metabolism that I laid out in section III.2. Thus, the whole ATP–ADP cycle speeds up during exercise. For this reason, the cycle has rightfully acquired a permanent place in the logos of the International Biochemistry of Exercise Conference (figure 9.2).

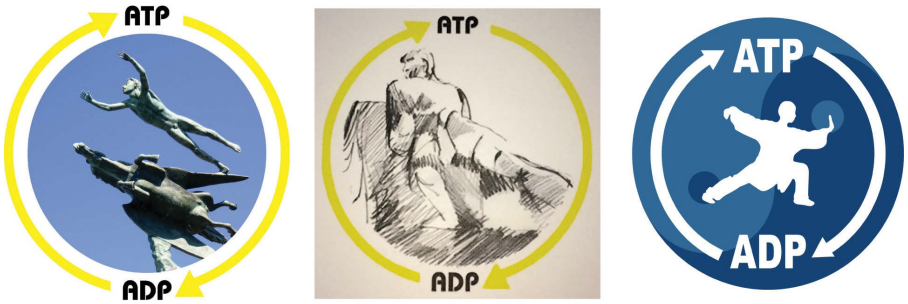


Figure 9.2 **Biochemistry of Exercise Conference.** The ATP–ADP cycle adorns the logos of the International Biochemistry of Exercise Conference (IBEC), held in different places around the world every three years. On the left is the logo of the 15th IBEC, held in Stockholm, Sweden, in 2012. Inside the cycle is the photograph of a sculpture full of movement, titled *Man and Pegasus*, created by Carl Milles, and located in the Millesgården. In the middle is the logo of the 16th IBEC, held in São Paulo, Brazil, in 2015. Inside the cycle is a drawing of part of the Monumento às Bandeiras, a gigantic sculpture created by Victor Brecheret and standing at the entrance of Ibirapuera Park. On the right is the logo of the 17th IBEC, held in Beijing, China, in 2018. Inside the cycle is a drawing of the graceful bow stance (gong bu), one of the stances of wushu, a Chinese martial art. The background depicts yin and yang, two complementary principles of Chinese philosophy that are in eternal movement and interaction. (Left) Courtesy of C. J. Sundeberg, (center) Courtesy of A. H. Lancha, and (right) Courtesy of Z. Yan.

The ATP content of a skeletal muscle at rest, measured in biopsy samples, is about 6 mmol · kg^{-1}. When the muscle is activated to contract, the cytosolic [ATP] in its fibers decreases as ATP is being hydrolyzed mainly by the myosin, Na^+–K^+, and Ca^{2+} ATPases. It is estimated that ATP would vanish in about 3 s of maximal exercise—and that would be the end of it—in the absence of sources and processes ensuring an almost instantaneous replenishment. Obviously, such sources and processes exist, and we will start considering them in the next paragraph. Thanks to them, the drop in the

[ATP] is limited. The largest decreases that have been reported are by approximately 50% after 30 s of maximal exercise. Exercises of lower intensity elicit smaller drops in the [ATP] because they have lower energy demands. This is also the case with exercises of longer duration, in which the processes that regenerate ATP have more time to balance its breakdown.

Researchers often prefer to report the amounts of ATP and other biomolecules in muscle per kilogram of dry muscle (denoted as *dm*, which happens to stand for both *dry muscle* and *dry mass*). Dry muscle is produced in the laboratory by lyophilization (section III.7) of the natural, "wet" muscle. Because 4 kg of wet muscle produces about 1 kg of dry muscle, we can convert the content of dry muscle into content of wet muscle by dividing by 4. Thus, 24 mmol ATP · (kg dry muscle)$^{-1}$ would be 6 mmol ATP · (kg wet muscle)$^{-1}$. In this book, contents will refer to wet muscle, the natural form.

In contrast to the [ATP] that decreases, the [ADP] and [P_i] increase in exercising muscles, because ADP and P_i are the products of ATP hydrolysis. The [AMP] also increases thanks to the reaction

$$2\ \mathrm{ADP} \rightleftharpoons \mathrm{AMP} + \mathrm{ATP} \qquad \Delta G^{\circ\prime} = 0.6\ \mathrm{kcal \cdot mol^{-1}} \qquad \text{(equation 9.3)}$$

In this reaction, the terminal phosphoryl group of one ADP is transferred to another ADP, converting the former into AMP and the latter into ATP (figure 9.3). Thus, we squeeze energy out of ADP, since it cannot serve as a direct energy source. To put it in numbers, one could write the equation 2 · 2 = 1 + 3 for the phosphoryl groups or 2 · 1 = 0 + 2 for the phosphoanhydride linkages.

As the cytosolic [ADP] increases and the cytosolic [ATP] decreases at the onset of exercise, reaction 9.3 is shifted to the right. Reaction 9.3 is a fast way of resynthesizing ATP. It has a maximal rate of 0.9 mmol ATP per kilogram of muscle per second, and it is anaerobic.

The enzyme that catalyzes reaction 9.3 is **adenylate kinase**. **Kinases** are enzymes that catalyze the phosphorylation of compounds; the phoshoryl donor is ATP. Kinases are specified by their substrates. Adenylate kinase is so named because, if you reverse reaction 9.3 (remember, enzymes catalyze reactions both ways; section 3.15), you will see that ATP phosphorylates adenylate (synonym of AMP, section 2.3). Adenylate kinase is also known as **myokinase** because it abounds in muscle.

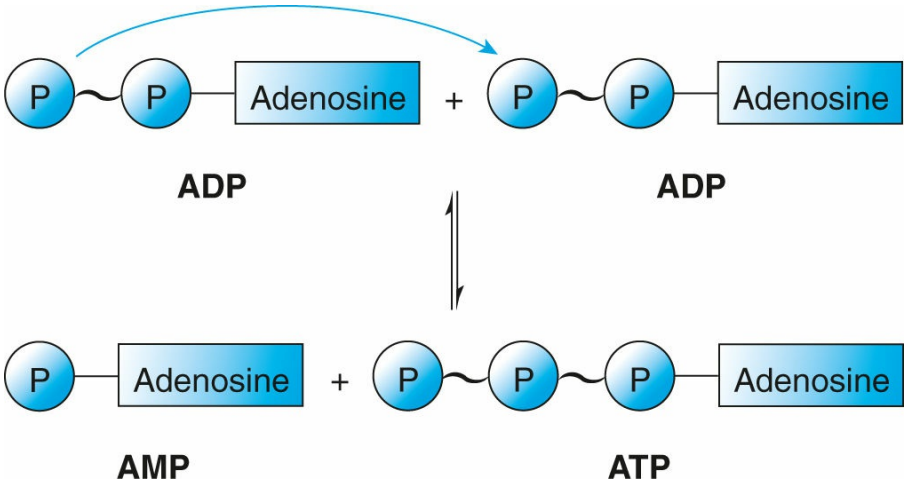


Figure 9.3 **Squeezing energy out of ADP.** Although ADP cannot serve as a direct energy source, one molecule of it can donate a ~P to another ADP molecule, thus producing ATP while being degraded into AMP.

9.3 Phosphocreatine

Skeletal muscles contain considerable quantities of **creatine** and **phosphocreatine**, or **creatine phosphate**. The structural formulas of the two compounds appear in figure 9.4. Creatine is an amino acid, though not one of the 20 amino acids that constitute proteins. Our bodies synthesize about one gram of creatine from three amino acids, that is, glycine, arginine, and methionine, daily. We obtain another gram from an average mixed diet (of animal and plant origin) daily. Meat of any kind (red meat, poultry, or fish) is the best source of creatine, hence the name, after *kréas*, the Greek word for meat. Creatine is absent from plant foods.

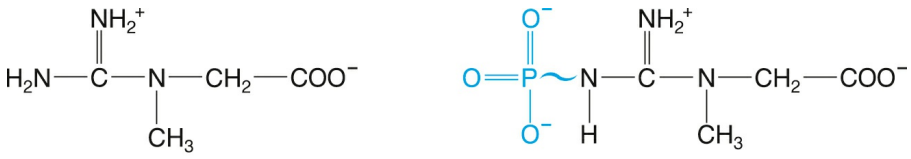


Figure 9.4 **Creatine and phosphocreatine.** These compounds aid in ATP homeostasis in muscle through their interconversion.

Phosphocreatine derives from the attachment of a phosphoryl group to a

terminal nitrogen of creatine through a phosphoanhydride linkage. A muscle at rest contains about 12 mmol of creatine and 20 mmol of phosphocreatine per kilogram. Thus, phosphocreatine outnumbers creatine at rest. It also outnumbers ATP by a factor of more than three.

Phosphocreatine is the fastest source for ATP resynthesis. Thus it is a valuable energy source during maximal exercise, in part because it regenerates ATP from ADP in a single reaction:

$$\text{Phosphocreatine} + \text{ADP} + \text{H}^+ \rightleftharpoons \text{creatine} + \text{ATP} \qquad \Delta G^{\circ\prime} = -3.2 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 9.4)}$$

In this reaction, phosphocreatine transfers its phosphoryl group to ADP, converting it into ATP, and, as for itself, becomes creatine (figure 9.5). This is a case of **substrate-level phosphorylation**, that is, the synthesis of ATP or another ribonucleoside triphosphate in a reaction in which a substrate is a compound of high phosphoryl-transfer potential. The term is used to contrast this manner of ATP formation with oxidative phosphorylation, introduced in section 2.6 and explained in section 10.15.

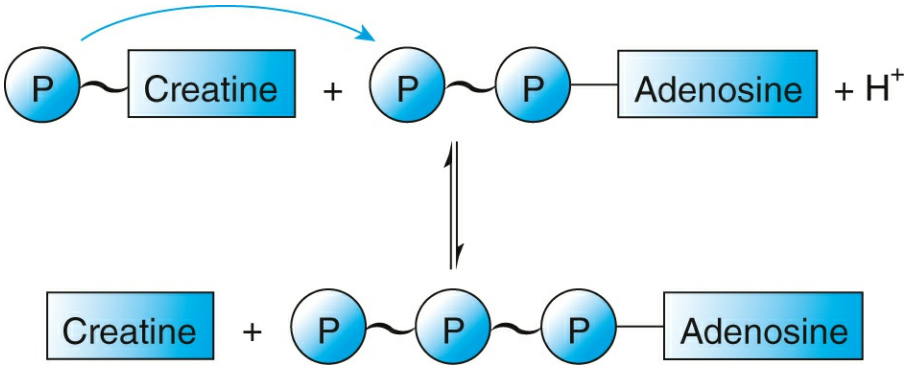


Figure 9.5 **Replenishing ATP from phosphocreatine.** Phosphocreatine can donate its ~P to ADP, thus producing ATP while being degraded into creatine.

The negative $\Delta G^{\circ\prime}$ of reaction 9.4 means that phosphocreatine has a higher phosphoryl-transfer potential than ATP (since the transfer of a phosphoryl group from phosphocreatine to ADP is favored, whereas the transfer of a phosphoryl group from ATP to creatine is not). This superiority of phosphocreatine is also clear from the fact that the $\Delta G^{\circ\prime}$ of phosphocreatine hydrolysis is more negative than that of ATP hydrolysis.

$$\text{Phosphocreatine} + \text{H}_2\text{O} \rightleftharpoons \text{creatine} + \text{P}_\text{i} \qquad \Delta G^{\circ\prime} = -9.5 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 9.5)}$$

Similarly to reaction 9.3, reaction 9.4 is shifted to the right as the cytosolic [ADP] increases and the cytosolic [ATP] decreases at the onset of exercise. The reaction rate is high owing to the high concentration of the catalyzing enzyme, which is called **creatine kinase (CK)** because it catalyzes the phosphorylation of creatine by ATP (reverse reaction 9.4). CK in the cytosol consists of two subunits; each can be either of two isoforms denoted by B for brain and M for muscle. This variation yields three combinations:

- CK-BB, or CK1, which dominates in the brain and smooth muscle
- CK-MB, or CK2, which dominates in the heart
- CK-MM, or CK3, which dominates in skeletal muscle

The term *creatine phosphokinase* (abbreviated CPK), used sometimes in the literature and in reports of biochemical laboratories, is redundant: *creatine kinase* will do.

Part of CK-MM is bound to the M line in the middle of the sarcomeres thanks to its high affinity for myomesin and, to a lesser degree, M-protein (section 8.1), as shown by Thorsten Hornemann and associates. Thus, CK-MM is appropriately positioned close to the myosin heads, ensuring the rapid replenishment of ATP from phosphocreatine and ADP during muscle activity.

ATP regeneration by phosphocreatine is an anaerobic process. Owing to the superiority of phosphocreatine over ATP in terms of both concentration and phosphoryl-transfer potential, the high activity of CK, and the proximity of CK to the myosin heads, ATP is efficiently resynthesized during the first seconds of a maximal effort. The maximal rate of ATP resynthesis in human muscle is estimated to be about 2.6 mmol $\cdot$ kg^{-1} $\cdot$ s^{-1} and to be attained within 1 to 2 s of maximal contraction. Thus, as mentioned in the previous section, ATP suffers a moderate decline as compared with its massive hydrolysis to support muscle activity. By way of contrast, phosphocreatine is decimated: Its concentration, from three to four times the ATP concentration at rest, may drop below the ATP concentration after half a minute of maximal exercise. An example of such changes is presented in figure 9.6.

Compounds of high phosphoryl-transfer potential provide a good starting

point for exploring the metabolic differences among muscle fiber types that I alluded to in section 8.9. In several elegant studies, researchers carefully isolated single muscle fibers from muscle biopsy samples taken at rest and after exercise. Then they characterized each fiber and measured its ATP and phosphocreatine contents. Most findings show that type II fibers have higher [ATP] and [phosphocreatine] as compared with type I fibers. What is more, maximal exercise causes larger drops in the [ATP] and [phosphocreatine] of type II fibers in both absolute and relative terms (that is, whether changes are expressed in mmol · kg^{-1} or as percentages of the concentrations at rest). In fact, the drops are so big that the postexercise ATP and phosphocreatine contents are lower in type II than in type I fibers. Table 9.1 summarizes these differences, which show that type II fibers are better suited than type I fibers to supporting maximal muscle activity.

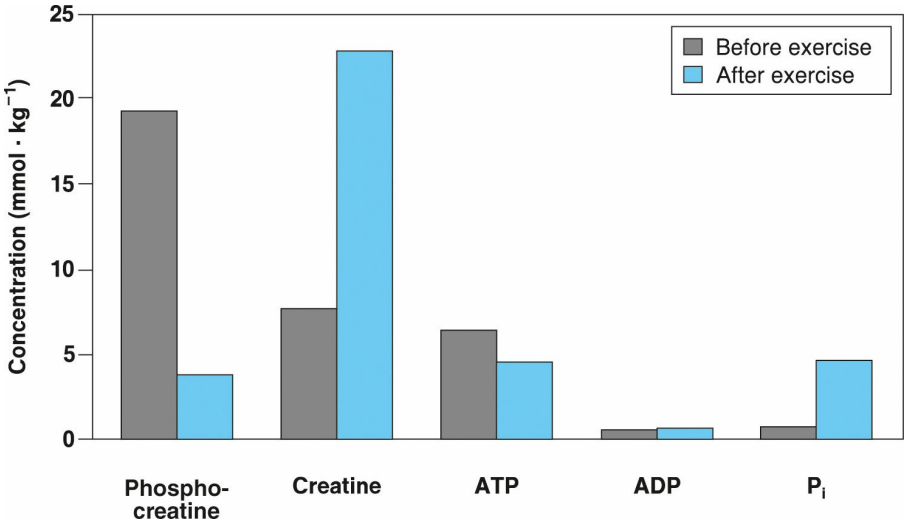


Figure 9.6 Changes in the concentrations of compounds related to energy production during maximal exercise. As determined in biopsy samples taken from human quadriceps muscle before and after 30 s of maximal cycling, phosphocreatine decreases by 80%, being converted into creatine, and ATP decreases slightly, while ADP and P_i increase.

The graph was constructed on the basis of data from Gregory Bogdanis and coworkers.

Table 9.1 Differences of the Main Muscle Fiber Types in Humans with Regard to ATP and Phosphocreatine

	Muscle fiber type

Property	I	II
[ATP] at rest	Lower	Higher
[Phosphocreatine] at rest	Lower	Higher
[ATP] decrease with maximal exercise	Lower	Higher
[Phosphocreatine] decrease with maximal exercise	Lower	Higher
[ATP] after maximal exercise	Higher	Lower
[Phosphocreatine] after maximal exercise	Higher	Lower

Because its amount is rather limited, phosphocreatine is the *major* source for ATP resynthesis (that is, it contributes more ATP than any other source does) in maximal sporting activities lasting only a few seconds. Exactly how many seconds? The answer is hard to determine because measuring the contribution of all potential sources to energy production during very short periods of exercise is experimentally difficult. At any rate, research findings indicate that the dominance of phosphocreatine does not exceed 7 s of maximal exercise. Maximal exercises of longer duration derive ATP mainly from carbohydrate breakdown, as we will see in the next chapter.

Thus, phosphocreatine is the major energy source in sports and events such as weightlifting, jumps, throws, and the 60 m sprint. It is also the major energy source during short maximal efforts in intermittent events, such as a sprint by a football or soccer player, an attack by a boxer, and a spike in volleyball.

Apart from phosphocreatine breakdown during maximal exercise, great interest is also taken in phosphocreatine replenishment during and after exercise. Because this process requires the prior resynthesis of ATP by aerobic mechanisms that we have not yet examined, I will defer the discussion of phosphocreatine replenishment until we reach section 14.26, where we will also discuss the hot issue of creatine supplementation.

9.4 Watching Exercise Metabolism

The measurement of metabolites in small muscle biopsy samples is the source of most of our knowledge about how exercise changes their concentrations (as was the case in figure 9.6). An alternative way of studying such changes is through noninvasive techniques like NMR spectroscopy (section III.7). This method can be applied not only to biological samples but

also to whole organisms, including humans placed inside powerful cylindrical magnets, since it is painless and harmless. In fact, a person may exercise while being monitored in an NMR spectrometer. Thus, NMR spectroscopy lets us watch muscle metabolism at work during exercise, much as we watch the life in an aquarium through one of its windows. However, the technique does have two drawbacks—the high cost of the equipment and the fact that one can perform only a limited number of exercises inside a magnet.

Because the predominant isotope of phosphorus, ^{31}P, is suitable for NMR spectroscopy, the technique has been extensively used to monitor changes in the muscle ATP, phosphocreatine, and P_i concentrations during exercise. As mentioned in section III.7, we can identify compounds based on the frequencies with which their nuclei resonate, and we can measure their concentrations from the amount of radiation absorbed. We do so by analyzing **NMR spectra**, that is, graphs of the absorbed radiation as a function of frequency (figure 9.7*)*. ^{31}P NMR spectra of muscle present five major peaks; three account mainly for the α, β, and γ phosphorus atoms of ATP (consult figure 2.4), and the other two belong to the phosphorus atoms of phosphocreatine and P_i.

The spectrum taken after exercise shows a dramatic drop in the [phosphocreatine] and an impressive rise in the [P_i]. On the other hand, the [ATP] has not changed. Such experiments let us watch in real time the movement of phosphoryl groups from one compound to another during exercise.

We can obtain additional information about muscle metabolism during exercise by studying the shift in the P_i peak along the frequency axis. The position of the peak is affected by the cytosolic pH, which determines the dominant ionic form of P_i. At physiological pH, the dominant form is hydrogen phosphate (HPO_4^{2-}, figure 2.5). However, if protons are produced, then formation of dihydrogen phosphate ($H_2PO_4^-$) is favored according to the reaction

$$HPO_4^{2-} + H^+ \rightleftharpoons H_2PO_4^- \qquad \text{(equation 9.6)}$$

Incidentally, the interconversion of the two phosphate ions constitutes an

important buffer system in the body—namely, the **phosphate system**. Now, because the P nucleus is surrounded by different atoms in HPO_4^{2-} and $H_2PO_4^-$, its resonance frequency depends on the proportion of the two ions and, hence, the cytosolic proton concentration.

The main reason for proton production in muscle during exercise is the anaerobic breakdown of carbohydrates into lactate. By using standard solutions of phosphate salts (that is, solutions of defined composition and pH), investigators can measure the drop in cytosolic pH and estimate lactate production from the shift in the P_i peak.

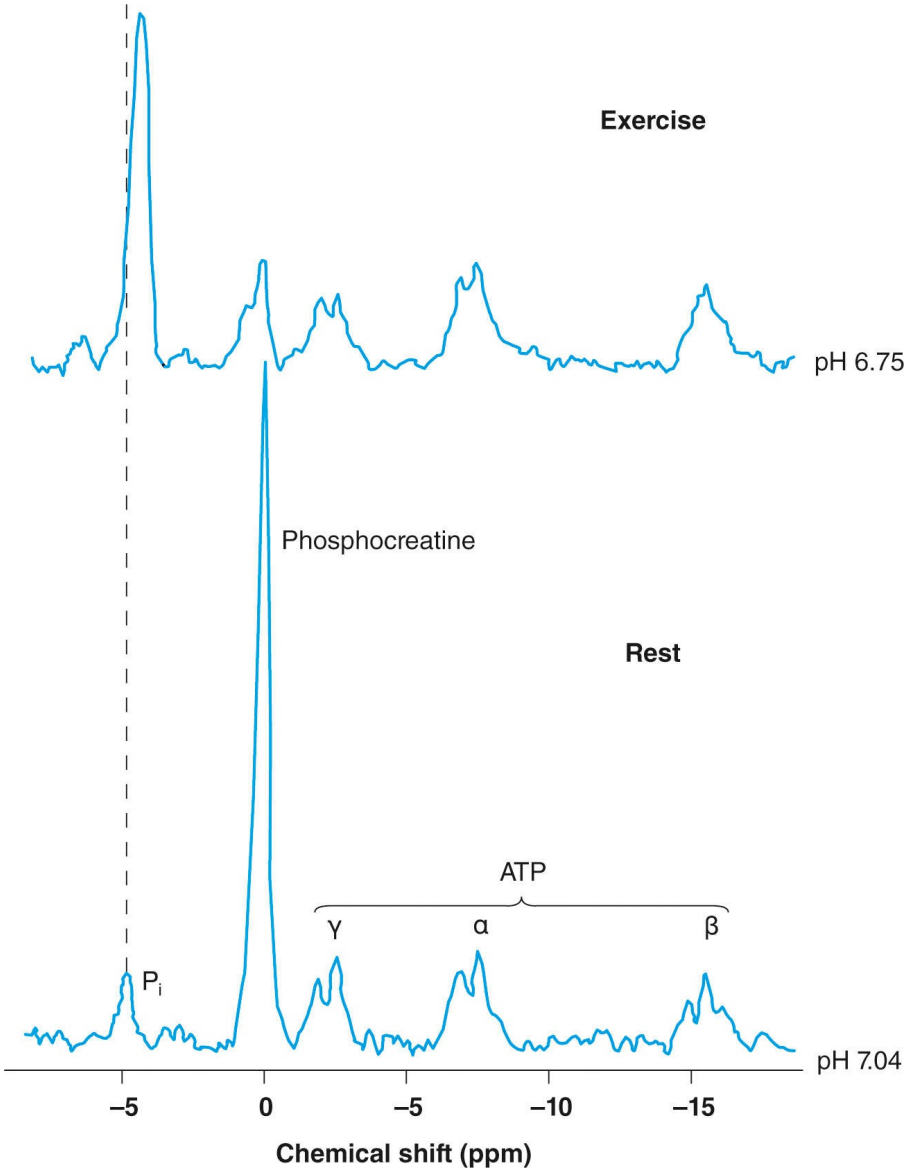


Figure 9.7 Watching exercise metabolism through NMR spectroscopy. Nuclear magnetic resonance spectroscopy affords the bloodless monitoring of metabolism in a human tissue. The ^{31}P NMR spectrum of the vastus medialis muscle shows spectacular—and inverse—changes in the [phosphocreatine] and [P_i] (proportional to the areas under their peaks) after 15 min of exercise on a horizontal step ergometer at 20%, 35%, and 45% of a person's maximal strength (5 min at each intensity in sequence). In contrast, the peaks corresponding to the α, β, and γ phosphorus atoms of ATP have remained practically unaltered. (To be exact, the peaks labeled α and β also contain the signals from the P atoms of ADP; the peak labeled α also contains the signal from the P atom of AMP, but the ADP and AMP concentrations are much lower than that of ATP.) Note how much more phosphocreatine there is as compared with ATP at rest. The P_i peak has moved slightly to the right after exercise (the dotted line shows the position at rest), which is a sign of a drop in pH. Based on the frequencies with which P_i resonated at rest and after exercise, the researchers calculated that pH decreased from 7.04 to 6.75. Frequency is expressed as deviation (or chemical shift) from the frequency of a reference compound and is measured in millionths (parts per million, or ppm) of the instrument's operating frequency; the frequency axis is arranged in an unusual way, decreasing from left to right.

Reprinted by permission from G. Bernus et al., "31P Nuclear Magnetic Resonance Spectra Of Human Vastus Medialis Muscle at Rest And Exercise," *Sandoz Sport Research Project,* (1992): 1988-1995. Unpublished data.

9.5 Loss of AMP by Deamination

In section 9.2, we saw that two ADP can yield one AMP and one ATP, thus providing some additional ATP for muscle activity. AMP can next be converted into **inosinate**, or **inosine monophosphate (IMP**, figure 9.8), through the highly exergonic reaction

$$AMP + H_2O + H^+ \rightarrow IMP + NH_4^+ \qquad \Delta G^{\circ\prime} = -7.6 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 9.7)}$$

This process is a **deamination**, that is, a loss of an amino group. The amino group appears in the products as **ammonium** cation (NH_4^+), which is the conjugate acid of the base **ammonia** (NH_3) and predominates over NH_3 at physiological pH. Reaction 9.7 is catalyzed by **adenylate deaminase**, an enzyme that is activated during hard exercise. The main reason for this activation is the decrease in cytosolic ATP.

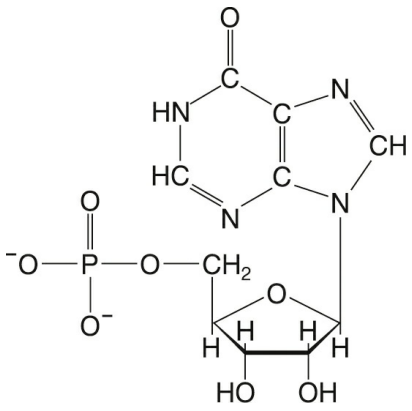


Figure 9.8 **Inosine monophosphate (IMP).**

ATP controls the activity of adenylate deaminase in an impressive way: As discovered by Ichiro Hisatome and colleagues, the enzyme has a domain that binds ATP and a domain that binds to the myosin heavy chains (precisely, S2; section 8.4). The presence of ATP in the first domain inhibits the binding to myosin. The enzyme then remains soluble in the cytosol and inactive. More than 90% of adenylate deaminase is found in this state at rest. When ATP decreases during hard exercise, 50% to 60% of the enzyme loses its ATP and binds to S2. Binding activates adenylate deaminase and speeds up AMP deamination. Another possible mechanism of activation is the decrease in cytosolic pH because of the anaerobic breakdown of carbohydrates, because adenylate deaminase exhibits maximal activity at pH 6.5.

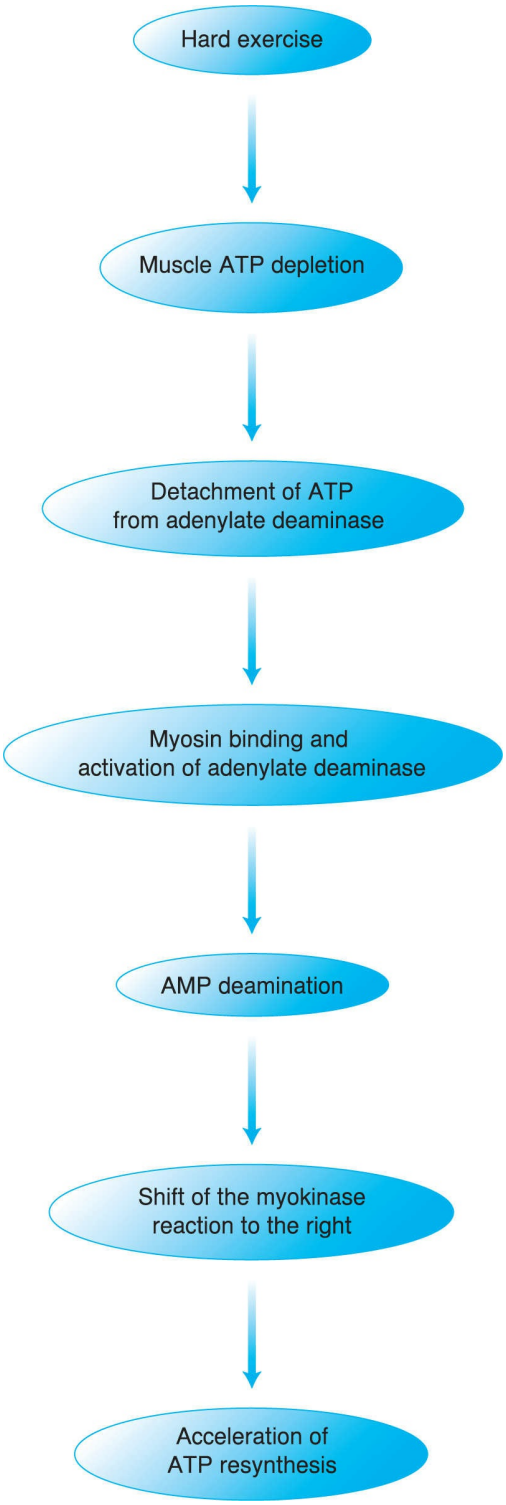


Figure 9.9 **A compound taking good care of itself.** The decrease in muscle ATP during hard exercise signals the acceleration of its resynthesis by the myokinase reaction through the activation of adenylate deaminase.

Why is AMP deamination important to such a degree that it is controlled by muscle activity? The answer lies in the shift of reaction 9.3 to the right as one of the products (AMP) is eliminated. The shift increases the amount of the other product (ATP), thus speeding up ATP regeneration. We see here another way in which ATP ensures its homeostasis during hard exercise: When it abounds, adenylate deaminase is relatively inactive; when it decreases, adenylate deaminase is activated and indirectly speeds up ATP resynthesis (figure 9.9).

This benefit comes at a price, though: AMP deamination reduces the pool of adenine ribonucleotides (ATP, ADP, and AMP), thus making ATP regeneration during recovery from exercise more time- and energy-consuming, as we will see in section 14.26. It seems, however, that the gain from the extra supply of ATP under the highly demanding conditions of hard exercise outweighs the extra burden during recovery.

9.6 Purine Degradation

The positive effect of ATP depletion on adenylate deaminase activity increases the IMP and ammonium concentrations in vigorously exercising muscles. Ammonium crosses the sarcolemma through an integral transport protein, appears in the interstitial fluid, and enters the bloodstream. Most of it is then taken up by the liver, where it is converted into urea, as I will describe in section 12.9.

IMP, on the other hand, may lose its phosphoryl group through **dephosphorylation** to become **inosine** (figure 9.10) in the sarcoplasm. Inosine may then lose its ribose through hydrolysis to become **hypoxanthine** (figure 9.11), which is considered a purine thanks to its structural similarity to adenine and guanine (remember purines from section 4.3). Hypoxanthine, in turn, may suffer two successive oxidations to **xanthine** (figure 9.12) and **urate** (figure 9.13), which is the anion, or conjugate base, of **uric acid**. Urate is the main product of purine degradation in us humans, although it too may suffer an oxidation, followed by **decarboxylation**, that is, loss of a carboxyl group in the form of carbon dioxide, to yield **allantoin** (figure 9.14).

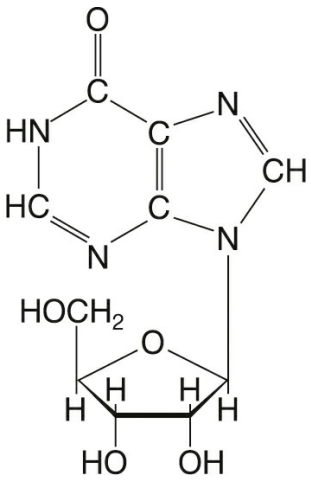


Figure 9.10 **Inosine.**

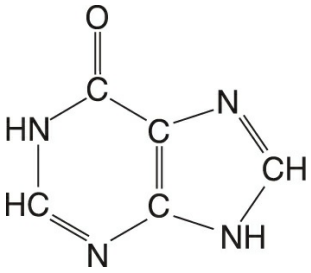


Figure 9.11 **Hypoxanthine.**

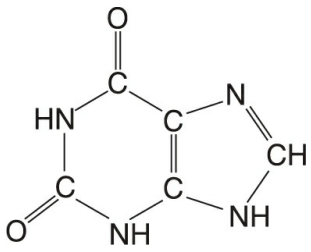


Figure 9.12 **Xanthine.**

IMP and its degradation products usually increase in muscle after hard exercise. The same compounds except IMP (which does not cross the sarcolemma readily) are also detected in increased concentrations in plasma and urine.

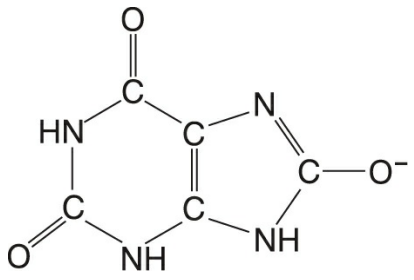


Figure 9.13 **Urate.**

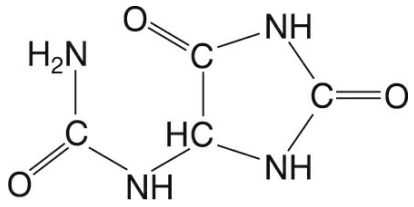


Figure 9.14 **Allantoin.**

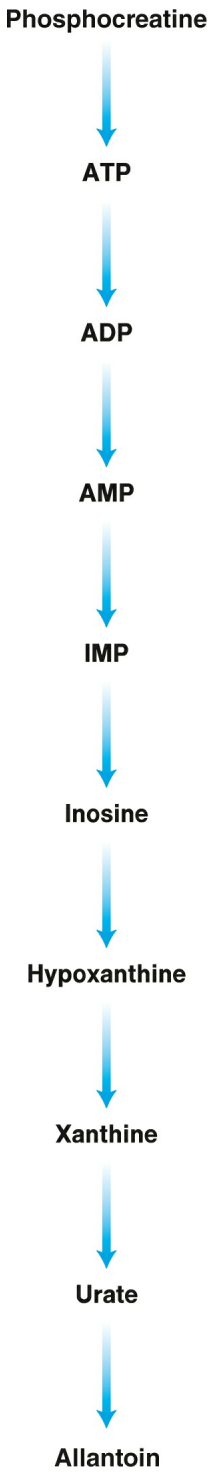


Figure 9.15 Conversions of compounds of high phosphoryl-transfer potential and their degradation products during exercise.

Of the conversions just described, two merit further discussion. These are the oxidations of hypoxanthine to xanthine and of xanthine to urate according

to the following reactions:

$$\text{Hypoxanthine} + 2\ O_2 + H_2O \rightarrow \text{xanthine} + 2\ O_2^{\bullet -} + 2\ H^+ \qquad \text{(equation 9.8)}$$

$$\text{Xanthine} + 2\ O_2 + H_2O \rightarrow \text{urate} + 2\ O_2^{\bullet -} + 3\ H^+ \qquad \text{(equation 9.9)}$$

Both reactions are catalyzed by **xanthine oxidase** and, as you can see, produce the superoxide radical introduced in section 1.5. $O_2^{\cdot -}$ and other radicals produced from it or through other routes tend to remove single electrons from compounds in their vicinity and pair those electrons with their own unpaired electrons. In doing so, they may damage DNA, proteins, and lipids, thus causing disease or speeding up aging. On the other hand, radicals may destroy invading pathogens, trigger signal transduction pathways, and mediate adaptations to exercise. Radical production increases during exercise and is therefore attracting the attention of many researchers in exercise science. Thus, I will return to this topic in chapters 14 and 15. For now, let me say that, because exercise augments the flux of metabolites along the purine degradation pathway, reactions 9.8 and 9.9 are considered substantial sources of radicals during exercise.

Figure 9.15 recaps the series of conversions considered in the present chapter.

Summary

Compounds of high phosphoryl-transfer potential constitute a class of energy sources that contain phosphoryl groups and release high amounts of energy when hydrolyzed. This class includes ATP, ADP, and phosphocreatine. ATP and ADP participate in the namesake cycle, in which ADP and P_i synthesize ATP with the energy released from catabolic processes, while ATP is broken down into ADP and P_i to provide energy for anabolism, movement, active transport, and signal amplification. ATP serves as a direct energy source for these processes, whereas ADP, phosphocreatine, carbohydrates, lipids, and proteins serve as indirect energy sources (they need to provide ATP first). The ATP–ADP cycle has a daily turnover

that exceeds half the weight of a person who is not particularly active and the entire weight of an athlete.

The ATP concentration in muscle is protected against excessive decline during exercise by a number of homeostatic mechanisms, one of which is the conversion of two ADP into ATP and AMP in a reaction catalyzed by adenylate kinase, or myokinase. This reaction is facilitated by the subsequent deamination of AMP to IMP and ammonium (a reaction favored during hard exercise), although this hinders ATP resynthesis during recovery. IMP is further degraded into inosine, hypoxanthine, xanthine, urate, and allantoin.

As compared with ATP and ADP, much more energy is stored in phosphocreatine, which has a higher phosphoryl-transfer potential than does ATP and generates it from ADP at a high rate during maximal exercise through the catalytic action of creatine kinase. The muscle-specific isoform of the enzyme is bound to the M line in the middle of the sarcomeres, close to the myosin heads. Phosphocreatine is decimated during maximal exercise and constitutes the major energy source in maximal efforts lasting up to about 7 s. Type II muscle fibers have higher ATP and phosphocreatine concentrations at rest than do type I fibers. In addition, they suffer higher declines in the [ATP] and [phosphocreatine] during maximal exercise.

Problems and Critical Thinking Questions

1. (Integrative problem) Of the three compounds of high phosphoryl-transfer potential discussed in this chapter, I have given the $\Delta G^{\circ\prime}$ of hydrolysis for two (ATP in reaction 2.5 and phosphocreatine in reaction 9.5) but not for the third (ADP). Based on reactions 2.5, 2.6, and 2.7, write down the reaction of ADP hydrolysis and calculate its $\Delta G^{\circ\prime}$.
2. (Integrative problem) Derive reaction 9.3 from reactions 2.5, 2.6, and 2.7 and verify its $\Delta G^{\circ\prime}$.

3. (Integrative problem) Derive reaction 9.5 from reactions 2.5 and 9.4 and verify its $\Delta G°'$.
4. The concentrations of ATP, phosphocreatine, creatine, and P_i in a muscle at rest are 6, 20, 12, and 1 mmol · kg^{-1}, respectively. The amount of ATP suffices for 3 s of maximal exercise. Assuming insignificant contribution from other energy sources, suggest probable concentrations of the four compounds after 3 s of maximal exercise.
5. Treatment of a muscle with the compound fluoro-2,4-dinitrobenzene causes a rapid decline in the [ATP], whereas the [phosphocreatine] does not change during a series of contractions. Propose an explanation.
6. Identify three ways in which muscle fibers ensure ATP homeostasis during hard exercise.
7. What changes does hard exercise cause in the concentrations of ATP, ADP, AMP, phosphocreatine, creatine, P_i, IMP, and ammonium in muscle?
8. The compounds of high phosphoryl-transfer potential treated in this chapter are not the only ones found in living organisms. Invertebrates, such as crustaceans and scallops, use phosphoarginine (that is, the amino acid arginine with a phosphoryl group at the end of its side chain), and annelids (segmented worms) use phospholombricine (a more complex compound). Both compounds serve to replenish ATP during exercise in these animals. Write down the reactions through which they do so.
9. Write down the reactions of hydrolysis of phosphoarginine and phospholombricine.
10. Based on the $\Delta G°'$ values of hydrolysis of ATP, phosphocreatine, and ADP (take the latter from problem 1)—and on the fact that the $\Delta G°'$ values of hydrolysis of phosphoarginine and phospholombricine are –7.8 and –4.7 kcal · mol^{-1}, respectively—rank the five compounds in order

of descending phosphoryl-transfer potential.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

compound of high phosphoryl-transfer potential
phosphagen
direct energy source
indirect energy source
ATP–ADP cycle
adenylate kinase, myokinase
kinase
creatine
phosphocreatine, creatine phosphate
substrate-level phosphorylation
creatine kinase, CK
NMR spectrum
phosphate system
inosinate, inosine monophosphate, IMP
deamination
ammonium, ammonia
adenylate deaminase
dephosphorylation
inosine
hypoxanthine
xanthine
urate, uric acid
decarboxylation
allantoin
xanthine oxidase

References and Suggested Readings

Bogdanis GC, Nevill ME, Boobis LH, Lakomy HK, Nevill AM (1995). Recovery of power output and muscle metabolites following 30 s of maximal sprint cycling in man. *Journal of Physiology* 482 (part 2): 467-480.

Hisatome I, Morisaki T, Kamma H, Sugama T, Morisaki H, Ohtahara A, Holmes EW (1998). Control of AMP deaminase 1 binding to myosin heavy chain. *American Journal of Physiology* 275: C870-C881.

Hornemann T, Kempa S, Himmel M, Hayess K, Fürst DO, Wallimann T (2003). Muscle-type creatine kinase interacts with central domains of the M-band proteins myomesin and M-protein. *Journal of Molecular Biology* 332: 877-887.

CHAPTER 10

Carbohydrate Metabolism in Exercise

Learning Objectives

After reading this chapter, you should be able to do the following:

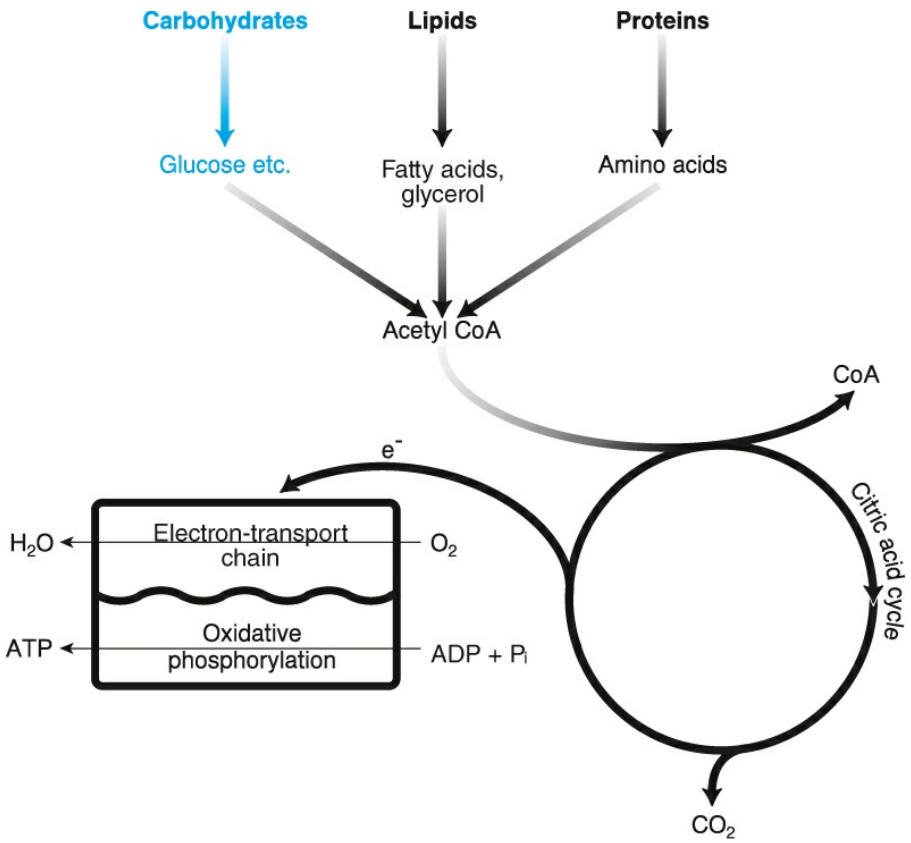
- Discuss how we digest and store carbohydrates in the body.
- Describe the process of glycogenolysis and the factors that speed it up during exercise.
- Explain glycolysis, describe the steps in this metabolic pathway, and explain how exercise speeds it up.
- List the processes involved in the aerobic breakdown of carbohydrates, describe what happens in each one, and explain how exercise speeds them up.
- Contrast the anaerobic with the aerobic breakdown of carbohydrates in terms of products, energy yield, speed of energy production, and exercises in which they predominate.
- Critically assess differing views on lactate as a cause of fatigue and why lactate production increases with increasing exercise intensity.
- Discuss how the liver helps muscle with carbohydrates during exercise.

- Identify the factors that control the plasma glucose concentration during exercise.
- Describe the kinetics of blood lactate during and after exercise.
- Evaluate the relevance of the various lactate, aerobic, and anaerobic thresholds.

In chapter 5, we explored the variety (in terms of both structure and function) of animal and plant carbohydrates. In the present chapter, we will use that information as a basis for building our knowledge of how we assimilate carbohydrates from food and exploit them for energy production during exercise. As you will see, carbohydrates provide ATP in quantities and rates capable of supporting a wide variety of exercise tasks. This capacity is why *carbohydrates constitute the most precious energy source in the majority of sporting activities.* It is also why the present chapter is the longest in the book.

10.1 Carbohydrate Digestion, Absorption, and Distribution

Most **carbohydrates** in our bodies derive from dietary carbohydrates, which can belong to any of the categories used by biochemists and described in sections 5.1 to 5.4 (that is, monosaccharides, oligosaccharides, and polysaccharides) or to any of the categories preferred by nutritionists and described in section 5.5 (that is, simple and complex carbohydrates). Before they can be absorbed by our digestive tract and nourish us, most oligosaccharides and polysaccharides need to be broken down into their constituent monosaccharides. This breakdown, which happens through enzyme-catalyzed hydrolysis of the glycosidic linkages joining the monosaccharide residues, constitutes the process of **carbohydrate digestion**.



You are here: carbohydrate digestion.

A reduction of figure 2.11 (an overview of catabolism) will be displayed throughout part III, with a different segment highlighted in color each time, to indicate the specific process discussed and to help you fit it into the overall picture.

The bulk of carbohydrates in a usual diet come in the form of **starch** (a polysaccharide, or complex carbohydrate, introduced in section 5.4). Starch digestion begins in the mouth by the action of **α-amylase**, an enzyme secreted in the saliva. Remember that starch is a mixture of **amylose** and **amylopectin** (section 5.4). α-Amylase hydrolyzes the α1 → 4 glycosidic linkages of both substances, producing oligosaccharides such as **maltose** (introduced in section 5.3 and figure 5.5), **maltotriose**, and **α-dextrin**, or **limit dextrin** (figure 10.1). Maltotriose consists of three glucosyl units in a row, whereas α-dextrin consists of several glucosyl units around a branch point (an α1 → 6 glycosidic linkage) of amylopectin.

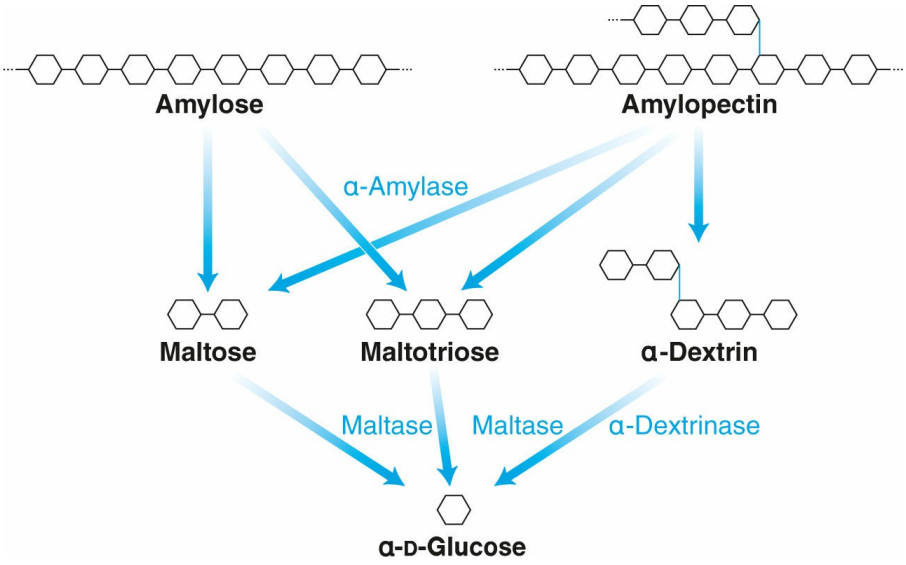


Figure 10.1 **Starch digestion.** The major carbohydrate in the human diet is broken down through the concerted action of three digestive enzymes. α-Amylase in the mouth and small intestine degrades amylose and amylopectin (the two constituent polysaccharides of starch) into maltose, maltotriose, and α-dextrin. Then maltase and α-dextrinase in the small intestine hydrolyze the three oligosaccharides to α-^{D}-glucose. Hexagons depict α-^{D}-glucose residues, as in figure 5.9. Horizontal connecting lines depict α1 → 4 glycosidic linkages. Vertical colored lines depict α1 → 6 glycosidic linkages.

Because food spends little time in the mouth, starch digestion in it is incomplete. No carbohydrate digestion takes place in the stomach, but when food enters the small intestine it is mixed with the pancreatic juice. This juice, secreted from the pancreas to the duodenum (the first section of the small intestine) through the pancreatic duct, contains α-amylase, which completes the breakdown of starch into maltose, maltotriose, and α-dextrin.

The final step in starch digestion involves the hydrolysis of these oligosaccharides to **α-D-glucose**. This process requires the catalytic action of two more enzymes, **maltase** and **α-dextrinase**, or **limit dextrinase**, which break down α1 → 4 and α1 → 6 glycosidic linkages, respectively. These two enzymes are integral glycoproteins of the plasma membrane of the microvilli (singular: **microvillus**)—microscopic fingerlike protrusions of cells that line the small intestine (**enterocytes**, figure 10.2), pointing to the interior of the intestine (the **intestinal lumen**). Enterocytes, in turn, cover larger fingerlike protrusions of the intestinal wall called *villi* (singular: **villus**, figure 10.3).

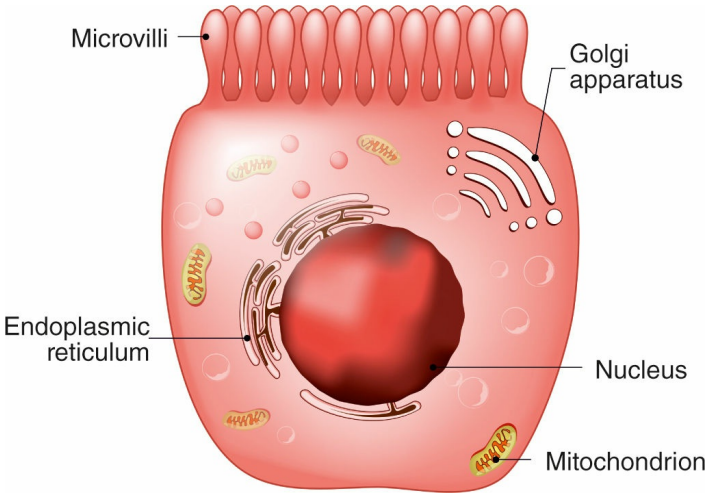


Figure 10.2 **Enterocyte.** Our small intestine is lined by enterocytes like the one shown here. Apart from the usual features of eukaryotic cells (such as the nucleus, mitochondria, endoplasmic reticulum, and Golgi apparatus), these cells feature numerous microvilli, that is, thin protrusions toward the intestinal lumen.

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Our diet usually contains two disaccharides, sucrose and lactose (section 5.3), which are present, respectively, in sweet foods (such as fruits and confectionery) and milk. Sucrose is hydrolyzed to α-D-glucose and β-D-fructose by **sucrase**, whereas lactose is hydrolyzed to β-D-galactose and α-D-glucose by **lactase**. Like maltase and α-dextrinase, both of these enzymes are integral glycoproteins of the plasma membrane of the microvilli.

The monosaccharides—primarily glucose and secondarily fructose and galactose—produced from the digestion of dietary carbohydrates in the lumen of the small intestine are absorbed into the cytosol of the enterocytes through transport proteins located in their plasma membrane. Finally, the monosaccharides exit the enterocytes through the other side—the one facing the capillaries of the villi. This exit completes **carbohydrate absorption**.

From the blood capillaries of the villi, monosaccharides enter the venous blood, which ultimately drains into the portal vein. This vein is the main supplier of blood to the liver, which serves as the distribution center of carbohydrates for the entire body. Once the liver receives dietary monosaccharides from the portal vein, it reserves a portion mainly to synthesize its **glycogen**. Then it releases the rest to the bloodstream, destined to nourish all other cells. This action completes **carbohydrate distribution**

to the body.

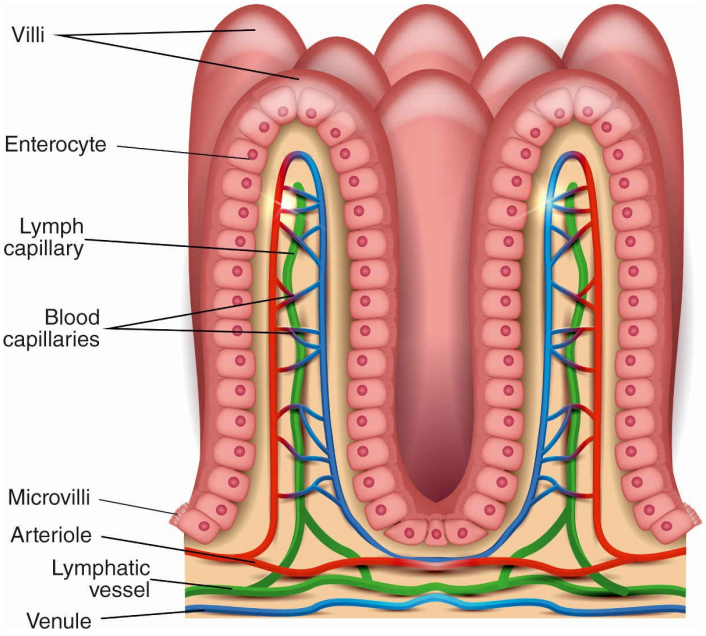

Figure 10.3 **Villi.** The inner surface of the small intestine is covered by thousands of villi, eight of which are depicted here (two in cross-section). Villi are covered by a single layer of enterocytes having tiny processes, the microvilli. The blood capillaries underneath the enterocytes carry dietary monosaccharides, amino acids, and minerals, whereas the lymph capillaries carry dietary lipids. Blood capillaries are connected to arterioles and venules, whereas lymph capillaries are connected to lymphatic vessels.

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A final word on carbohydrate digestion and absorption addresses a carbohydrate that we can neither digest nor absorb: **cellulose**. Indeed, the human genome lacks genes that encode **cellulase**, the enzyme that catalyzes the hydrolysis of the β1 → 4 glycosidic linkages in cellulose (section 5.4). Thus, cellulose in the cereals, fruits, and vegetables that we eat exits the body undigested. We see here another example of enzyme specificity: A difference between starch and cellulose in the configuration of the glycosidic linkages (α1 → 4 versus β1 → 4) necessitates different enzymes for their breakdown.

The fact that we cannot make use of cellulose does not render it useless. Scientific evidence suggests that consumption of dietary fiber (part of which is cellulose) can contribute to a number of health benefits, such as maintaining a healthy body weight and lowering the risk of diabetes and heart disease.

10.2 Glycogen Content of the Human Body

As mentioned in the previous section, the liver uses part of the dietary monosaccharides it receives to synthesize glycogen. The glycogen content of the liver is influenced by diet and exercise. If these factors are not extreme, then glycogen ranges between 3% and 7% of the liver mass. How many grams does this translate into? Let our models be a lean man weighing 75 kg and a lean woman weighing 62 kg. Their livers will weigh roughly 1.7 and 1.4 kg, respectively. If we adopt 5% as an average liver glycogen content, then we can calculate that the man will have about 85 g and the woman 70 g of liver glycogen.

Let's extend the calculations to the other glycogen depot, the skeletal muscles, which, in addition to the liver, use part of the glucose they receive from blood to synthesize our polysaccharide. Again, the muscle glycogen content is influenced by diet and exercise; if they are not extreme, then glycogen content ranges from 1% to 1.5%. Because the total muscle mass is disproportionately larger in males than in females—roughly 40% versus 30%—our model male and female will have 30 and 18.6 kg of muscle, respectively. If we adopt 1.25% as an average muscle glycogen content, then we can calculate that the male will have about 375 g and the female 233 g of muscle glycogen.

In scientific papers the glycogen content of tissues is often expressed in terms of millimoles of glucosyl units (equivalent to glucose residues) per kilogram of tissue. To convert this measure into percentage content, we must first multiply by the "molecular mass" of the glucose residue in glycogen (I place *molecular mass* between quotation marks because a residue is not an independent molecule), which depends on how a given glucose residue connects with its neighboring ones. Most often, a glucose residue connects with two other residues, in which case it has a "molecular mass" of 162 Da. Thus, for example, 80 mmol glucosyl units per kilogram muscle would be 80 · 162 mg, or 12,960 mg, or roughly 13 g per kilogram, or 1.3 g per 100 g, or 1.3% by weight.

Note that the liver contains proportionally more glycogen than muscles do. However, because our muscle mass is higher than our liver mass, we end up having more glycogen stored in the muscles than in the liver.

Glycogen is found in the cytosol of **hepatocytes** (liver cells) and muscle

fibers in the form of granules (figures 10.4 and 10.5). In the liver, glycogen granules can reach 290 nm in diameter, as determined by Glendon Parker and coworkers. In muscle, they are smaller, not exceeding 44 nm, as Ines Marchand and coworkers reported in 2002 and 2007. In addition to glycogen, the granules (in both the liver and muscle) contain the enzymes catalyzing the reactions of its synthesis and breakdown and even enzymes controlling these processes. (We will examine all of these enzymes later.) There is no glycogen in blood.

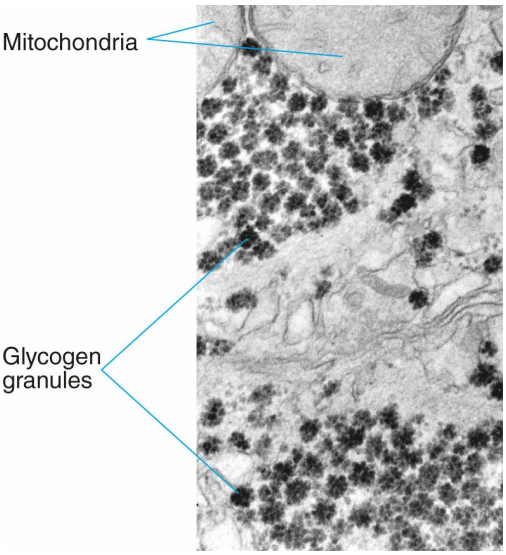


Figure 10.4 **Glycogen in hepatocytes.** Glycogen granules, looking like lead shot under high resolution in this electron micrograph, abound in hepatocytes. Half of the granules are clustered near two mitochondria at the top.

© Prof. E.R. Weibel, Institute of Anatomy, University of Bern, Switzerland.

Other animals have glycogen contents similar to those of us humans. From this similarity, you can understand that food of animal origin contributes little to our carbohydrate needs. Indeed, a 100 g portion of liver would provide just about 5 g of glycogen, whereas a 30 g slice of bread contains about 15 g of starch. Meat, on the other hand, contains no glycogen, which is fully degraded in the days between slaughter and consumption.

10.3 Glycogenesis

Glycogen synthesis, or **glycogenesis**, in hepatocytes and muscle fibers consists in the successive addition of α-D-glucose residues to a growing chain. Being a biosynthetic process, chain elongation requires an input of energy, which comes from two compounds of high phosphoryl-transfer potential: ATP and **UTP (uridine triphosphate)**. ATP is used to produce **α-D-glucose 1-phosphate**, or plain *glucose 1-phosphate*—that is, glucose carrying a phosphoryl group at C1 (figure 10.6).

Glucose 1-phosphate and many other monosaccharides carrying phosphoryl groups are often referred to as **sugar phosphates**.

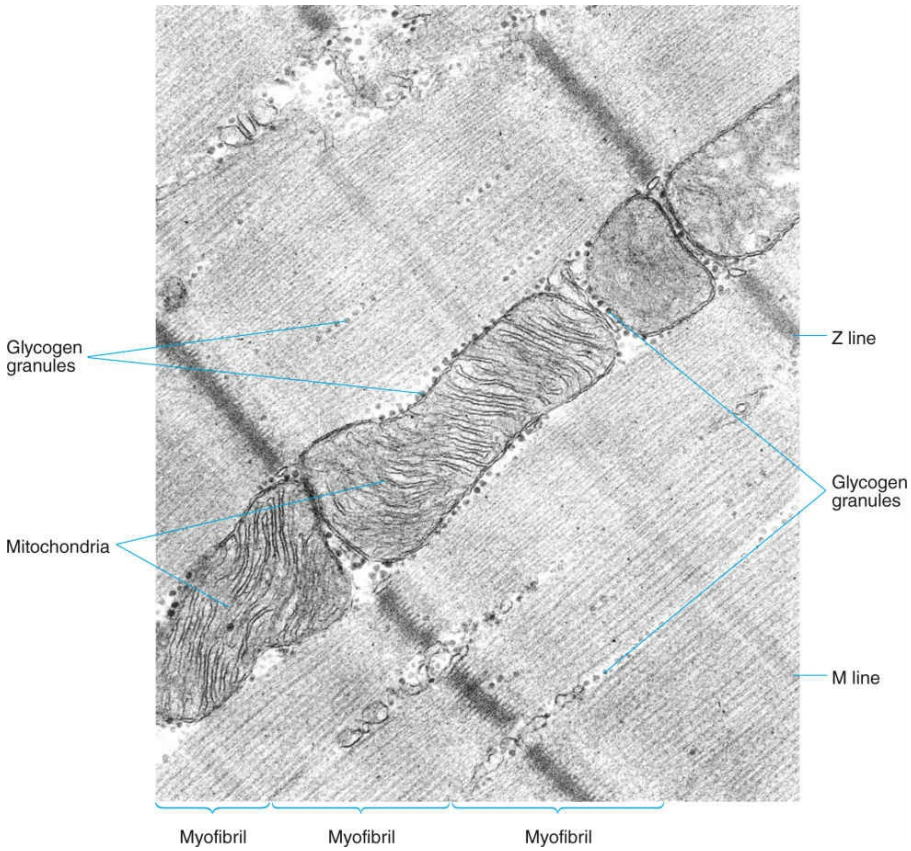


Figure 10.5 Glycogen in a skeletal muscle fiber. An electron micrograph of the sarcoplasm reveals plenty of glycogen granules, seen as dark dots squeezed among thick and thin filaments in the myofibrils, between different myofibrils, between myofibrils and mitochondria, and between mitochondria.

Courtesy of Dr. H. Hoppeler, University of Bern. Photographers H. Claassen and F. Graber.

$$\text{Glucose} + \text{ATP} \rightarrow \text{glucose 1-phosphate} + \text{ADP} \qquad \Delta G^{\circ\prime} = -2.3 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 10.1)}$$

Glucose 1-phosphate then reacts with UTP to produce **uridine diphosphate glucose**, or **UDP-glucose** (figure 10.7), and pyrophosphate in a reaction catalyzed by **UDP-glucose pyrophosphorylase**.

$$\text{Glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP-glucose} + PP_i \qquad \Delta G^{\circ\prime} = 0 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 10.2)}$$

The zero value of $\Delta G^{\circ\prime}$ reflects the equivalence of the phosphoanhydride linkages broken (in UTP) and formed (in UDP-glucose). The subsequent exergonic hydrolysis of PP_i (having a $\Delta G^{\circ\prime}$ of –3.8 kcal · mol^{-1}; reaction 2.7), catalyzed by **pyrophosphatase**, makes the synthesis of UDP-glucose favorable.

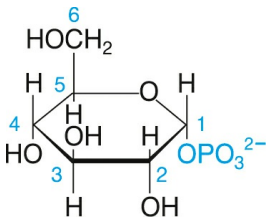


Figure 10.6 **α-D-Glucose 1-phosphate.**

Finally, UDP-glucose participates in the reaction of glycogen elongation, catalyzed by **glycogen synthase**.

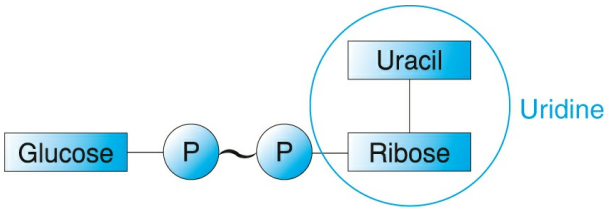


Figure 10.7 **Uridine diphosphate glucose (UDP-glucose).**

$$\text{UDP-glucose} + \text{glycogen } (n \text{ glucose residues}) \rightarrow \text{glycogen } (n+1 \text{ glucose residues}) + \text{UDP} + H^+ \qquad \Delta G^{\circ\prime} = -2.7 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 10.3)}$$

In this reaction, the UDP unit, acting as a vehicle for glucose, adds it to a nonreducing end (section 5.4) of glycogen through an α1 → 4 glycosidic linkage. The products are a glycogen molecule, longer by one glucose residue, and UDP.

In glycogenesis, glycogen is synthesized from UDP-glucose.

In glycogenesis, glycogen synthase is unable to start the process from scratch; rather, it requires a primer of at least four glucose residues. The primer is made by **glycogenin**, the very protein that forms the core of a glycogen molecule (figure 5.9). Glycogenin initiates glycogenesis by catalyzing the addition of a glucose residue from UDP-glucose to the hydroxyl group of the side chain of a tyrosine residue in its own molecule, as reviewed by Elton Zeqiraj and Frank Sicheri. Thus, glycogenin participates in this remarkable reaction as both substrate and enzyme. It adds seven glucose residues in a row to the initial one (although four residues in all are sufficient), at which point glycogen synthase takes over.

Which other enzyme requires a primer? (*Hint:* Refer to section 4.7.)

Another weakness of glycogen synthase is that it creates only linear chains. However, glycogen is branched. Another enzyme, named **glycogen-branching enzyme**, or simply **branching enzyme**, detaches segments of the nascent chain from the nonreducing end and transfers them to the interior of the molecule as branches by forming α1 → 6 glycosidic linkages (figure 10.8).

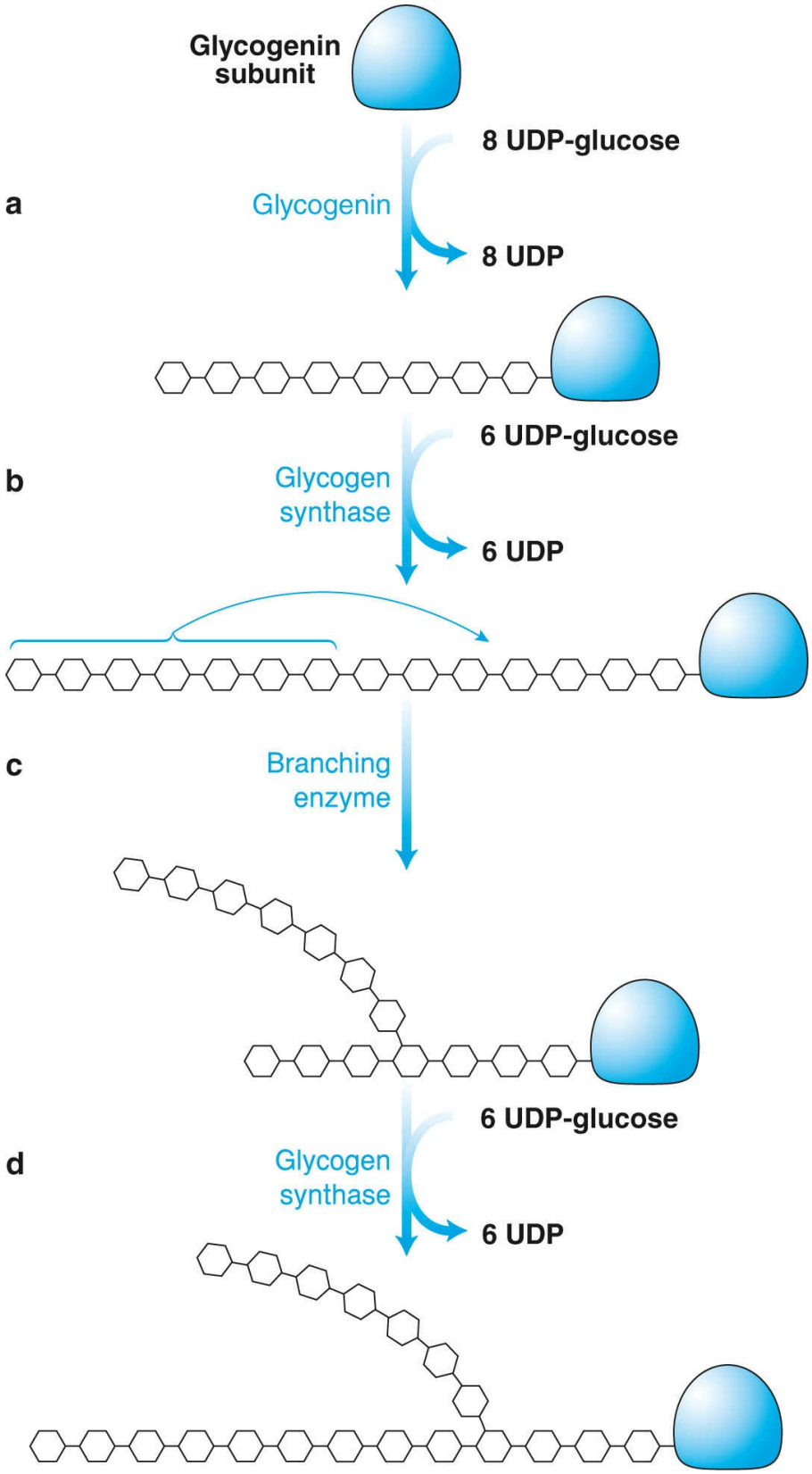


Figure 10.8 **Glycogenesis.** Three enzymes—glycogenin (also acting as the glycogen core),

glycogen synthase, and the branching enzyme—cooperate in glycogen synthesis, with UDP-glucose serving as the source of glucose residues. *(a)* Glycogenin adds eight residues in a row to itself. *(b)* Glycogen synthase adds more residues (six in this example) to the growing chain. *(c)* The branching enzyme moves seven residues to an inner position and forms a branch. *(d)* The synthase resumes work.

10.4 Glycogenolysis

We turn now to the degradation of glycogen, termed **glycogenolysis**. In this process, the α1 → 4 glycosidic linkages of glycogen are broken down by P_i according to the reaction

$$\text{Glycogen } (n \text{ glucose residues}) + P_i \rightarrow \text{glycogen } (n - 1 \text{ glucose residues}) + \text{glucose 1-phosphate} \qquad \text{(equation 10.4)}$$

The breakdown of a compound by P_i is termed **phosphorolysis**, in accordance with hydrolysis.

Through this reaction, glycogen loses, one by one, glucose residues from its nonreducing ends. At the same time, molecules of glucose 1-phosphate (the same compound used in glycogenesis) are produced. The reaction is catalyzed by **glycogen phosphorylase**, or simply **phosphorylase**, an enzyme containing pyridoxal phosphate (deriving from vitamin B_6; section 6.1) in its active site.

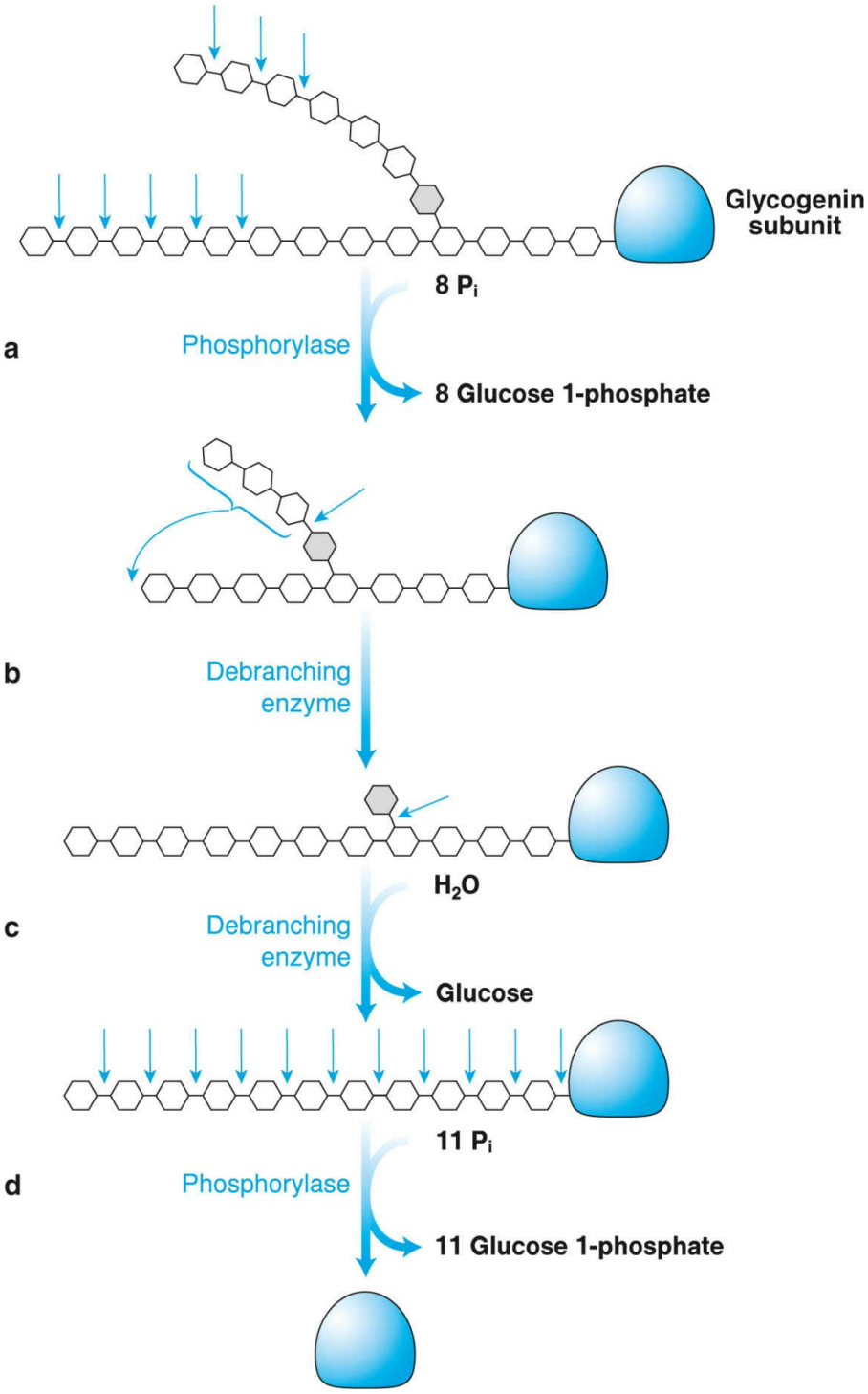
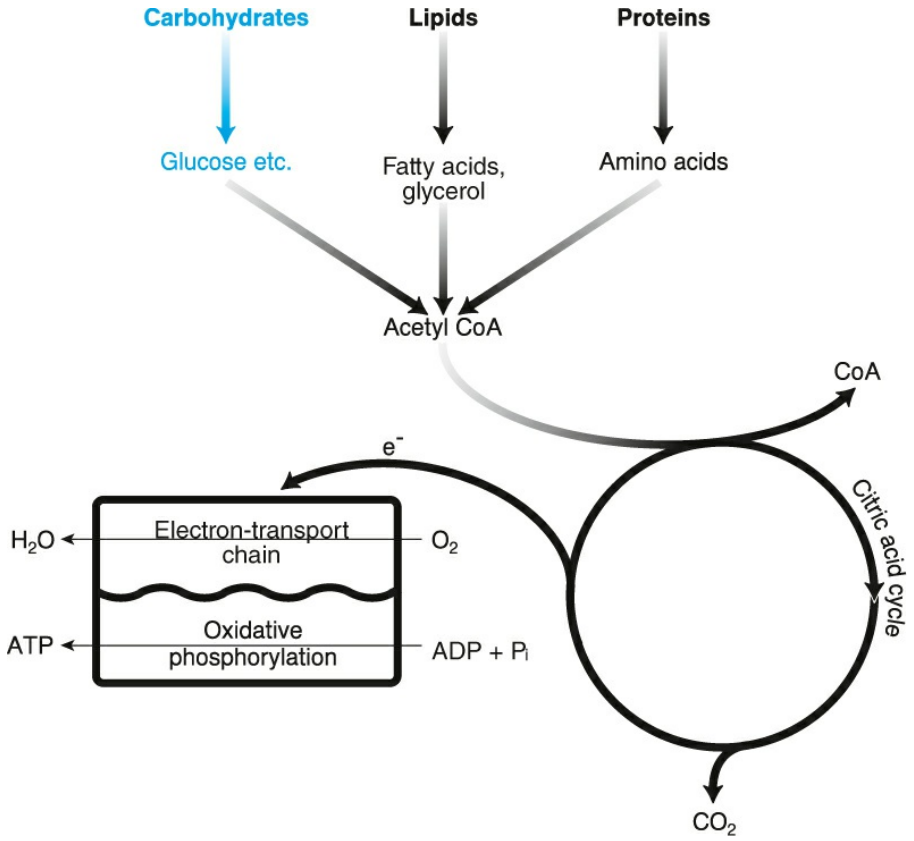


Figure 10.9 **Glycogenolysis.** Two enzymes, that is, phosphorylase and the debranching enzyme, are needed to degrade glycogen. *(a)* Starting with the product of figure 10.8, phosphorylase breaks down glycosidic linkages (vertical thin arrows) and detaches molecules of glucose 1-phosphate (eight in this example) until it gets four glucose residues away from a branch. *(b)* The debranching enzyme (acting at the oblique arrow) carries three glucose residues from one branch to another. *(c)* The same enzyme hydrolyzes the glycosidic linkage

at the branch point. *(d)* This leaves a linear chain at the disposal of phosphorylase. In the end, of the twenty glucose residues that constituted the segment of glycogen shown at the top, nineteen (the ones in white) are converted into glucose 1-phosphate, and one (in gray) is converted into glucose.



You are here: glycogenolysis.

Phosphorylase stars in glycogenolysis but does not suffice to complete the process because it is unable to remove glucose residues lying four or fewer places away from a branch point. When a chain becomes this short, another enzyme, named **glycogen-debranching enzyme**, or simply **debranching enzyme**, comes into play. The debranching enzyme catalyzes two reactions (figure 10.9): the transfer of three of the four glucose residues of a branch to the end of another branch and the of the polypeptide chain, counting from the free removal of the remaining residue by hydrolysis of the α1 → 6 glycosidic linkage joining it to the rest of the molecule. The product of the latter reaction is plain glucose.

In glycogenolysis, glycogen is degraded into glucose 1-phosphate and glucose.

Thanks to the debranching enzyme, the branch is obliterated, letting phosphorylase act once again. Molecules of glucose 1-phosphate are produced until the enzyme gets four glucose residues away from the next branch. The debranching enzyme eliminates this one too, and glycogenolysis continues through the concerted action of the two enzymes.

Glycogenesis and glycogenolysis are anaerobic processes.

10.5 Exercise Speeds Up Glycogenolysis in Muscle

The rate of glycogenolysis is low in a resting muscle. However, if the muscle is activated to contract, the rate of glycogenolysis increases thanks to changes in the concentration of certain compounds. The actions of these compounds fit into four of the means of metabolic control in exercise that we discussed in section III.4: allosteric regulation, covalent modification, changing substrate concentration, and hormonal control. Thus, glycogenolysis offers a first-rate opportunity to apply the knowledge you acquired in that section to a specific situation.

The first change that speeds up glycogenolysis in muscle during exercise is the increase in the P_i concentration because of ATP hydrolysis in the cytosol. Since P_i is a substrate in reaction 10.4, its rise speeds up glycogenolysis. In fact, it does so from the very first seconds of exercise.

The other changes that speed up glycogenolysis result in direct or indirect activation of its leading enzyme, phosphorylase. To understand how this activation happens, we need to consider some of the enzyme's properties. Phosphorylase consists of two identical 97 kDa subunits (it is, as we say, a **homodimer**) and exists in two interconvertible forms: a usually inactive *b* form and a usually active *a* form. The latter bears a phosphoryl group covalently attached to the side chain of serine 14 (14th amino acid of the polypeptide chain, counting from the free amino group) in each subunit.

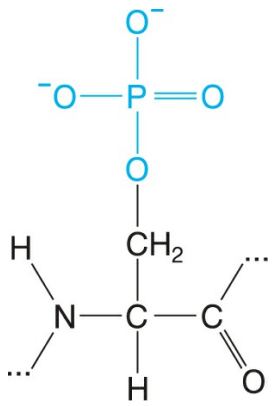


Figure 10.10 **Phosphoserine residue in a protein.**

The phosphoryl groups on phosphorylase derive from ATP and become attached to the hydroxyl group of serine through the catalytic action of **phosphorylase kinase**, a large (1,200 kDa) protein consisting of four different subunits in quadruplicate, that is, $(\alpha\beta\gamma\delta)_4$.The **phosphoserine** residue formed (figure 10.10) is another case of post-translational modification, next to the ones we considered in figures 6.4 and 6.7. Phosphorylase *b* is thus converted into phosphorylase *a* (figure 10.11).

The reverse conversion (phosphorylase *a* to *b*) is achieved through **dephosphorylation**, catalyzed by **protein phosphatase 1 (PP1)**. **Phosphatases** are enzymes catalyzing the dephosphorylation of compounds by hydrolysis. Like kinases, phosphatases are specified by their substrates. PP1 was so named because it was the first to be discovered among a group of enzymes that dephosphorylate proteins.

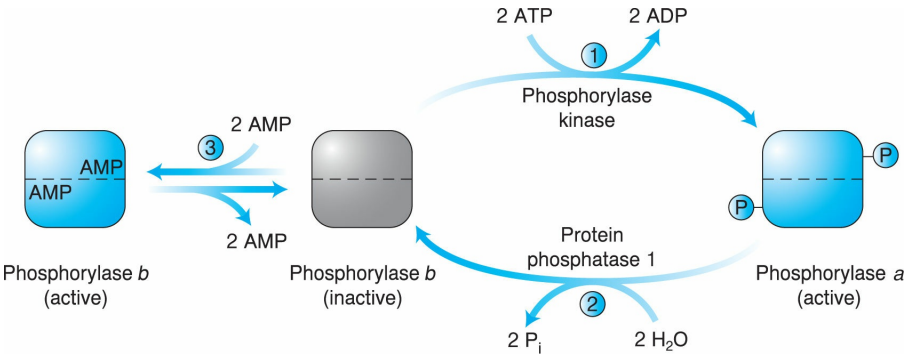


Figure 10.11 **Regulation of phosphorylase activity.** Phosphorylase activity is controlled by reversible phosphorylation thanks to the action of a specific kinase *(1)* and a specific phosphatase *(2)*, as well as by AMP binding *(3)*. The active forms of phosphorylase are

shown in color.

Following this acquaintance with the two forms of phosphorylase, we are ready to explore the changes that activate it during exercise. One change is the increase in the proportion of AMP to ATP. (Remember that the former increases while the latter decreases in an exercising muscle, as discussed in section 9.2.) AMP activates the (usually inactive) phosphorylase *b* by binding to a site in each of its subunits that is different from the active site (figure 10.11). Hence, AMP is an allosteric activator of phosphorylase. The binding of ATP to the same site inhibits the activation by AMP. Thus, the increase in AMP and the decrease in ATP during exercise lead to activation of phosphorylase *b*. In addition, phosphorylase *b* is activated by IMP, which increases during hard exercise (section 9.5).

Another change that affects phosphorylase activity is the increase in the cytosolic [Ca^{2+}] because of Ca^{2+} release from the sarcoplasmic reticulum during muscle activation (section 8.11). Ca^{2+} binds to phosphorylase kinase—in particular, to its four δ subunits, which have a specific name: **calmodulin**. Calmodulin is a 17 kDa protein, which is also present independently in cells and resembles TnC, the calcium sensor in the thin filaments (section 8.10). Ca^{2+} binding changes the conformation of calmodulin (as it does with TnC) and activates phosphorylase kinase when the [Ca^{2+}] reaches 10^{-6} mol · L^{-1}, a concentration well within the range of the cytosolic [Ca^{2+}] during contraction (figure 8.15). The activated kinase phosphorylates phosphorylase *b*, converting it into phosphorylase *a*, as already described.

Finally, phosphorylase is activated by the increase in the blood concentration of the hormone epinephrine (first mentioned as a neurotransmitter in section 6.1). This effect is mediated by a series of molecular interactions termed the *cyclic-AMP cascade* and constituting the culmination of complexity in the control of glycogenolysis. The cascade also controls other processes that will concern us later. For these reasons, it warrants treatment in a separate section.

10.6 The Cyclic-AMP Cascade

Epinephrine, or **adrenaline** (figure 10.12), is a relatively small biomolecule that belongs to the group known as **catecholamines**, as does the closely related compound **norepinephrine**, or **noradrenaline**. The two compounds are secreted into the bloodstream by the **adrenal medulla**, that is, the interior of the adrenal gland, one of which is located on top of each kidney. The adrenal medulla secretes about five times more epinephrine than norepinephrine.

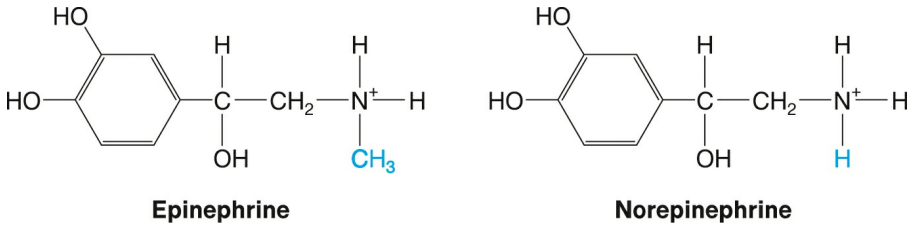


Figure 10.12 Epinephrine and norepinephrine. These two compounds, acting as hormones and neurotransmitters, differ by just one methyl group (in color).

In addition, the two catecholamines are synthesized in neurons of the sympathetic branch of the autonomic nervous system and are released from their terminal branches. Here, norepinephrine predominates over epinephrine. Thus, a division of labor exists between the two catecholamines: Epinephrine acts primarily as a hormone, whereas norepinephrine acts primarily as a neurotransmitter.

Exercise—or even its anticipation—excites the sympathetic system, which, among other organs, innervates the adrenal glands. Sympathetic excitation stimulates the adrenal glands to secrete catecholamines to the bloodstream. In fact, the plasma concentrations of epinephrine and norepinephrine relate to exercise intensity. Epinephrine molecules are transported through the circulation to the surface of muscle fibers, where they bind to specific receptors that are integrated in the sarcolemma (as is the acetylcholine receptor). The ones in question are termed **adrenergic receptors** (after *adrenaline*) and are divided into types differing in function and specificity for epinephrine and norepinephrine. The type participating in the cyclic-AMP cascade is characterized as **β-adrenergic receptor**.

Due to the close relationship between the sympathetic system and the adrenal glands,

they are often referred to collectively as the **sympathoadrenergic system**.

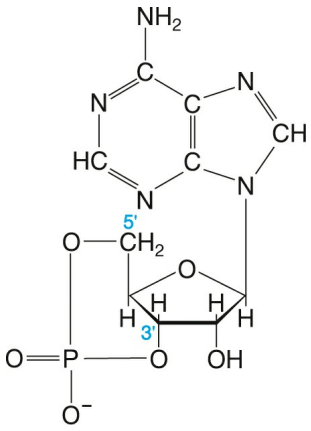


Figure 10.13 **Cyclic AMP.** Cyclic AMP differs from AMP (figure 2.4) in that its phosphoryl group connects with not only the 5' but also the 3' carbon of ribose, thus forming one more ring (hence the term *cyclic*). This small structural difference endows cAMP with a biological role that is quite different from that of AMP.

The β-adrenergic receptor has a molecular mass of 64 kDa and features a binding site for epinephrine on its extracellular surface. Binding of the hormone changes the receptor's conformation and activates the enzyme **adenylate cyclase**. This is another integral protein of the plasma membrane; it has a molecular mass of 120 kDa and catalyzes the synthesis of **cyclic adenylate**, or **cyclic AMP** (**cAMP**, figure 10.13). Cyclic AMP is omnipresent in eukaryotic cells and holds a central position in the control of many biological processes. It is synthesized from ATP with the concomitant production of pyrophosphate.

$$\text{ATP} \rightleftharpoons \text{cAMP} + \text{PP}_\text{i} \qquad \Delta G^{\circ\prime} = 1.7\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.5)}$$

The reaction is slightly endergonic and thus not favored. However, like reaction 10.2, it is followed by the hydrolysis of PP_i (reaction 2.7), which has a $\Delta G^{\circ\prime}$ of –3.8 kcal · mol^{-1}. This linking of reactions renders the overall process exergonic, with a $\Delta G^{\circ\prime}$ of –2.1 kcal · mol^{-1}.

The activation of adenylate cyclase by the β-adrenergic receptor is not direct. Rather, it is mediated by a **G protein** belonging to a family of proteins involved in signal transduction. The protein that mediates the activation of adenylate cyclase is denoted by G_s (*s* for *stimulatory*). G_s is attached to the

cytosolic side of the plasma membrane and consists of three subunits, α, β, and γ. The α subunit has a binding site for the guanine ribonucleotides GTP and GDP (hence the name *G protein*). When the β-adrenergic receptor is vacant, $G_{s\alpha}$ is occupied mainly by GDP and is inactive. However, epinephrine binding causes the attachment of the receptor to G_s (figure 10.14), the substitution of GTP for GDP in $G_{s\alpha}$, and the separation of $G_{s\alpha}$ from the other subunits, which remain united as $G_{s\beta\gamma}$. The now-free $G_{s\alpha}$ diffuses across the membrane, binds to adenylate cyclase, and activates it.

Because of these events, cAMP increases in the cytosol of muscle fibers during exercise. In turn, cAMP binds to and activates a **protein kinase** specified by the letter **A** and abbreviated as **PKA** for distinction from similar enzymes. In the absence of bound cAMP, PKA is composed of two pairs of identical subunits (that is, four subunits in all). Two subunits (38 kDa each) are catalytic and are denoted by C, whereas the other two (49 kDa each) are regulatory and are denoted by R. The resulting tetramer, R_2C_2, is inactive. However, when the [cAMP] rises to 10 nmol · L^{-1}, two cAMP molecules bind to each R, dismantling R_2C_2 into R_2 and two C (figure 10.15).

Protein kinases phosphorylate proteins at the expense of ATP.

The liberated C subunits of PKA are now active and able to phosphorylate cellular proteins. Phosphoryl groups are attached to the side chains of serine (figure 10.10) or threonine residues (figure 10.16), which are flanked by amino acid sequences that fit the active site of C. One of the substrates of PKA is phosphorylase kinase. Phosphorylation at its α and β subunits activates the enzyme; in fact, it acts in an additive manner to the activation caused by Ca^{2+} binding to its δ subunits, which we discussed in the previous section. Phosphorylase kinase then, through its catalytic γ subunits, phosphorylates and activates glycogen phosphorylase.

This action concludes a “cascade” of molecular interactions (figure 10.17) that begins with the binding of epinephrine to the β-adrenergic receptor, involves cAMP as a key molecule, and ends in the acceleration of glycogenolysis. This **cAMP cascade** accounts for other actions of epinephrine as well, two of which we will explore in sections 10.26 and 11.6.

In addition, the cAMP cascade mediates the action of many other hormones. Cyclic AMP is usually labeled a second messenger, because the hormone is considered the first messenger.

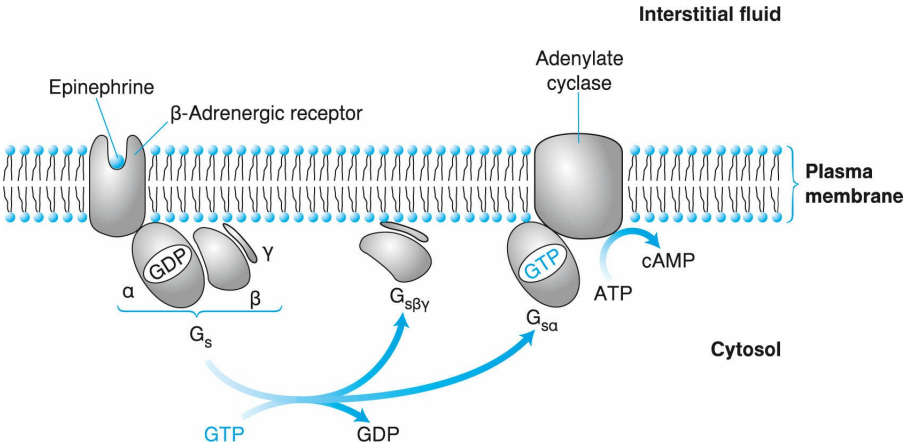


Figure 10.14 **The beginning of the cAMP cascade.** The binding of epinephrine to the β-adrenergic receptor in the plasma membrane causes the replacement of GDP by GTP in the α subunit of G_s protein and the detachment of the α from the β and γ subunits. Next, $G_{s\alpha}$ binds to adenylate cyclase and activates it to form cAMP.

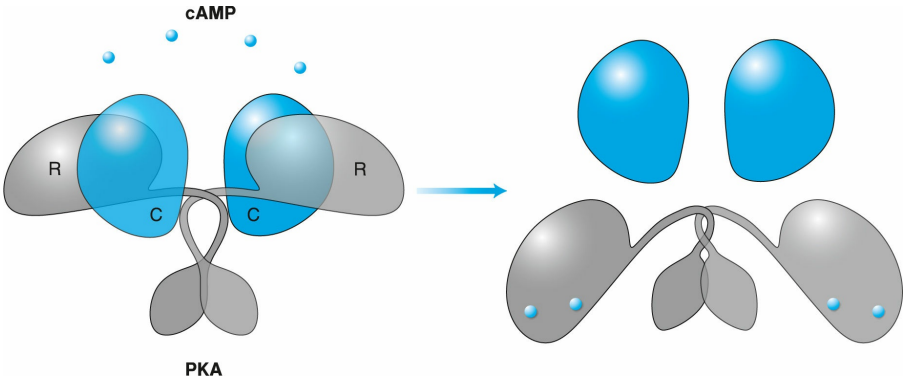


Figure 10.15 **Activation of protein kinase A.** The binding of cAMP to PKA is the key to stimulation of the cAMP cascade. When four cAMP molecules bind to the two regulatory subunits (R), the catalytic subunits (C) are liberated and can phosphorylate target proteins.

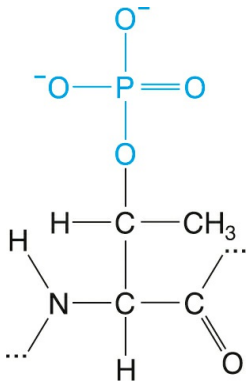


Figure 10.16 **Phosphothreonine residue in a protein.**

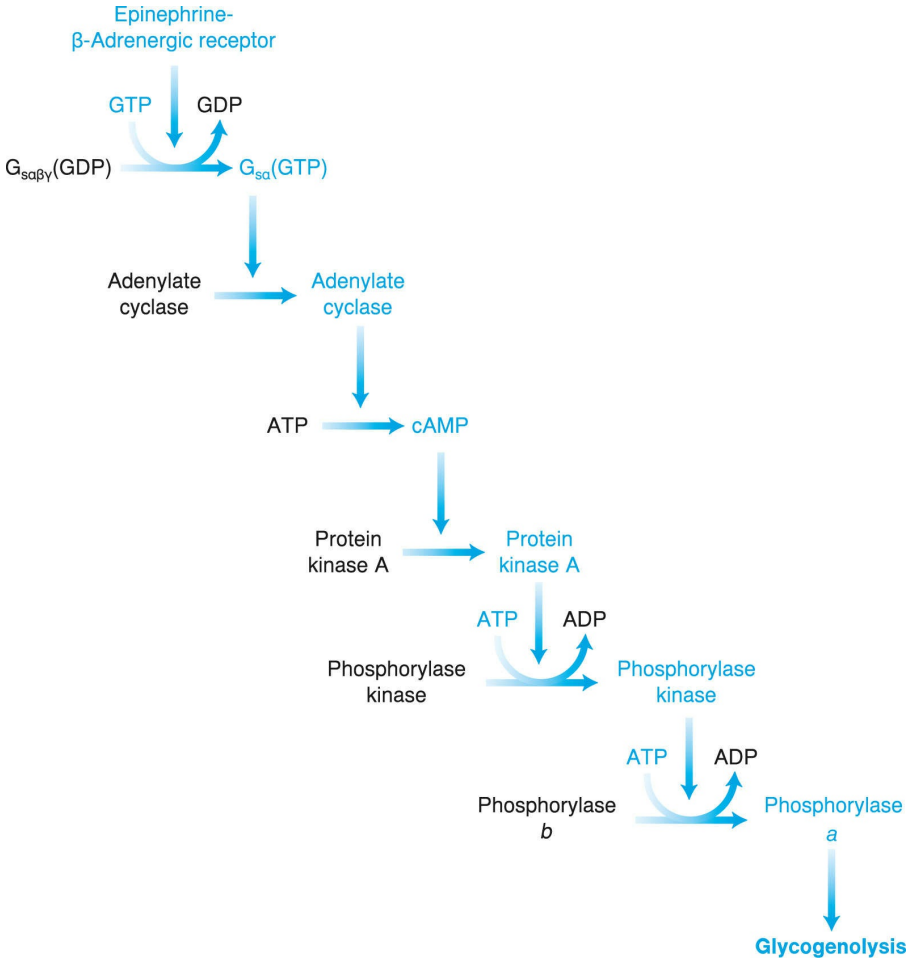


Figure 10.17 **Control of glycogenolysis by the cAMP cascade.** Thanks to a series of interactions, epinephrine leads to a great acceleration of glycogenolysis by binding to the β-adrenergic receptor in the plasma membrane. The hormone-receptor complex activates $G_{s\alpha}$, which activates adenylate cyclase, which synthesizes cAMP, which activates protein kinase A, which phosphorylates and activates phosphorylase kinase, which, finally, phosphorylates and activates phosphorylase. In this and subsequent signal transduction pathways, the more

active forms of proteins are depicted in color.

Why are so many steps involved in this control mechanism? Remember that enzymes are very efficient catalysts. Therefore, one enzyme molecule participating in a step of the cAMP cascade catalyzes the formation of many product molecules, which amplify its effect. Successive amplifications are multiplied, resulting in the release of an enormous number of glucose residues from glycogen by just a few epinephrine molecules.

On the negative side, epinephrine does not speed up glycogenolysis as fast as the substances discussed in the previous section do. This difference exists mainly because it takes several minutes for exercise to raise the plasma epinephrine concentration. In addition, the amplification of the hormone signal is not free of charge, because the cell spends ATP for both cAMP synthesis and enzyme phosphorylation. This spending of ATP corroborates the position of signal amplification on the side of the ATP-consuming processes in figure 9.1.

10.7 Recapping the Effect of Exercise on Muscle Glycogen Metabolism

In the last two sections, we witnessed the control of a single reaction, the basic one of glycogenolysis (reaction 10.4), by a plethora of factors. The existence of such tight control must have given you an idea of how important glycogenolysis is. Indeed, reaction 10.4 sets a valuable energy source on a course of degradation, thus weakening the body's reserves. It is therefore essential, on one hand, to avoid any purposeless breakdown of glycogen and, on the other, to ensure its rapid and massive mobilization in case of need. As far as the latter point is concerned, note that the rate of glycogenolysis in human quadriceps muscle during maximal exercise (about 1 mmol glucosyl units per kilogram per second) approaches the V_{max} of phosphorylase.

The importance of glycogen for exercise metabolism (although not for life) is manifest in McArdle disease, a rare genetic disorder involving mutations that result in fully defective forms of muscle phosphorylase. Patients experience exercise intolerance, with early fatigue and painful muscle cramps.

Figure 10.18 summarizes the regulatory mechanisms of glycogenolysis that we have examined.

Discussion of how exercise affects muscle glycogen metabolism would be incomplete without an examination of what happens to the antipode of glycogenolysis—namely, glycogenesis. The effect of exercise on glycogenesis is more complicated and less straightforward than its effect on glycogenolysis. This difference results mainly from the fact that glycogen synthase activity is affected by phosphorylation at multiple sites and by multiple kinases, resulting in opposing effects. Thus, increased, decreased, and unchanged glycogen synthase activity during exercise have all been reported in the literature. Jakob Nielsen and Jørgen Wojtaszewski have proposed that the net effect of exercise on glycogenesis in muscle depends on the relative strength of stimulatory and inhibitory signals, which may vary according to the exercise parameters and muscle glycogen content.

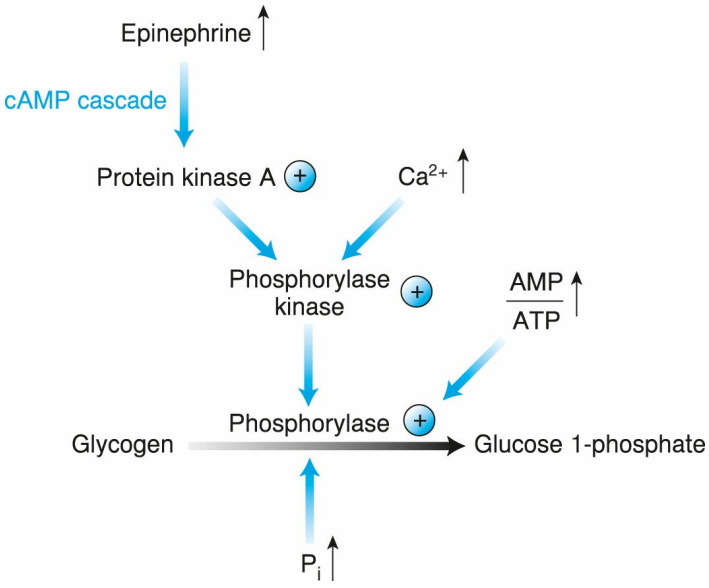


Figure 10.18 Control of glycogenolysis in muscle during exercise. Exercise speeds up glycogenolysis in muscle through several collaborative interactions. Upward thin arrows denote increase, whereas the + symbol in the circles denotes enzyme activation.

Regarding the exercise parameters, vigorous or maximal exercise of short duration seems to lower glycogen synthase activity, whereas prolonged moderate-intensity exercise raises it (though to a lesser degree as compared with phosphorylase activity). As for the muscle glycogen content, it appears

that glycogen synthase activation is related to glycogen depletion. In other words, the using up of glycogen during exercise signals the activation of glycogen synthase, probably as a homeostatic mechanism. Glycogen synthase remains activated for several hours after exercise (contrary to phosphorylase, which is quickly deactivated, as we will see in section 14.26, under glycogen replenishment). This activation ensures the rapid resynthesis of muscle glycogen.

The degree of muscle glycogen depletion with exercise depends primarily on the initial glycogen content, exercise intensity, and exercise duration. As an indication, José Areta and Will Hopkins, in a meta-analysis of the relevant literature report average reductions ranging from 40% (with high glycogen content) to 50% (with normal glycogen content) after one hour of hard exercise. Muscle glycogen metabolism may also be affected by two additional parameters: fiber type and subcellular location.

Regarding muscle fiber type, researchers have found either higher glycogen content in type II fibers as compared to type I fibers or no difference between them. The same is the case with phosphorylase content. Glycogen synthase content either does not depend on muscle fiber type or is higher in type I fibers. Such contradictory findings imply that *muscle fiber type might not be a strong determinant of glycogen metabolism*.

Regarding subcellular location, research has revealed that glycogen is segregated in three places within muscle fibers. Most of it (about 80%) is located between myofibrils or between myofibrils and mitochondria. These locations are termed **intermyofibrillar**. The rest can be found either within the myofibrils or between the sarcolemma and the outermost myofibrils in approximately equal amounts. These locations are termed **intramyofibrillar** and **subsarcolemmal**, respectively. During exercise, glycogen is used from all three of these subcellular locations (figure 10.19), but it appears that

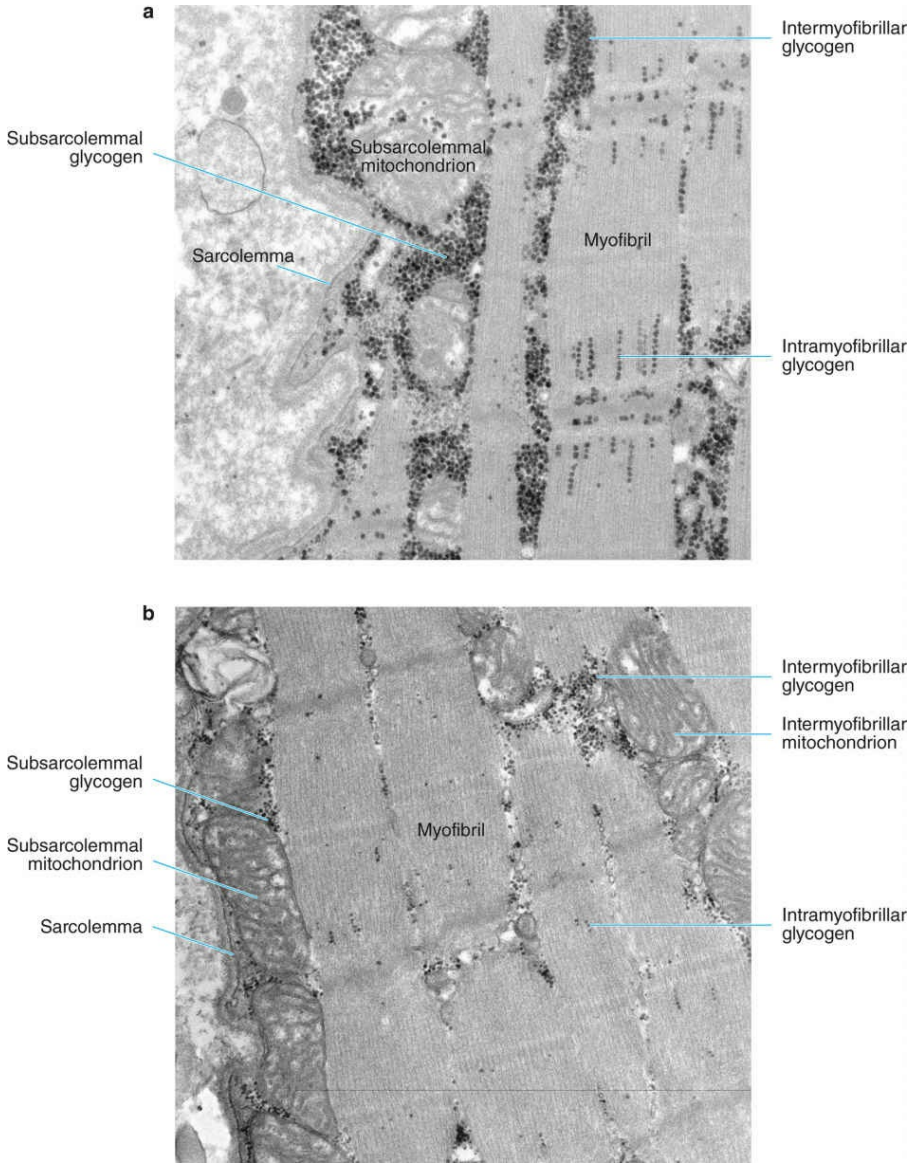


Figure 10.19 **Subcellular localization of muscle glycogen.** Glycogen granules can be seen in three distinct locations in these electron micrographs of muscle fibers from the triceps brachii at rest *(a)* and after a simulated sprint race in cross-country skiing *(b)*: intermyofibrillar (between myofibrils or between myofibrils and mitochondria), intramyofibrillar (within the myofibrils), and subsarcolemmal (between the sarcolemma and the outermost myofibrils).The fewer granules in *b* as compared with *a* show that all three depots are depleted after exercise.

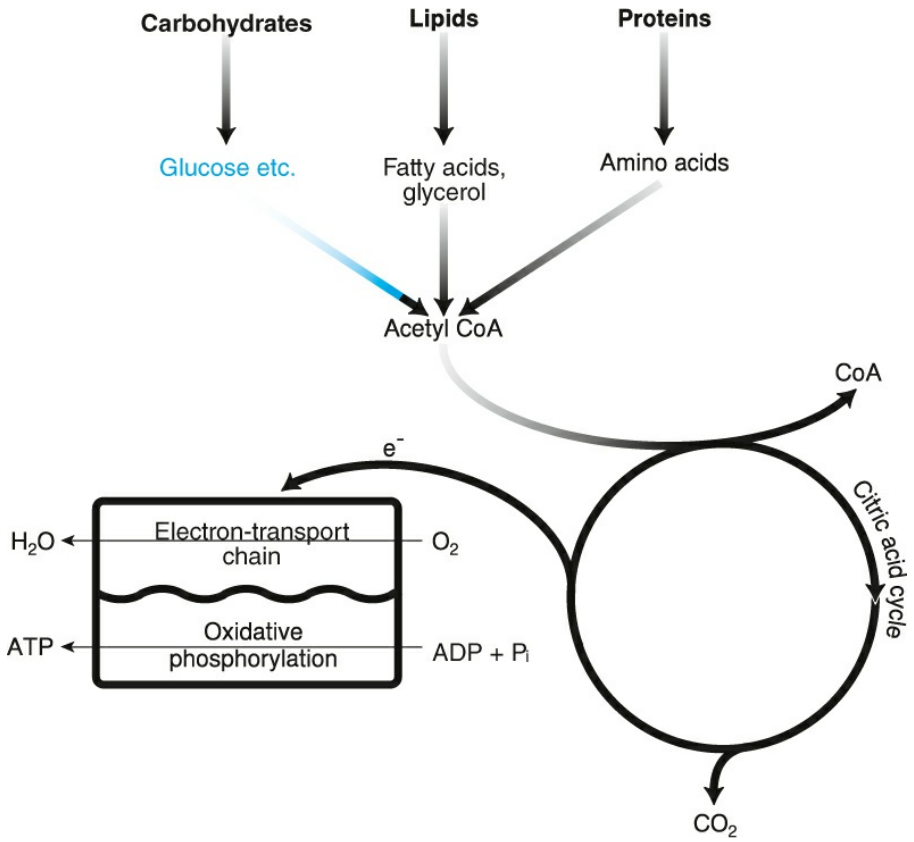
Courtesy of Joachim Nielsen and Kasper Gejl, Department of Sports Science and Clinical Biomechanics, University of Southern Denmark. Transmission electron microscopy was conducted at the Department of Pathology, Odense University Hospital, Denmark.

intramyofibrillar glycogen suffers the greatest loss, as reviewed by Niels Ørtenblad and Joachim Nielsen. This fact may be important for the

emergence of fatigue, since the depletion of glycogen from this location seems to compromise Ca^{2+} release from the sarcoplasmic reticulum.

10.8 Glycolysis

As we saw in section 10.4, glycogenolysis produces glucose 1-phosphate and, to a lesser extent, glucose. Cells also contain glucose originating in the diet and arriving through the bloodstream. Another source of glucose is biosynthesis from compounds that are not carbohydrates, as we will see in section 10.24.



You are here: glycolysis.

Glucose of any origin and glucose 1-phosphate from glycogen can yield energy through **glycolysis**, which is the breakdown of a glucose molecule into two **pyruvate** molecules. Glycolysis is the most common metabolic pathway, because it operates in all cells in the body. What is more, it is the

sole energy source for erythrocytes, as I will explain in section 10.29. Thus, we are dealing with a process of fundamental importance for metabolism and, in particular, exercise metabolism, as we will see soon.

In glycolysis, glucose is broken down into two pyruvates.

Glycolysis is an anaerobic catabolic process. It consists of 10 reactions that take place in the cytosol and are catalyzed by different enzymes. The full sequence is shown in figure 10.20. The pathway begins with glucose undergoing a phosphorylation to become **glucose 6-phosphate** at the expense of ATP. In the second reaction, glucose 6-phosphate isomerizes to **fructose 6-phosphate**, which is subsequently phosphorylated at the expense of another ATP to become **fructose 1,6-bisphosphate**. In the fourth reaction, this compound splits into two isomeric triose phosphates: **dihydroxyacetone phosphate** and **D-glyceraldehyde 3-phosphate** (cf. figure 5.1). The former is converted into the latter in the fifth reaction. From this point onward, glycolysis continues with two molecules of glyceraldehyde 3-phosphate per molecule of glucose.

You might wonder why fructose carrying two phosphoryl groups is a *bis*phosphate, whereas adenosine carrying two phosphoryl groups is a *di*phosphate. The prefix *bis-* is used to describe two groups attached to a molecule at different positions, whereas *di-* is reserved for two groups attached in a row to a single position of a molecule.

In the sixth reaction of glycolysis, D-glyceraldehyde 3-phosphate accepts a second phosphoryl group, this time from P_i, while being oxidized to **1,3-bisphosphoglycerate**. NAD^+ (section 2.5) serves as the oxidant, getting reduced to NADH. This is an important reaction, as we will see in subsequent sections. 1,3-Bisphosphoglycerate has sufficient phosphoryl-transfer potential to donate one of its two phosphoryl groups to ADP in the seventh reaction, thus becoming **3-phosphoglycerate** and synthesizing ATP (for more on this, see problem 3). This is another case of substrate-level phosphorylation (introduced in section 9.3). The eighth reaction is an isomerization of 3-phosphoglycerate to **2-phosphoglycerate**, which loses water to become **phosphoenolpyruvate** in the ninth reaction. Finally, phosphoenolpyruvate

transfers its phosphoryl group to another ADP, thus yielding ATP and pyruvate through yet another substrate-level phosphorylation.

How much energy is produced in glycolysis? Two ATP are spent initially, and two ATP are produced toward the end. However, the latter derive from one glyceraldehyde 3-phosphate, whereas the breakdown of glucose yields two triose phosphates, one of which is converted into the other. Thus one glucose yields $2 \cdot 2 = 4$ ATP in the second half of glycolysis. As a result, the net gain from glycolysis is $4 - 2 = 2$ ATP per glucose. Let me point out in advance that this quantity is minimal as compared with what pyruvate can yield in the ensuing steps of its catabolism.

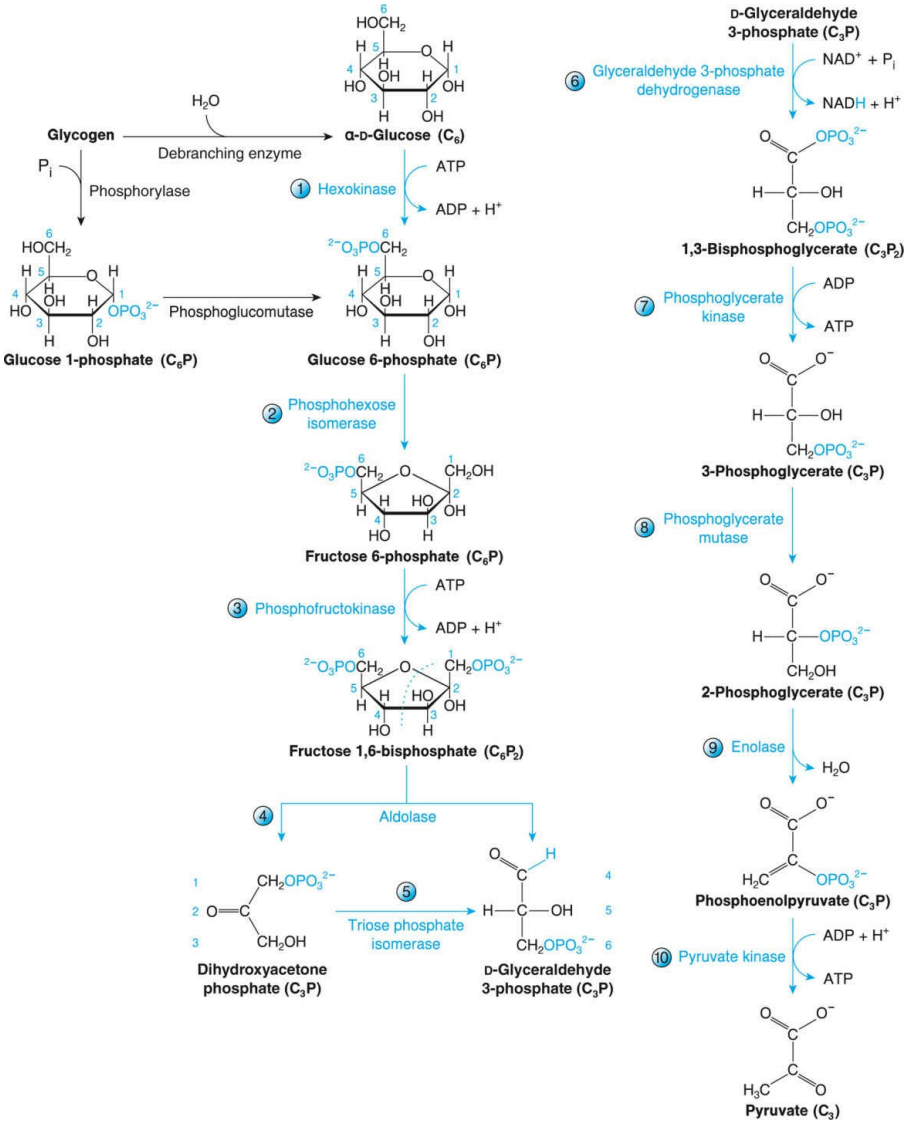


Figure 10.20 Glycolysis. A pathway of fundamental importance to carbohydrate metabolism, glycolysis transforms glucose and glucose 1-phosphate (the main product of glycogenolysis) into pyruvate while resynthesizing ATP from ADP. The glycolytic reactions are noted with numbers and colored arrows; feeder reactions are noted with black arrows. To help you follow the sequence of transformations, the numbers of carbon and phosphorus atoms of each compound are indicated in parentheses next to its name. Phosphoryl groups are noted in color for clarity. Also noted in color is the hydrogen of D-glyceraldehyde 3-phosphate (product of the fourth and fifth reactions) that is incorporated in NADH (in the sixth reaction). The broken curved line across fructose 1,6-bisphosphate (product of the third reaction) shows how it splits in the fourth reaction. The numbers next to the two triose phosphates (products of the fourth reaction) are the numbers of fructose 1,6-bisphosphate's carbons.

The overall reaction of glycolysis, excluding the accompanying synthesis of ATP, is

$$\text{Glucose} + 2\ \text{NAD}^+ \rightarrow 2\ \text{pyruvate} + 2\ \text{NADH} + 4\ \text{H}^+ \qquad \Delta G^{\circ\prime} = -31.9\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 10.6)}$$

Notice that the $\Delta G^{\circ\prime}$ of glycolysis is the lowest we have encountered so far, making this the most exergonic process (again, so far). In fact, the process is so exergonic that it can fuel the synthesis of two ATP (having a $\Delta G^{\circ\prime}$ of 12.6 kcal for two moles; see reaction 2.10) and remain highly exergonic:

$$\text{Glucose} + 2\ \text{NAD}^+ + 2\ \text{ADP} + 2\ \text{P}_\text{i} \rightarrow 2\ \text{pyruvate} + 2\ \text{NADH} + 2\ \text{ATP} + 2\ \text{H}_2\text{O} + 2\ \text{H}^+ \qquad \Delta G^{\circ\prime} = -19.3\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 10.7)}$$

Thus, glycolysis exemplifies what we discussed in section 2.4—namely, the fact that part of the energy released in catabolic processes is used in the synthesis of ATP.

Glucose 1-phosphate, the main product of glycogenolysis, enters the glycolytic pathway after isomerizing to glucose 6-phosphate (the product of the first glycolytic reaction) through a shift of its phosphoryl group from C1 to C6 (figure 10.20). In this way, glucose 1-phosphate avoids the expense of one ATP in the first glycolytic reaction. Thus, the gain from the conversion of glucose 1-phosphate into pyruvate is three ATP. By analogy to reaction 10.7, the overall reaction of the conversion of glucose 1-phosphate into pyruvate is

$$\text{Glucose 1-phosphate} + 2\ \text{NAD}^+ + 3\ \text{ADP} + 2\ \text{P}_\text{i} \rightarrow 2\ \text{pyruvate} + 2\ \text{NADH} + 3\ \text{ATP} + 2\ \text{H}_2\text{O} + \text{H}^+ \qquad \Delta G^{\circ\prime} = -17\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 10.8)}$$

10.9 Exercise Speeds Up Glycolysis in Muscle

Exercise can augment the glycolytic rate in a muscle by 100 fold and in more than one way. First, substrate availability increases. As glycogenolysis speeds up, there is a rise in the concentrations of its products: glucose and glucose 1-phosphate; the latter isomerizes to glucose 6-phosphate, as mentioned earlier. Thus, the substrates of both the first and second glycolytic reactions increase.

Glucose 6-phosphate inhibits **hexokinase**, the enzyme that catalyzes its formation, thus constituting a case of feedback inhibition (section III.4). It is believed that this inhibition becomes important when there is an overproduction of glucose 1-phosphate and, hence, glucose-6-phosphate as a result of high glycogenolytic rate during hard or maximal exercise. Under such conditions, glycogen utilization may prevent glucose utilization. During exercise of lower intensity, however, glucose utilization becomes important. This brings us to the next means by which exercise augments the glycolytic rate.

Exercising muscles increase their uptake of glucose from blood by as much as 50 fold, mainly because of two factors. The first is the enhanced blood flow to the active muscles (up to 20 times the flow at rest), which can be detected within as little as one second from the onset of muscle activity. This increase is a characteristic corollary of exercise, although how it happens is not known with certainty.

The second factor responsible for increased glucose uptake by exercising muscles is a rise in the number of glucose transporters in the plasma membrane. Let's explore what this rise means and how it happens. Cells take up glucose through integral proteins of the plasma membrane called simply **glucose transporters** and abbreviated as **GLUT**. Muscle fibers contain several forms of these proteins, which carry out passive transport of glucose from the interstitial fluid into the cytosol.

GLUT4, the most abundant GLUT in muscle fibers, is distinct in that it is not always present in the plasma membrane. Instead, it commutes between a population of intracellular vesicles and the sarcolemma or the transverse tubule membrane (figure 10.21). Remember that the transverse tubules

constitute an extension of the sarcolemma (section 8.11). For brevity, I will refer to the two membranes as the plasma membrane in the ensuing discussion. Type I fibers contain more GLUT4 than do type II fibers, as reviewed by Erik Richter and Mark Hargreaves.

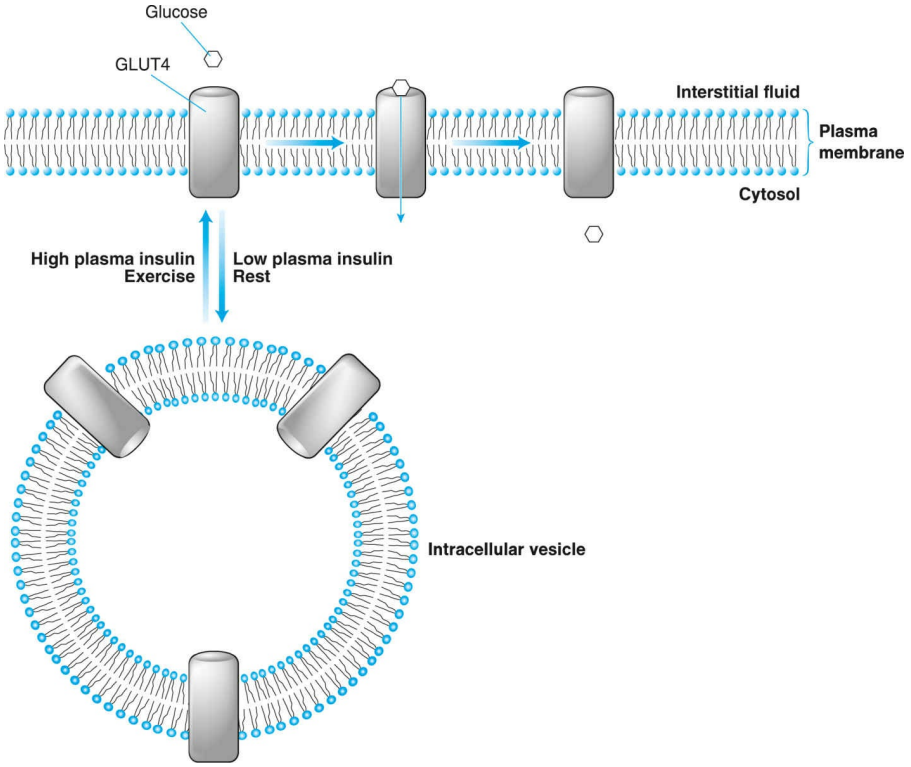


Figure 10.21 The shuttling of GLUT4. The main glucose transporter in muscle fibers commutes between intracellular vesicles and the plasma membrane. The vesicle membrane fuses with the plasma membrane in much the same way as neurotransmitter-filled vesicles do during the chemical transmission of nerve signals (see figure 7.10), although more slowly. Only when present in the plasma membrane can GLUT4 transport glucose from the interstitial fluid into the cytosol. The movement of the vesicles to the plasma membrane is stimulated independently by exercise and by the hormone insulin. When exercise ends, or the concentration of insulin in plasma drops, parts of the plasma membrane are sequestered in the cytoplasm as vesicles, thus withdrawing GLUT4.

The movement of GLUT4 from the intracellular vesicles to the plasma membrane provides a means of controlling glucose uptake by a muscle fiber: If more GLUT4 molecules are present in the plasma membrane, then more glucose enters the cell; if fewer GLUT4 molecules are present in the membrane, then less glucose enters. Exercise augments the movement of GLUT4 to the plasma membrane, resulting in higher glucose uptake. The

biochemical mechanism of this event is not known with certainty. When exercise ends, GLUT4 returns to its intracellular location. Note that the hormone insulin (which we will get to know better in section 10.29) also augments glucose uptake by muscle fibers through the reversible movement of GLUT4 to the plasma membrane. However, this movement occurs through a different mechanism, which is known to some degree and will be presented in section 10.29.

Another way by which exercise speeds up glycolysis is through the allosteric activation of **phosphofructokinase**, the enzyme catalyzing the third reaction, which happens to be the committed step (section III.4) of glycolysis. Phosphofructokinase is inhibited by ATP. The inhibition is enhanced by phosphocreatine and relieved by AMP. Thus the enzyme is relatively inactive in a resting muscle, in which the ATP and phosphocreatine concentrations are high, whereas the AMP concentration is low. However, when ATP and phosphocreatine begin to decrease at the onset of exercise, AMP increases, and phosphofructokinase is activated.

Another activator of phosphofructokinase is ammonium, whose muscle concentration increases during exercise because of AMP deamination (section 9.5) and amino acid deamination (as we will see in chapter 12). However, ammonium seems to play a minor role in the regulation of phosphofructokinase. In addition, the enzyme is inhibited at acidic pH. As shown in figure 9.7 and explained further in section 10.20, the sarcoplasmic pH may become acidic during exercise. It has been proposed that a decrease in the glycolytic rate because of phosphofructokinase inhibition at low pH may protect the muscle fibers and blood (to which the acidity diffuses) from an excessive and hazardous fall in pH. However, no phosphofructokinase inhibition by acidic pH has been found in vivo. It seems that other compounds produced in an exercising muscle (such as AMP and ammonium) compensate for any inhibitory action of acidity.

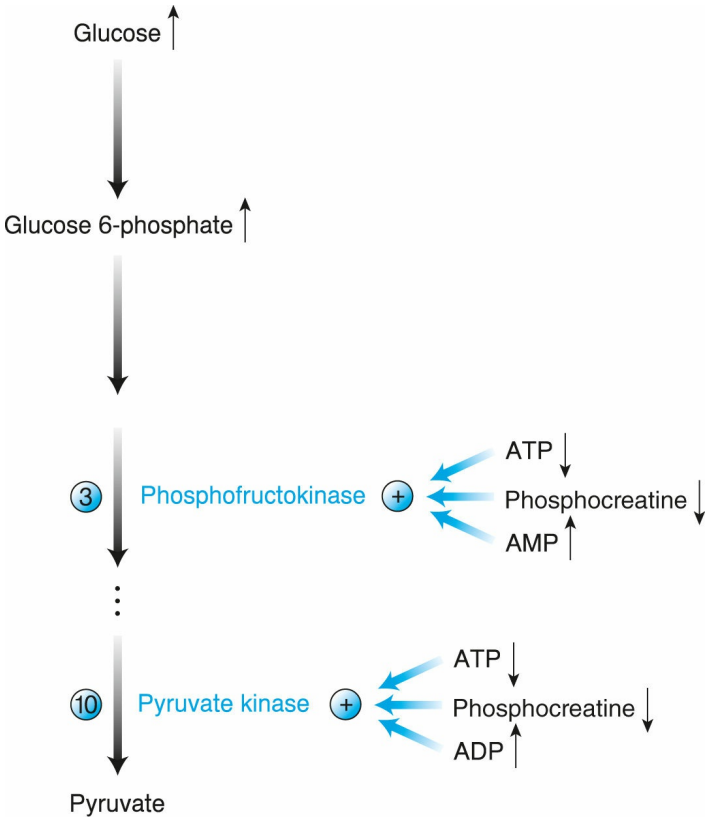


Figure 10.22 **Control of glycolysis by exercise.** Exercise speeds up glycolysis in muscle thanks to the concentration changes shown here. Numbers in circles correspond to glycolytic steps as in figure 10.20. In addition to the symbols introduced in figure 10.18, downward thin arrows denote decrease.

A final point of interest involves the regulation of **pyruvate kinase**, the enzyme that catalyzes the last reaction of glycolysis. The enzyme is inhibited by ATP and phosphocreatine but activated by ADP. Changes in the concentrations of these compounds during exercise favor the activation of the enzyme.

The main factors contributing to the acceleration of glycolysis during exercise are summarized in figure 10.22.

Muscle fiber types differ in their contents of glycolytic enzymes (phosphofructokinase is the one most frequently measured by researchers): Type II fibers have higher contents than do type I fibers. As a result, type II fibers achieve higher glycolytic power; that is, they synthesize ATP from glycolysis faster than do type I fibers. This metabolic difference contributes to the functional specialization of muscle fiber types, as I will explain in

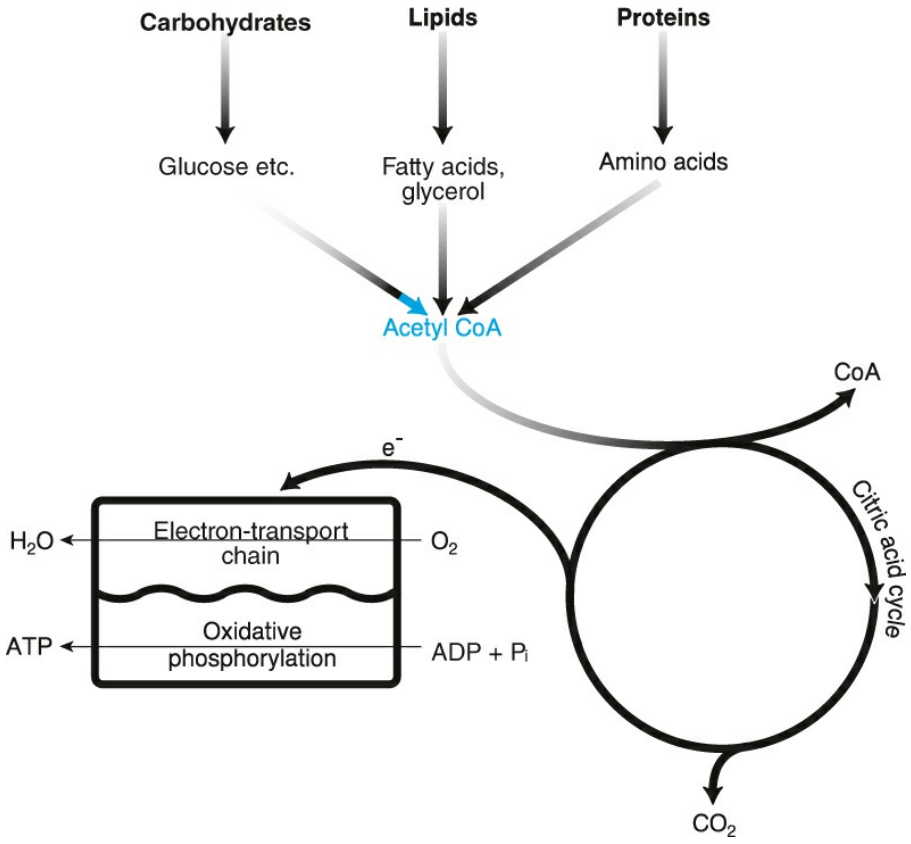
section 14.19.

10.10 Pyruvate Oxidation

Pyruvate can yield much more ATP than the amount produced in glycolysis. To do so, it must pass from the cytosol to the mitochondria, where most of the biological oxidations and most of ATP resynthesis take place.

Mitochondria are elliptical organelles that measure about 2 by 0.5 μm in size and are wrapped in a double membrane (figure 10.23). The outer membrane features pores that let most metabolites pass freely. The inner membrane serves as the actual permeability barrier by separating the mitochondrial interior, or **mitochondrial matrix**, from the cytosol. The inner membrane has many folds, or *mitochondrial cristae* (singular: **mitochondrial crista**), which greatly increase its area. This characteristic is important because the electron-transport chain and oxidative phosphorylation—the two processes introduced in section 2.6 and accounting for the synthesis of most of our ATP—take place exactly in the inner mitochondrial membrane.

Under the electron microscope, mitochondria can be seen in two distinct locations within a muscle fiber. These positions coincide with two of the three subcellular locations of glycogen granules discussed in section 10.7 and viewed in figure 10.19: intermyofibrillar and subsarcolemmal. As with glycogen, about 80% of the mitochondria are intermyofibrillar (and such are the mitochondria shown in figure 10.5). Mitochondria in each location are not isolated and static structures, as usually thought; rather, they communicate with each other through fusion and fission of their membranes to such a degree that they form a **mitochondrial reticulum**. What is more, there may be physical continuity between intermyofibrillar and subsarcolemmal mitochondria, as Martin Picard and colleagues have suggested.



You are here: pyruvate oxidation.

Type I muscle fibers have the highest mitochondrial content, type IIA fibers have intermediate mitochondrial content, and type IIX fibers have the lowest. Thus the content of mitochondrial enzymes and other mitochondrial proteins follows the order I > IIA > IIX.

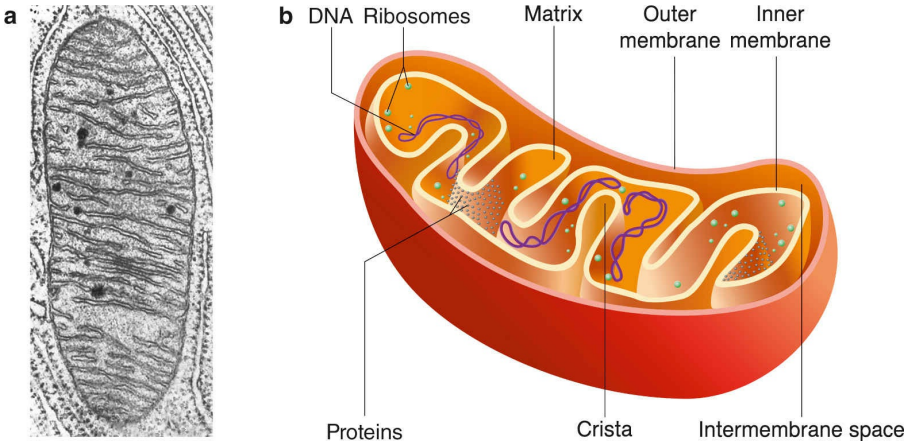


Figure 10.23 Mitochondria. Mitochondria are places of aerobic energy production in

eukaryotic cells. *(a)* Electron micrograph of a longitudinally sliced mitochondrion shows its two membranes and the deep folds of the inner membrane. The organelle is surrounded by endoplasmic reticulum studded with ribosomes. *(b)* A diagram of a cutaway mitochondrion shows the two membranes and the intermembrane space better. The inner membrane is less permeable than the outer one, exhibits numerous folds (or cristae), houses the proteins of the electron-transport chain and oxidative phosphorylation, and surrounds the matrix, where multiple copies of the circular mitochondrial DNA and ribosomes for protein synthesis are found.

Figure 10.23a: Photo courtesy of Dr. H. Hoppeler, University of Bern. Photographers H. Claassen and F. Grabe. Figure 10.23b: ©Snapgalleria | Dreamstime.com.

Upon entering the mitochondrial matrix, pyruvate reacts with **coenzyme A (CoA)** to yield **acetyl coenzyme A**. We met these two compounds during the overview of catabolism (figure 2.11), and now it is necessary to learn more about them. Coenzyme A (figure 10.24) is a rather complex compound, in the molecule of which one can discern 3'-phosphoadenosine diphosphate (consisting of adenine, β-D-ribose, and three phosphoryl groups), the vitamin pantothenate (section 6.1), and 2-mercaptoethylamine. A sulfhydryl group at the end of the molecule is the reactive part of CoA, which is why it is symbolized as CoA-SH in biochemical equations. The sulfhydryl group serves as a carrier of the **acetyl group** (figure 10.25) and, in general, **acyl groups** (section 5.8), which are attached to S by replacing H.

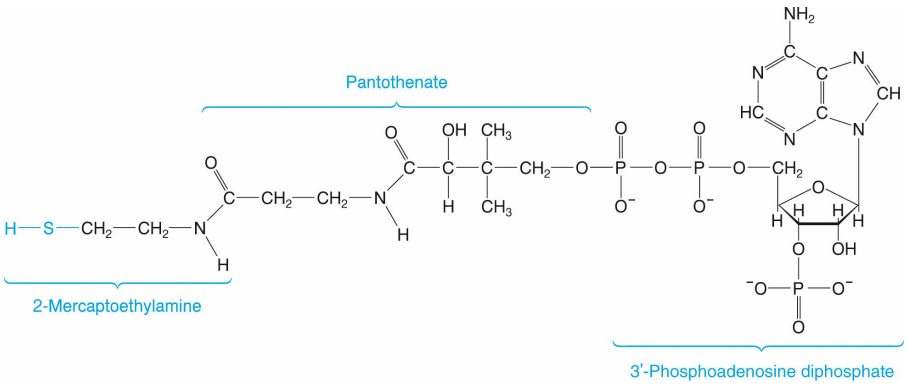


Figure 10.24 **Coenzyme A.** CoA stars in carbohydrate, lipid, and protein metabolism thanks to its ability to carry acyl groups. It bears a terminal sulfhydryl group (in color), through which it bonds with acyl groups after losing one H.

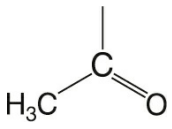
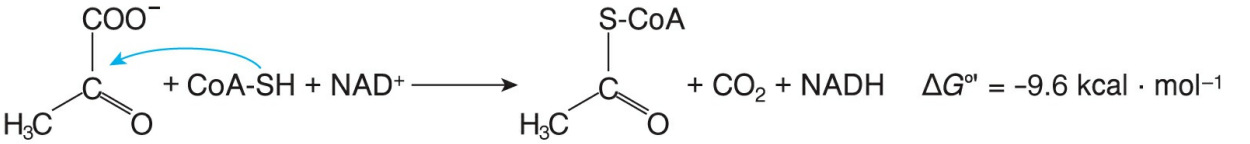


Figure 10.25 **Acetyl group.**

Acetyl CoA derives from the attachment of an acetyl group to CoA. The acetyl group is what remains from pyruvate after it is decarboxylated. At the same time, pyruvate is oxidized by NAD^+. The entire reaction is



Pyruvate **Acetyl CoA** (equation 10.9)

Pyruvate oxidation is irreversible because of the very negative $\Delta G^{\circ\prime}$ value of the reaction and the experimentally demonstrated inability of CO_2 to be reattached to the acetyl group. Thus, it is impossible to convert acetyl CoA back into pyruvate. Because acetyl CoA subsequently enters a route of aerobic breakdown, reaction 10.9 commits carbohydrates to aerobic catabolism. The importance of reaction 10.9 is indicated by the fact that it is catalyzed by no fewer than three enzymes forming the **pyruvate dehydrogenase complex**, a huge assembly of dozens of polypeptide chains located in the mitochondrial matrix.

The enzyme that has lent its name to the entire complex—**pyruvate dehydrogenase**—hosts thiamine pyrophosphate (deriving from vitamin B_1; section 6.1) in its active site. Vitamin B_1 deficiency prevents pyruvate from entering the realm of aerobic breakdown and producing most of the energy stored in carbohydrates. Because the nervous system depends mainly on the aerobic breakdown of carbohydrates for ATP resynthesis, it is particularly susceptible to vitamin B_1 deficiency, as described in section 6.1.

10.11 Exercise Speeds Up Pyruvate Oxidation in Muscle

Because the pyruvate dehydrogenase complex catalyzes a reaction of pivotal importance, it is subject to strict control. Key to this control is the reversible phosphorylation of pyruvate dehydrogenase, the component of the complex mentioned at the end of the previous section. Phosphorylation by a specific

kinase inhibits pyruvate dehydrogenase, whereas dephosphorylation by a specific phosphatase restores its activity (figure 10.26). At rest, most of pyruvate dehydrogenase in muscle is phosphorylated and relatively inactive (*b* form).

The situation is reversed at the onset of exercise, as pyruvate dehydrogenase phosphatase is activated and pyruvate dehydrogenase kinase is inhibited. The phosphatase is activated by Ca^{2+} released from the sarcoplasmic reticulum. It is also activated by Mg^{2+}, the concentration of which, in free form, increases. This increase happens because ATP, which binds Mg^{2+} (section 8.5), decreases, whereas ADP (produced from ATP hydrolysis) does not bind Mg^{2+} as tightly. Although these changes take place in the cytosol, they are transmitted by cellular mechanisms to the mitochondrial matrix, where pyruvate oxidation occurs. On the other hand, the kinase is inhibited by pyruvate, which increases because of increased glycolytic rate. In addition, pyruvate speeds up reaction 10.7, being a substrate. The bottom line of all of these effects is an increase in the dephosphorylated, active, *a* form of pyruvate dehydrogenase.

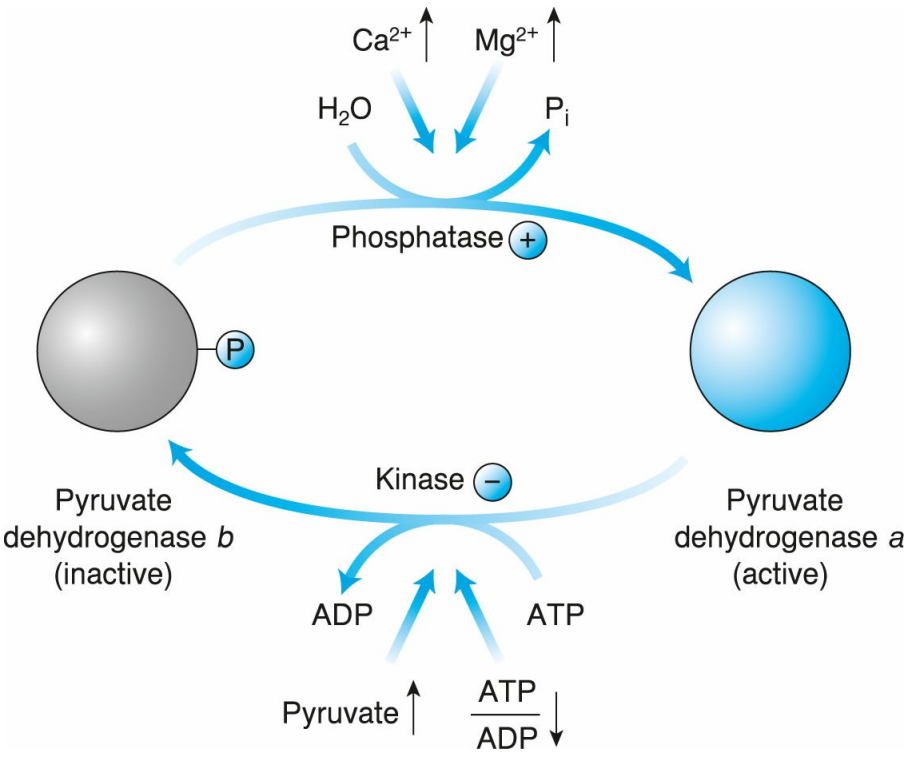


Figure 10.26 Control of muscle pyruvate dehydrogenase by exercise. Pyruvate

dehydrogenase is present in either phosphorylated (and inactive) or dephosphorylated (and active) form thanks to the actions of pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase. During exercise, increases in Ca^{2+} and Mg^{2+} activate the phosphatase, while an increase in pyruvate and a drop in the ATP/ADP ratio inhibit the kinase, all of which leads to activation of pyruvate dehydrogenase in muscle. In addition to the symbols used in figure 10.22, the – sign in a circle denotes enzyme inhibition.

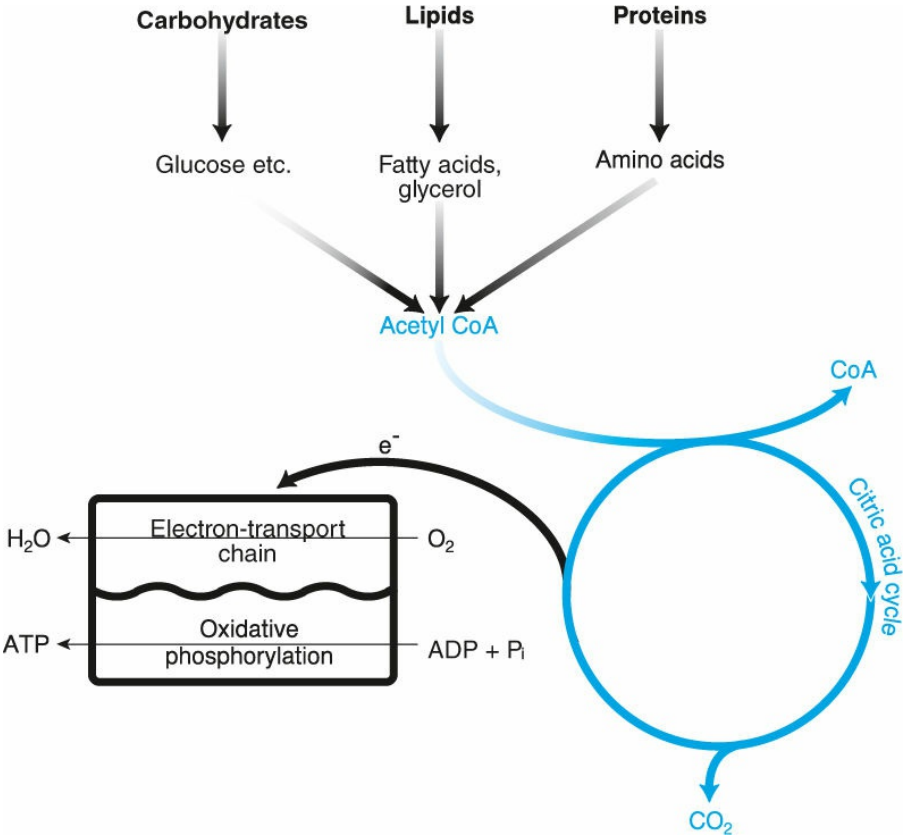
Pyruvate dehydrogenase kinase is subject to three more regulatory influences: It is activated by high concentration ratios of ATP to ADP, NADH to NAD^+, and acetyl CoA to CoA. The [ATP]/[ADP] ratio decreases during exercise in muscle, thus contributing to the inhibition of the kinase. [NADH]/[NAD^+] does not change in a firm way and is not believed to exert a major influence on the activity of the kinase. As for [acetyl CoA]/[CoA], it generally increases during exercise because of the conversion of CoA into acetyl CoA in reaction 10.9 and in fatty acid oxidation (section 11.9). This change favors the slowing down of pyruvate oxidation, although in section 14.5 we will see how this inhibitory effect is attenuated.

10.12 The Citric Acid Cycle

The **citric acid cycle** is a series of nine enzyme reactions that take place in the mitochondria and process the acetyl group of acetyl CoA, deriving from the oxidation of carbohydrates, lipids, and proteins. The acetyl group is oxidized to two molecules of carbon dioxide, and the metabolites of the cycle are restored after a full turn. Several of these metabolites serve as precursors for the synthesis of other biomolecules. In the process, considerable energy is released.

> “Whatever returns is good, not what passes and is done with. The easiest way to return from where you’ve been without retracing your steps is to walk in a circle.”—Umberto Eco, *Foucault’s Pendulum*

In the citric acid cycle, the acetyl group of acetyl CoA is oxidized to two CO_2.



You are here: citric acid cycle.

Let's take a closer look at the cycle (figure 10.27). In its first reaction, the acetyl group is linked with **oxaloacetate**, a four-carbon compound, to form **citrate**, a six-carbon compound that gave the pathway its name. The pathway is also known as the **Krebs cycle**, after Hans Krebs, the German-born British physician and biochemist who discovered it in 1937. It is also referred to as the **tricarboxylic acid cycle**, because citrate and the products of the second and third reactions, *cis*-aconitate and isocitrate, bear three carboxyl groups each. The two reactions consist of **dehydration** and **hydration** (that is, removal and addition of water, respectively), catalyzed by a single enzyme, which removes and returns the two parts of H_2O (that is, OH^- and H^+) from and to different carbons, so that an isomer of citrate, **isocitrate**, is produced.

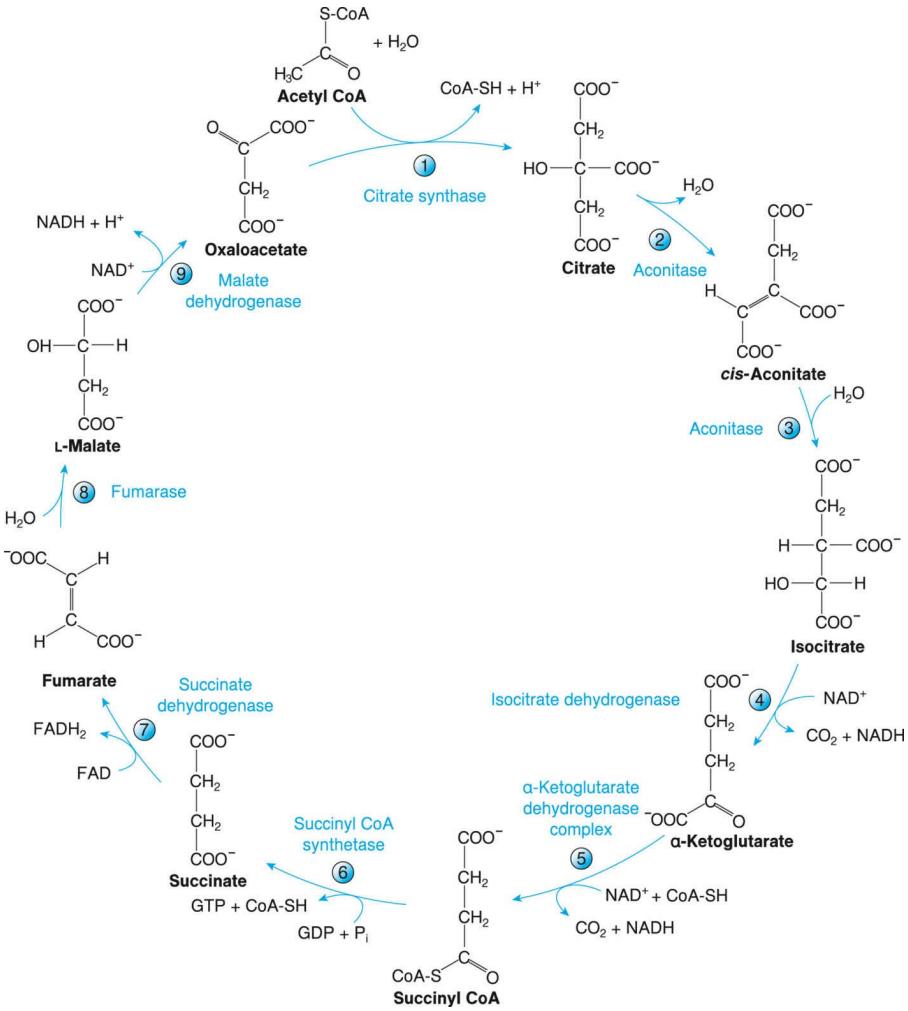


Figure 10.27 **The citric acid cycle.** The cycle is a metabolic pathway ending in the very compound from which it starts: oxaloacetate. The acetyl group, coming from the catabolism of carbohydrates, lipids, and proteins, enters the cycle as acetyl CoA and is finally oxidized to carbon dioxide, producing energy on the way.

The most interesting events start with the fourth reaction of the cycle. In this cycle and the following one, two carbons depart as two CO_2. At the same time isocitrate is oxidized to **α-ketoglutarate** (also known as **2-oxoglutarate**), and α-ketoglutarate is oxidized to **succinyl CoA** (with reentry of CoA into the cycle). In both reactions, NAD^+ serves as the oxidant.

In the sixth reaction, succinyl CoA splits into CoA and **succinate**. This splitting releases sufficient energy to feed GTP synthesis from GDP and P_i. GTP synthesis is another case of substrate-level phosphorylation (sections 9.3 and 10.8). The seventh reaction is an oxidation of succinate to **fumarate**, in

which FAD (section 2.5) is reduced to $FADH_2$. In the eighth reaction, fumarate is hydrated to **L-malate**, which undergoes a final oxidation by NAD^+ to regenerate oxaloacetate in the ninth reaction.

Because I defined substrate-level phosphorylation as the synthesis of ATP or another ribonucleoside triphosphate (such as GTP, in this case) in a reaction in which a substrate is a compound of high phosphoryl-transfer potential, you may wonder: Given that succinyl CoA bears no phosphoryl group, which is the compound of high phosphoryl-transfer potential in this reaction? The answer is succinyl phosphate, formed transiently from succinyl CoA in the enzyme's active site through the replacement of CoA by phosphate and thanks to the high energy inherent in succinyl CoA.

The term *cycle* is used metaphorically in biochemistry to denote metabolic pathways that end at the compound from which they start. The term does not imply any circular arrangement of metabolites or enzymes.

How would we sum up the citric acid cycle? Let's recap the net changes that accompany it.

- An acetyl group is converted into two CO_2 in the fourth and fifth reactions.
- Three NAD^+ are converted into three NADH in the fourth, fifth, and ninth reactions.
- One FAD is converted into $FADH_2$ in the seventh reaction.
- One GDP is converted into GTP in the sixth reaction.

All other biomolecules participating in the pathway, from citrate to oxaloacetate, are recycled. Thus, the overall reaction describing the citric acid cycle is

$$\begin{aligned}&\text{Acetyl CoA} + 3\ \text{NAD}^+ + \text{FAD} + \text{GDP} + \text{P}_\text{i} + 2\ \text{H}_2\text{O} \rightarrow 2\ \text{CO}_2 \\ &+ \text{CoA} + 3\ \text{NADH} + \text{FADH}_2 + \text{GTP} + 2\ \text{H}^+ \qquad \Delta G^{\circ\prime} = 3.3\ \text{kcal}\cdot\text{mol}^{-1}\end{aligned} \qquad \text{(equation 10.10)}$$

You may be surprised to see that reaction 10.10 is endergonic and that, nonetheless, I have used a unidirectional, rightward-pointing arrow. The reason is that the citric acid cycle is coupled to the highly exergonic electron-transport chain, which removes the NADH and $FADH_2$ produced in the cycle, as we will see in section 10.14. By doing so, the electron-transport chain

"pulls" the citric acid cycle. Remember that removing the products of a reaction constitutes a means of making it favored (section 2.2).

Notice that no ATP is produced in the citric acid cycle, although GTP is almost equivalent to ATP. GTP is used as a compound of high phosphoryl-transfer potential in several cases, such as translation (section 4.17) and signal transduction through G proteins (section 10.3). In addition, GTP can replenish ATP according to the reaction

$$\text{GTP} + \text{ADP} \rightleftharpoons \text{GDP} + \text{ATP} \qquad \Delta G^{\circ\prime} = 0.6\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.11)}$$

Is then one GTP or ATP the energy yield of the entire cycle? No, the energy produced is much greater, but it is hidden in NADH and $FADH_2$. We will discover it shortly as we consider the processes of the electron-transport chain and oxidative phosphorylation.

No oxygen is directly involved in the citric acid cycle. However, the NAD^+ and FAD that are consumed in its reactions can be regenerated inside the mitochondria (to be used again as oxidants) only if NADH and $FADH_2$ transfer their electrons to O_2 through the electron-transport chain. Thus, oxygen is indirectly required for the operation of the citric acid cycle, which is therefore considered an aerobic pathway.

10.13 Exercise Speeds Up the Citric Acid Cycle in Muscle

The rate of acetyl group oxidation through the citric acid cycle may go up by as much as 100 fold in muscle during hard exercise. This increase results primarily from the rise in acetyl CoA concentration because of the accelerated pyruvate oxidation. In addition, the cycle is accelerated during exercise through the allosteric regulation of three of its enzymes—**citrate synthase**, **isocitrate dehydrogenase**, and **α-ketoglutarate dehydrogenase** (part of the **α-ketoglutarate dehydrogenase complex**)—which catalyze the first, fourth, and fifth reactions, respectively. The former two are inhibited by a high [ATP]/[ADP] ratio, and the drop in this ratio during exercise activates the enzymes. Isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are activated by Ca^{2+}, the mitochondrial concentration of which increases with

prolonged exercise. The two enzymes are also inhibited by NADH.

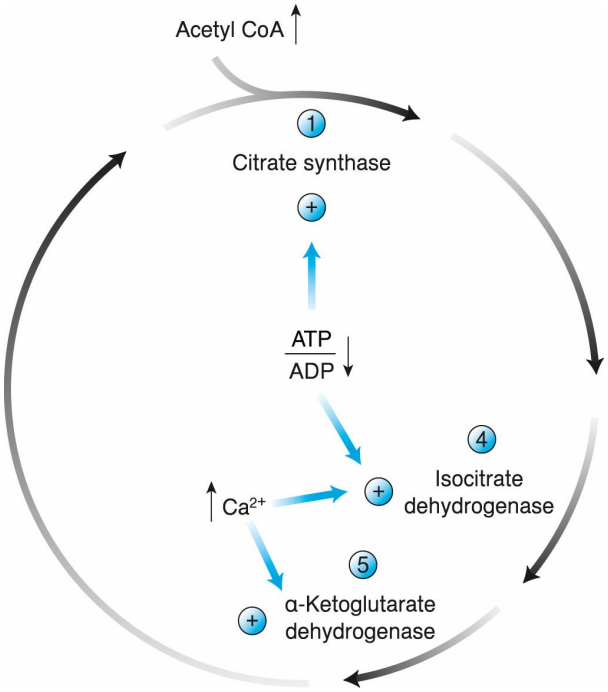


Figure 10.28 **Control of the citric acid cycle by exercise.** Exercise speeds up the cycle in muscle by increasing the substrate (acetyl CoA) concentration, decreasing the [ATP]/[ADP] ratio, and increasing the Ca^{2+} concentration. Numbers in circles correspond to reactions as in figure 10.27.

The regulation of the citric acid cycle in muscle during exercise is summarized in figure 10.28.

10.14 The Electron-Transport Chain

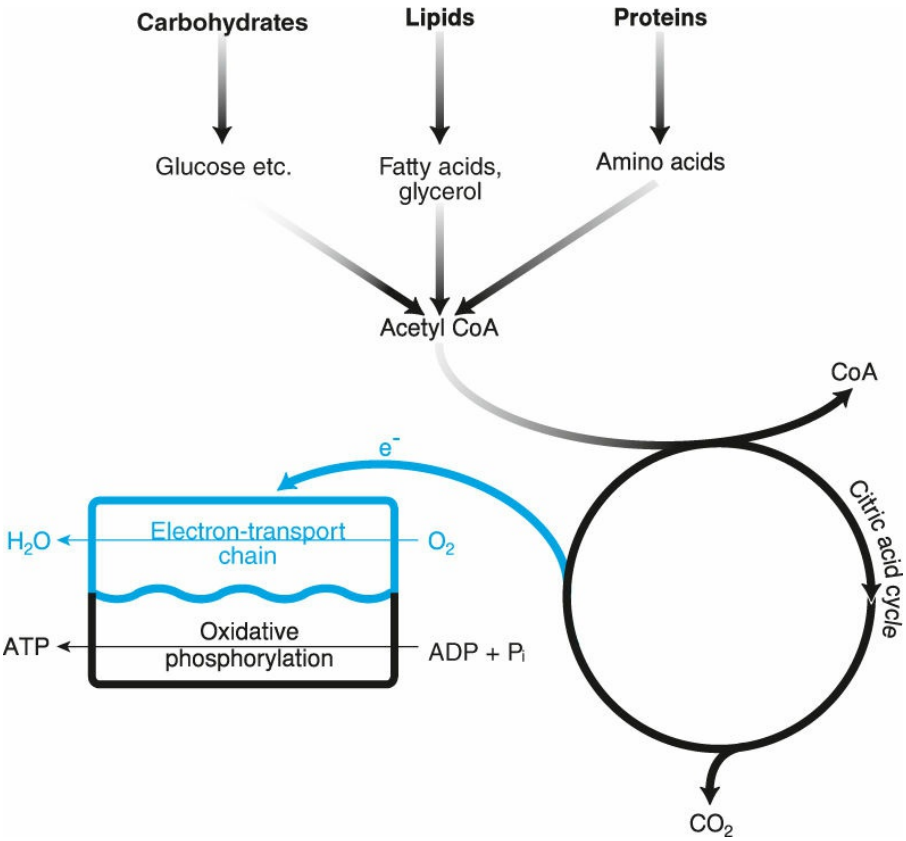
As mentioned in previous sections, the NADH and $FADH_2$ produced in the mitochondria during pyruvate oxidation and the citric acid cycle can be oxidized to NAD^+ and FAD by transferring their electrons (along with hydrogens) to oxygen. This transfer is accomplished through the redox reactions

$$NADH + H^+ + \tfrac{1}{2}O_2 \rightarrow NAD^+ + H_2O \qquad \Delta G^{\circ\prime} = -55 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.12)}$$

$$FADH_2 + \tfrac{1}{2}O_2 \rightarrow FAD + H_2O \qquad \Delta G^{\circ\prime} = -50.9 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.13)}$$

In the electron-transport chain, electrons flow from NADH and $FADH_2$ to O_2, yielding NAD^+, FAD, and H_2O.

Electrons are transferred not in one step but through an intricate system involving four large protein complexes and two mobile electron carriers. The protein complexes are **NADH-Q oxidoreductase** (or **NADH dehydrogenase**), **succinate dehydrogenase**, **Q-cytochrome *c* oxidoreductase** (or **cytochrome reductase**), and **cytochrome *c* oxidase**. For brevity, they are known as **complexes I**, **II**, **III**, and **IV**, respectively. Notice that complex II (succinate dehydrogenase) is also the enzyme catalyzing the seventh reaction in the citric acid cycle (section 10.12 and figure 10.27). The mobile electron carriers are a lipid-like compound named **ubiquinone** (or **coenzyme Q**, or just **Q**) and a small protein named **cytochrome *c***.



You are here: electron-transport chain.

The whole system of protein complexes and mobile electron carriers is

called the **electron-transport chain**, or **respiratory chain**, because the destination of the electrons is respiratory oxygen, carried to the cells by blood. The components of the electron-transport chain are embedded in the inner mitochondrial membrane. Several of the components contain heme as their prosthetic group, which underlines the suitability of this compound for handling oxygen.

A diagram of the electron-transport chain is shown in figure 10.29. A pair of electrons from NADH is transferred initially to NADH-Q oxidoreductase and from there to ubiquinone. $FADH_2$, produced at the active site of succinate dehydrogenase during the seventh reaction of the citric acid cycle, also donates a pair of electrons to ubiquinone. Then the electrons from the two donors follow a common route, leaping from one component of the chain to another until they reach O_2. Addition of electrons to a component of the chain reduces it, but the subsequent transfer of the electrons to the next component restores the former to its oxidized form so that it can accept new electrons. Thus, complexes I to IV function as enzymes catalyzing redox reactions. In the end, the only net changes are the oxidation of NADH and $FADH_2$ to NAD^+ and FAD, as well as the reduction of O_2 to two H_2O by the addition of four H^+ and two electron pairs.

The electron-transport chain is part of the relay race depicted in figure 2.10. It's just that more runners (the components of the chain) intervene between NADH or $FADH_2$ and O_2. Also, an electron pair, rather than hydrogens, is the baton. Be a sportscaster of the event!

Cells gain two important things from the electron-transport chain:

- They regenerate NAD^+ and FAD.
- They produce high amounts of energy, as evidenced by the extremely negative $\Delta G^{\circ\prime}$ values of reactions 10.12 and 10.13. Part of this energy is then used to regenerate ATP in oxidative phosphorylation.

Through the electron-transport chain, cells can also regenerate the cytosolic NAD^+ that is reduced to NADH in the sixth reaction of glycolysis (section 10.8). This regeneration is not as simple as it seems, because NADH cannot cross the inner mitochondrial membrane. There are, however, compounds in

the cytosol that accept an electron pair from NADH (thus converting it into NAD^+), travel to the mitochondria, and transfer the electron pair to either NAD^+ or FAD (thus converting them into NADH and $FADH_2$, respectively). The electron carriers themselves, now devoid of the electron pair, return to the cytosol, where they can take part in electron transport again. These biomolecules are called **molecular shuttles**, because they go back and forth between the cytosol and the mitochondria.

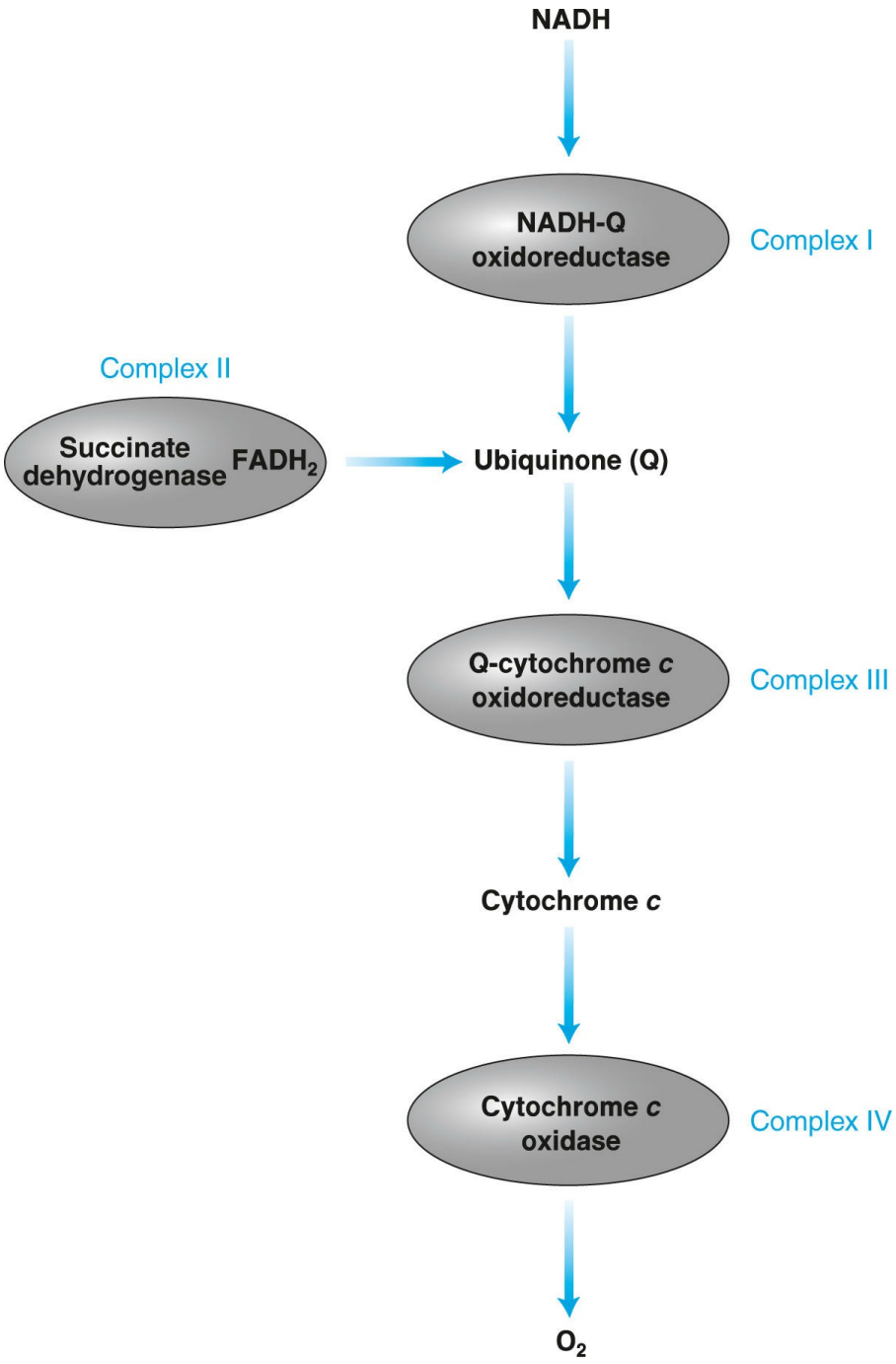


Figure 10.29 **Electron-transport chain.** The chain consists of four protein complexes (gray ellipses) and two mobile electron carriers in between. NADH and $FADH_2$ donate their electrons to different components of the chain. However, after the ones from NADH have converged with those from $FADH_2$ on ubiquinone, all flow together along the chain toward their destination: oxygen.

As a side reaction in the final step of the electron-transport chain, some

oxygen molecules receive one electron instead of four, thus giving rise to the superoxide radical introduced in section 1.5 and discussed in section 9.6. However, it is now believed that this is a minor source of $O_2^{\cdot -}$ during exercise. In fact, evidence suggests that that $O_2^{\cdot -}$ production in the electron-transport chain decreases when muscles pass from the resting to the exercising state, as reviewed by Feng He and associates.

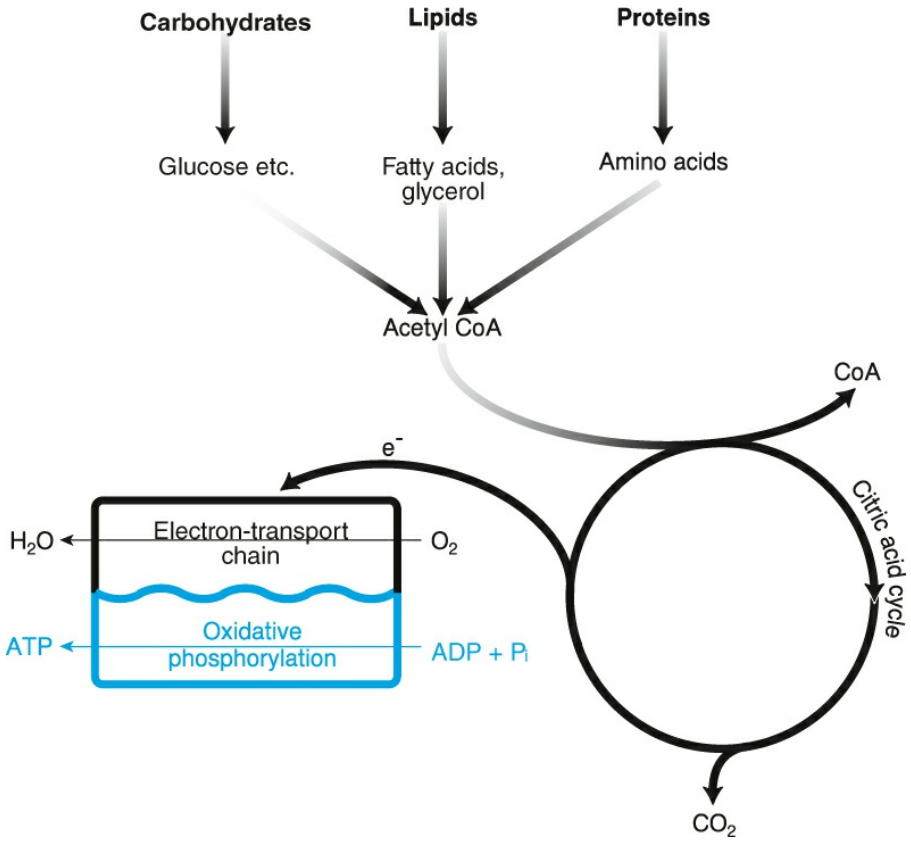
10.15 Oxidative Phosphorylation

Eukaryotic cells have evolved a way of channeling part of the energy released in the electron-transport chain to ATP synthesis, which is carried out according to the familiar endergonic reaction

$$ADP + P_i + H^+ \rightleftharpoons ATP + H_2O \qquad \Delta G^{\circ\prime} = 6.3 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 10.14)}$$

Because this process involves the phosphorylation of ADP with the aid of the energy liberated from the oxidation of NADH and $FADH_2$, we call it **oxidative phosphorylation**. It is an aerobic process, because it depends on oxygen utilization in the electron-transport chain. ATP synthesis is catalyzed by **ATP synthase** (also known as **F_0F_1 ATPase**), an integral protein of the inner mitochondrial membrane. ATP synthase consists of about 20 subunits forming a cylindrical part, which is embedded in the membrane, and a knob protruding into the mitochondrial matrix (figure 10.30).

In oxidative phosphorylation, ATP is synthesized from ADP and P_i with the energy of the electron-transport chain.



You are here: oxidative phosphorylation.

The active site of ATP synthase is in the mitochondrial matrix, which, therefore, is where ATP is synthesized. ATP then passes to the intermembrane space with the help of another integral protein of the inner mitochondrial membrane, **ATP–ADP translocase**. The protein is so called because for every ATP that it exports, it imports one ADP. This fact ensures that the ADP produced from ATP hydrolysis in the cytosol is "recharged" in the mitochondria. Both ATP and ADP cross the outer mitochondrial membrane freely. Finally, one more integral protein of the inner mitochondrial membrane, the **phosphate carrier**, imports the phosphate needed for ATP synthesis along with a proton (H^+).

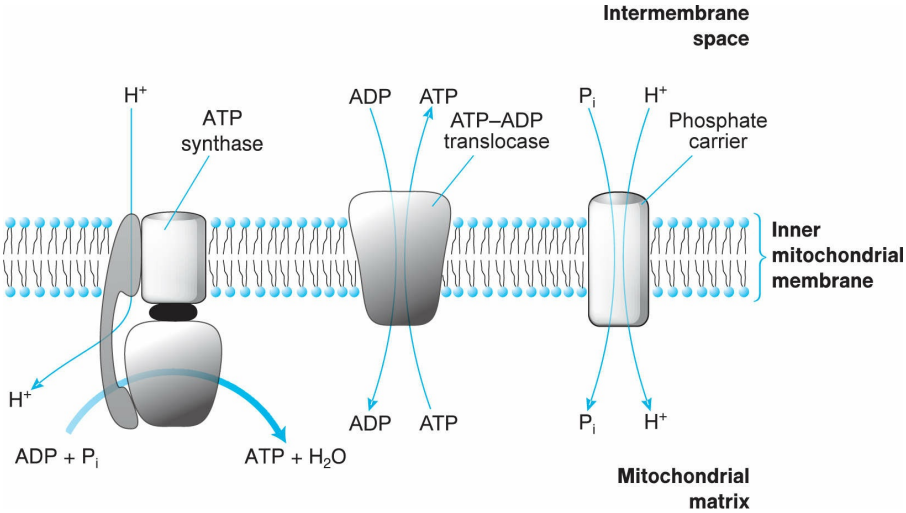


Figure 10.30 **Proteins dedicated to mitochondrial ATP synthesis.** Three integral proteins of the inner mitochondrial membrane cooperate in ATP synthesis. ATP synthase catalyzes ATP formation from ADP and P_i with the energy provided by the influx of H^+ (to be explained in section 10.16). ATP–ADP translocase brings ADP into the mitochondrial matrix in exchange for ATP. Finally, the phosphate carrier imports the P_i needed for ATP synthesis.

10.16 Energy Yield of the Electron-Transport Chain

Because the electron-transport chain and oxidative phosphorylation are distinct processes, the question arises as to how the energy produced by the former is channeled to the latter. This question has constituted one of the most tantalizing problems in biochemistry. The prevalent view is expressed by the **chemiosmotic hypothesis**, proposed in 1961 by the British biochemist Peter Mitchell. The hypothesis states that *the link between the electron-transport chain and oxidative phosphorylation consists of protons that are expelled from the mitochondrion as the electron-transport chain operates, only to return through ATP synthase.*

Let's take a closer look at the chemiosmotic hypothesis (figure 10.31). As an electron pair from NADH or $FADH_2$ runs along the protein complexes of the electron-transport chain, each complex except complex II uses part of the energy produced to actively transport H^+ from the mitochondrial matrix to the intermembrane space. Thus, a surplus of H^+ builds up in the intermembrane space, as opposed to a deficit of H^+ in the mitochondrial matrix. This surplus

results in a proton electrochemical gradient across the inner mitochondrial membrane, which forces the protons back to the matrix. The protons enter through ATP synthase, and the energy of their flow fuels ATP synthesis.

How much ATP is generated by oxidative phosphorylation with the energy of the electron-transport chain? Because the motive force for ATP synthesis is the influx of H^+ to the mitochondrion, answering this question requires us to know two things:

- How many H^+ does each protein complex of the electron-transport chain drive out of the mitochondrial matrix when an electron pair from NADH or $FADH_2$ flows along the chain?
- How many H^+ need to pass through ATP synthase for one ATP to be formed?

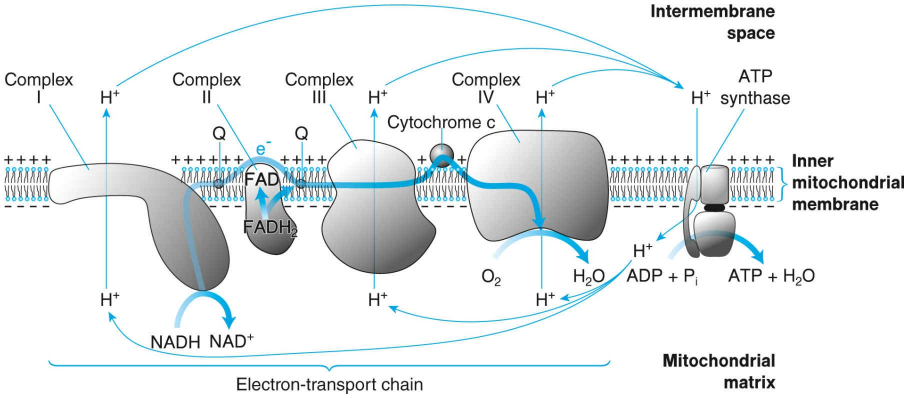


Figure 10.31 **The chemiosmotic hypothesis.** The hypothesis explains the coupling of the electron-transport chain to oxidative phosphorylation by postulating that protons driven out of the mitochondrial matrix by the flow of electrons toward oxygen return through ATP synthase and cause the linking of ADP and P_i to form ATP. The six components of the electron-transport chain have been placed in the order shown in figure 10.29. In reality, they are scattered all over the inner mitochondrial membrane.

We are currently uncertain about the answers to these questions. The best available estimates of the numbers of protons expelled by complexes I, III, and IV of the electron-transport chain per electron pair are four, two, and four in sequence. On the other hand, the synthesis of one ATP requires the passage of approximately three H^+ through ATP synthase. In addition, as mentioned in the previous section, one H^+ enters the mitochondrial matrix

with phosphate through the phosphate carrier. This H^+ needs to be added to the cost of synthesizing ATP; thus, a total of four H^+ are needed to generate one ATP.

Based on the figures just given, we can conclude that the 10 (that is, 4 + 2 + 4) H^+ removed from the mitochondrial matrix during the oxidation of one NADH produce about 10/4 = 2.5 ATP. The gain from $FADH_2$ oxidation is less. Because it transfers its electrons to ubiquinone rather than NADH-Q oxidoreductase, its oxidation results in the removal of only 6 (that is, 2 + 4) H^+ from the mitochondrial matrix. Thus, approximately 6/4 = 1.5 ATP are formed as one $FADH_2$ is oxidized.

10.17 Energy Yield of Carbohydrate Oxidation

At this point, carbohydrate oxidation—which started anaerobically (through glycogenolysis and glycolysis) and continued aerobically (through pyruvate oxidation, the citric acid cycle, the electron-transport chain, and oxidative phosphorylation)—has come to an end. With the aid of figure 10.32, let's recap what we get per glucose molecule.

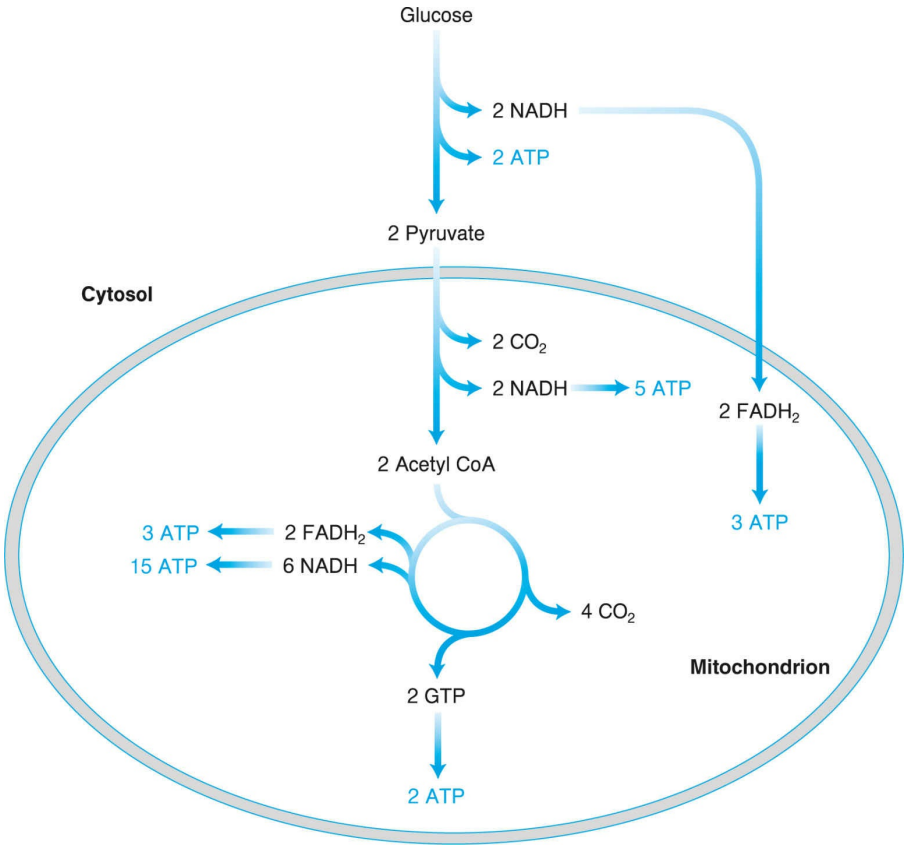


Figure 10.32 **Energy yield of glucose oxidation.** The complete oxidation of glucose to six CO_2 yields 30 ATP.

- Glycolysis yields two pyruvates, two ATP, and two NADH (one of each per glyceraldehyde 3-phosphate; section 10.8) in the cytosol. To yield ATP, the two cytosolic NADH must transfer their electrons to either mitochondrial NAD^+ or mitochondrial FAD by means of molecular shuttles (section 10.14). When the former happens, one cytosolic NADH gives rise to one mitochondrial NADH; when the latter happens, one cytosolic NADH gives rise to one mitochondrial $FADH_2$. The former seems to prevail in the heart and liver, the latter in skeletal muscle. Thus, two NADH in the cytosol of muscle fibers are equivalent to two $FADH_2$ in the mitochondria.
- Oxidation of the two pyruvates to two acetyl CoA in the mitochondrial matrix yields two CO_2 and two NADH.
- Oxidation of the two acetyl CoA in the citric acid cycle yields four CO_2, six NADH, two $FADH_2$, and two GTP. The latter are almost equivalent

to two ATP.

Summing up, the oxidation of one glucose in muscle yields six CO_2, four ATP, eight NADH, and four $FADH_2$. The eight NADH and four $FADH_2$ are oxidized in the electron-transport chain by six O_2 (four and two O_2, respectively, according to reactions 10.12 and 10.13). The eight NADH fuel the synthesis of approximately twenty (that is, 8 · 2.5) ATP, and the four $FADH_2$ fuel the synthesis of approximately six (that is, 4 · 1.5) ATP in oxidative phosphorylation. The sum of all the ATP produced from glucose oxidation is thirty.

The value of 30 ATP as the energy yield of glucose oxidation is based on knowledge that is almost three decades old. Being the most accurate estimate currently possible, it should be preferred over the older one, 36, that is still widely used and is based on estimated yields of 3 ATP per NADH and 2 ADP per $FADH_2$, which were made about six decades ago.

The overall reaction of the complete oxidation (or combustion) of glucose, excluding the accompanying synthesis of ATP, is

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O \qquad \Delta G^{\circ\prime} = -707\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.15)}$$

Reaction 10.15 is the reversal of photosynthesis (reaction 3.1). The huge amount of free energy released makes it the most exergonic process we have encountered so far. That is why the process can feed the synthesis of 30 ATP (having a $\Delta G^{\circ\prime}$ of 189 kcal for 30 moles according to reaction 10.14) while remaining extremely exergonic:

$$\text{Glucose} + 6\ O_2 + 30\ \text{ADP} + 30\ P_i + 30\ H^+ \rightarrow 6\ CO_2 + 30\ \text{ATP} + 36\ H_2O \qquad \text{(equation 10.16)}$$
$$\Delta G^{\circ\prime} = -518\ \text{kcal} \cdot \text{mol}^{-1}$$

The yield per glucose residue of glycogen, released as glucose 1-phosphate in glycogenolysis, is one ATP more, since glucose 1-phosphate yields three rather than two ATP in glycolysis (section 10.8). Thus, the combustion of most glucose residues of glycogen yields 31 ATP. By analogy to reaction 10.16, the overall reaction of the combustion of glucose 1-phosphate is

$$\text{Glucose 1-phosphate} + 6\ O_2 + 31\ \text{ADP} + 30\ P_i + 31\ H^+ \rightarrow 6\ CO_2 + 31\ \text{ATP} + 36\ H_2O \qquad \text{(equation 10.17)}$$
$$\Delta G^{\circ\prime} = -516\ \text{kcal} \cdot \text{mol}^{-1}$$

10.18 Exercise Speeds Up Oxidative Phosphorylation in Muscle

The most important determinant of the rate of oxidative phosphorylation is the concentration of its substrate, ADP. As ADP increases in an active muscle, it speeds up oxidative phosphorylation and ATP resynthesis. Oxidative phosphorylation requires an energy input from the electron-transport chain. Because the two processes are usually coupled tightly (that is, electrons do not flow through the electron-transport chain unless ATP is synthesized at the same time), as reviewed by David Wilson, the acceleration of oxidative phosphorylation "pulls" the electron-transport chain. This pulling, in turn, has three effects.

- It raises oxygen consumption, which is one of the most evident corollaries of exercise. *Oxygen consumption rises with exercise intensity because so does the rate of ATP breakdown into ADP and P_i.*
- It prevents an excessive rise in the [NADH], which would block pyruvate oxidation and the citric acid cycle (as discussed in sections 10.11 and 10.13).
- It speeds up the regeneration of NAD^+ and FAD, which are indispensable oxidants in glycolysis, pyruvate oxidation, and the citric acid cycle.

Thus, *the last link in the long chain of ATP production from carbohydrate oxidation ensures the supply of materials that are necessary for the continued operation of the preceding links.*

10.19 Lactate Production in Muscle During Exercise

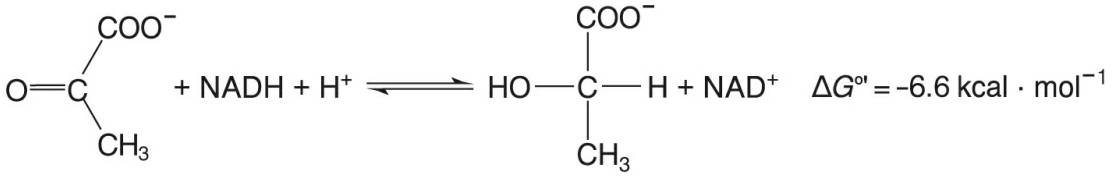
As we saw in sections 10.5 to 10.7 and 10.9, exercise speeds up glycogenolysis and glycolysis in muscle. The increase in the glycogenolytic and glycolytic rates relates to exercise intensity: Harder exercise brings larger changes—some positive, some negative—in the concentrations of

phosphocreatine, ATP, ADP, AMP, IMP, P_i, Ca^{2+}, and epinephrine, which affect one or both rates. Now remember that glycolysis is accompanied by the conversion of NAD^+ into NADH in its sixth reaction. To maintain a high glycolytic rate, muscle fibers need to regenerate NAD^+. If NAD^+ is depleted, glycolysis will shut down, and the flow of metabolites to subsequent pathways will stop.

The only means of regenerating cytosolic NAD^+ that we have considered so far (in section 10.14) is the transfer of an electron pair from NADH to a molecular shuttle, entry of the shuttle into the mitochondria, delivery of the electron pair to NAD^+ or FAD, and channeling of the electron pair to O_2 through the electron-transport chain. Completion of this process is mandatory for the regeneration, in oxidized form, of all the cellular components mediating the flow of electrons from cytosolic NADH to O_2.

Because of the multitude of steps involved, the aerobic conversion of cytosolic NADH into NAD^+ is time consuming. Thus, as exercise intensity increases, the rate of NADH formation in glycolysis begins to exceed the rate of aerobic NAD^+ regeneration, resulting in an increase in the [NADH]/[NAD^+] ratio. This increase slows down the conversion of pyruvate into acetyl CoA (reaction 10.7) for two reasons: first, because NADH is a product and NAD^+ is a reactant, and second, because a high [NADH]/[NAD^+] ratio results in inhibition of pyruvate dehydrogenase, as mentioned in section 10.11.

The resulting accumulation of pyruvate and NADH favors a seminal reaction in which both are substrates—specifically, the *anaerobic* conversion of pyruvate into **L-lactate**.



Pyruvate **L-Lactate** (equation 10.18)

Reaction 10.18 takes place in the cytosol and is catalyzed by **lactate dehydrogenase**. The reaction introduces pyruvate in place of oxygen as the oxidant of NADH. It also introduces the rapid regeneration of NAD^+, which

can then go back and help with glycolysis (figure 10.33).

Type II muscle fibers have higher lactate dehydrogenase activity than do type I fibers. This activity, along with their higher content of glycolytic enzymes (mentioned in section 10.9), results in their producing more lactate as compared with type I fibers. Another factor that may contribute to this difference is the different subunit composition of lactate dehydrogenase in type I and type II fibers. Let's see what this means.

Lactate dehydrogenase is a tetramer composed of a combination of two kinds of similar subunits, denoted by H for heart and M for muscle. This composition produces five combinations, H_4, H_3M, H_2M_2, HM_3, and M_4, which are also referred to as LD1, LD2, LD3, LD4, and LD5, respectively, or LDH1 through LDH5. The H and M subunits differ in their affinities for pyruvate and lactate. As a result, LD isoforms in which H predominates (that is, LD1 and LD2) catalyze reaction 10.18 faster in the reverse direction (lactate to pyruvate) than in the forward direction. By contrast, LD isoforms in which M predominates (that is, LD4 and LD5) catalyze reaction 10.18 faster in the forward than in the reverse direction.

LD and LDH are the new and old abbreviations, respectively, for lactate dehydrogenase.

LD1, LD2, and LD3 abound in type I muscle fibers, whereas LD5 abounds in type II fibers, in which small amounts of LD4 and LD3 can also be found, as reviewed by Stefano Schiaffino and Carlo Reggiani. It is believed that these differences produce two effects. On one hand, they increase the suitability of type II fibers to produce large amounts of lactate, thus ensuring high glycolytic power. On the other hand, they increase the suitability of type I fibers to use lactate in a way that we will explore in section 10.23.

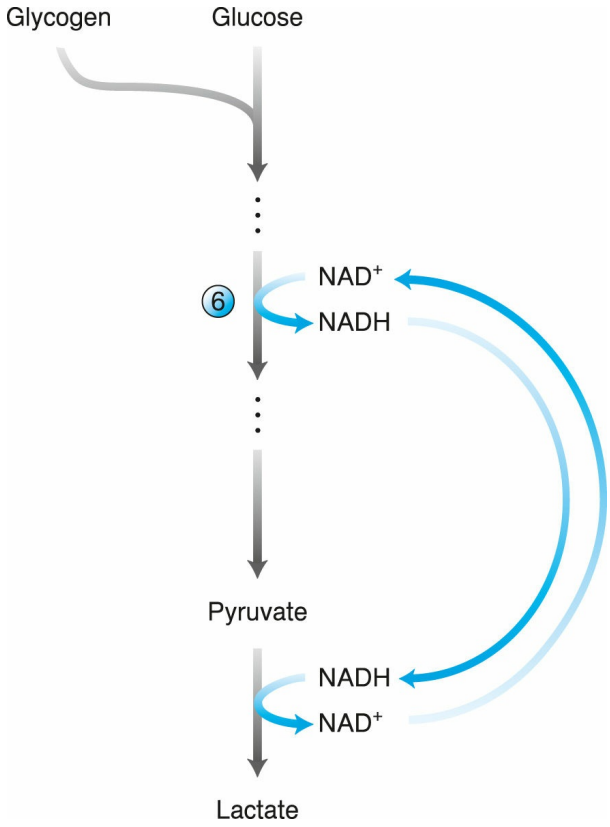


Figure 10.33 The benefit from lactate production. The anaerobic breakdown of carbohydrates into lactate requires no NAD^+, because NADH produced in the sixth reaction of glycolysis is rapidly converted back into NAD^+ when pyruvate is reduced to lactate.

The muscle lactate concentration is approximately 1 mmol · kg^{-1} at rest, which reflects a low rate of anaerobic catabolism of carbohydrates, since the majority are broken down aerobically into CO_2. The lactate concentration gradually increases with exercise intensity and can reach 30 mmol · kg^{-1} during maximal exercise, thus indicating massive anaerobic carbohydrate degradation. In fact, this is the most impressive change brought about by exercise in the concentration of a metabolite. Thus, it is no wonder that lactate has been measured numerous times in studies on exercise metabolism and is used widely as an index of the body's response to exercise. Unfortunately, just because of this popularity, lactate has fallen victim to a number of misconceptions that I will tackle in sections 10.20, 10.21, and 10.32. Before doing that, however, let me tackle a misuse of terms.

The conversion of glucose (or even glycogen) into lactate is sometimes termed *anaerobic glycolysis*. However, for the sake of accuracy—and every

science requires accurate use of its terminology in order to enable accurate transfer of information—I must note that glycolysis is a specific metabolic pathway (the one leading from glucose to pyruvate), not just any pathway of carbohydrate breakdown. In addition, glycolysis is solely anaerobic; thus, the term *anaerobic glycolysis* is redundant. Moreover, use of this term leaves room for another, inaccurate, term—*aerobic glycolysis*. This term is usually meant to denote the combustion of glucose or glycogen to CO_2, although some scholars, particularly in the field of cancer research, use the term quite differently, that is, to denote the conversion of glucose into lactate in the presence of oxygen, thus further baffling the reader.

Table 10.1 Recommended Terminology for Pathways of Carbohydrate Catabolism

Pathway	Recommended name
Glucose to pyruvate	Glycolysis (not anaerobic glycolysis)
Glucose to lactate	Anaerobic glucose catabolism (not anaerobic glycolysis)
Glycogen to lactate	Anaerobic glycogen catabolism (not anaerobic glycolysis)
Glucose to CO_2	Aerobic glucose catabolism (not aerobic glycolysis)
Glycogen to CO_2	Aerobic glycogen catabolism (not aerobic glycolysis)

To avoid confusion, I believe that the right thing is to refer to the conversion of carbohydrates into lactate as **anaerobic carbohydrate catabolism** (or *anaerobic carbohydrate degradation*, or *anaerobic carbohydrate breakdown*). My suggestions are summarized in table 10.1.

10.20 Lactate Production Is Probably Not a Cause of Fatigue

Lactate production from the anaerobic catabolism of carbohydrates in exercising muscles is accompanied by an acidification (increase in the acidity) of the cytosol of their fibers (type II fibers in particular), because the net conversion of glucose or glycogen into lactate goes hand in hand with proton production.

$$C_6H_{12}O_6 \rightarrow 2\ C_3H_5O_3^- + 2\ H^+ \qquad \text{(equation 10.19)}$$

$$\text{Glycogen } (n \text{ glucose residues}) + H_2O \rightarrow \text{glycogen } (n-1 \text{ glucose residues}) + 2 \text{ lactate} + 2\ H^+ \qquad \text{(equation 10.20)}$$

In contrast, there is no net H^+ production when carbohydrates are broken down aerobically, as you can see in reaction 10.15.

As a result of massive proton production in an intensely exercising muscle, the cytosolic pH may drop from 7 to 7.2 at rest to as low as 6.2. The presence of both lactate and H^+ among the products of reactions 10.19 and 10.20 does not mean that lactate is the cause of proton formation during hard exercise (a widely held notion). Rather, proton formation and the drop in cytosolic pH should be viewed as results of the entire process of anaerobic carbohydrate degradation.

Besides, if lactate were to be blamed for the drop in cytosolic pH, then pyruvate should be blamed twice as much, since the conversion of glucose into pyruvate produces four rather than two H^+ (reaction 10.6). If anything, lactate formation from pyruvate absorbs H^+ (reaction 10.18). Pinning the acidification of muscle on lactate is like pinning the defeat of a relay team on the last runner because she was just that, even though she ran faster than her teammates.

Let me now address the point posited in the title of this section. As I will analyze in section 14.25, the acidification of muscle during exercise is considered a cause of fatigue. This view and the unfortunate association of lactate production with muscle acidification have led to the notion that lactate is a fatigue-causing substance. However, the evidence that lactate production during exercise causes fatigue is less than the evidence for the opposite, as reviewed by Brian Ferguson and associates. Moreover, there are cases in which both lactate production and exercise performance increase, as I will also analyze in section 14.25.

Likewise, there is little support for the frequent association of lactate production with the burning sensation in muscles during hard exercise, with muscle pain, or with the soreness (termed *delayed-onset muscle soreness*) that we usually experience for a couple of days after hard or unaccustomed exercise. Blaming lactate for the undesirable effects of exercise resembles the ancient practice of laying the sins of the people upon a scapegoat.

Misconceptions about the role of lactate in exercise often extend from theory to training practice. For example, the ability of an athlete to maintain high speed for a long time (an activity accompanied by high lactate production) is sometimes termed **lactate tolerance**, as if lactate is what one must train to tolerate. In such cases, it would be safer to use a performance-oriented term such as *speed endurance*.

10.21 Lactate Production Is Not Due to a Lack of Oxygen

As mentioned in section 10.19, muscle lactate concentration exhibits a spectacular increase during exercise. Why this happens has been the subject of hot debates among researchers for decades. As you have seen, the explanation that I gave was essentially this: Since NAD^+ is converted into NADH in glycolysis, a reactant increases and a product decreases in reaction 10.18. These changes shift the reaction to the right, resulting in increased lactate production. This explanation, however, is not shared by all scholars, several of whom maintain that the cause of lactate production in a hardworking muscle is a lack of oxygen. In fact, this view is widespread, both within and beyond sport science.

The view is founded on the fact that the conversion of carbohydrates into lactate is an anaerobic process. Therefore, the reasoning goes, there has to be a shortage of oxygen for the process to be favored. However, the fact that a process is anaerobic does not mean that it takes place *because* or *only when* oxygen is lacking; nor does being anaerobic preclude the process from being accelerated in the presence of abundant oxygen if other factors can speed it up.

Let me make this clearer with an example before I treat it in detail. Suppose you own a motorcycle and a bicycle. Obviously, the motorcycle requires fuel to run, whereas the bicycle does not. Thus you can ride the motorcycle only when there is gas in the tank. But does this mean that you will ride the bicycle *only* when there is no gas in the motorcycle's tank? Of course not! You can ride the bicycle whenever you wish just because you own one, regardless of whether you have the alternative of riding the

motorcycle.

Now substitute the aerobic and anaerobic breakdown of carbohydrates for the motorcycle and the bicycle, respectively, and substitute oxygen for the fuel. Your muscles can run the anaerobic breakdown to lactate even in the presence of ample oxygen, just because the process is feasible both thermodynamically and kinetically. It is feasible thermodynamically because the conversion of pyruvate into lactate is exergonic (see the negative $\Delta G^{\circ\prime}$ value in reaction 10.18) and kinetically because there is ample lactate dehydrogenase in muscle fibers.

Now let me get to the details of why lactate production in a normally oxygenated muscle does not seem to be due to a lack of oxygen. To begin with, muscles produce lactate even at rest (resulting in a concentration of about 1 mmol · kg^{-1}, as mentioned in section 10.19), although they are as oxygenated as can be. In other words, there is no shortage of oxygen, but still there is lactate production just because it is feasible or, I should say, inevitable.

Next, let's consider what happens during exercise at intensities that cause a rise in lactate production. Most of the available experimental data show that, although the sarcoplasmic oxygen concentration decreases when we exercise, there is still sufficient oxygen to support a maximal rate of ATP resynthesis in the mitochondria even during maximal exercise. This conclusion was drawn, for example, by Russell Richardson and collaborators in a study published in 1998, in which they used NMR spectroscopy (section III.7) to measure the SO_2 of myoglobin (section 3.12) in human muscle at various exercise intensities. The researchers then used the SO_2 values to deduce the pO_2 inside the muscle fibers, based on the relationship established in figure 3.16.

As expected, high lactate production was found during hard, near-maximal, and maximal exercise. However, the pO_2 did not fall below the critical level at which the maximal rate of ATP resynthesis in the mitochondria is compromised. What is more, lactate production was unrelated to pO_2 across a range of exercise intensity, from moderate to maximal. In contrast, lactate production was related to O_2 consumption. These findings show that lactate production during exercise is not due to the

muscle's running out of oxygen.

Using NMR spectroscopy again, Richardson and collaborators showed in 2015 that human skeletal muscle myoglobin did not begin to lose its O_2 until after about 6 s of hard exercise. However, the processes of anaerobic ATP resynthesis (such as phosphocreatine breakdown and carbohydrate breakdown into lactate) are accelerated from the very first second of hard exercise. This fact suggests that the activation of anaerobic catabolism is due not to a lack of oxygen but, rather, to the slower activation of aerobic catabolism, which renders it unable to match the high rate of ATP hydrolysis with a high rate of ATP resynthesis at the onset of hard exercise.

Thus, it seems that the true cause of the augmented lactate production, or accumulation, in the cytosol of active muscle fibers is the overproduction of NADH and pyruvate. The fast regeneration of NAD^+ resulting from the conversion of pyruvate into lactate ensures the continuation of glycolysis and of ATP resynthesis at a high rate, although no ATP is produced in the reaction itself.

10.22 Features of Anaerobic Carbohydrate Catabolism

Anaerobic carbohydrate catabolism is uneconomical: It yields only two ATP per glucose (all that is produced in glycolysis) against some thirty ATP produced in the aerobic breakdown. The corresponding figures for most of the glucose residues in glycogen are three against thirty-one. Nevertheless, the anaerobic route is rapid. In fact, it is so rapid that, despite the low ATP yield, it can produce three times as much ATP as the aerobic route at a given time.

Indeed, the maximal rate of ATP resynthesis from the conversion of glycogen into lactate in human muscle is estimated at 1.5 mmol $\cdot$ kg^{-1} $\cdot$ s^{-1} and is reached within 5 s of maximal exercise. In contrast, the maximal rate of ATP resynthesis from the conversion of glycogen into CO_2 is estimated at 0.5 mmol $\cdot$ kg^{-1} $\cdot$ s^{-1} and, to be reached, it requires more than 1 min of maximal exercise. *Thus the high rate of ATP regeneration constitutes the edge of the anaerobic carbohydrate breakdown.*

When catabolized anaerobically, glycogen is the fastest source for ATP resynthesis next to phosphocreatine. In fact, it surpasses phosphocreatine in the amount of ATP it can regenerate: While the phosphocreatine content of a muscle is, as we have said, about 20 mmol · kg^{-1} (yielding an equal amount of ATP, according to reaction 9.4), an average glycogen content of 1.25% translates into 77 mmol glucosyl units per kilogram, which can yield close to 231 (that is, 3 · 77) mmol ATP · kg^{-1}. (Note that 1.25% glycogen means 1.25 g per 100 g of muscle, or 12.5 g · kg^{-1}, or 12,500 mg · kg^{-1}. Divide this by the "molecular mass" of the glucosyl unit [162 Da], and you will get 77 mmol · kg^{-1}.)

Thanks to this combination of rate and quantity, the anaerobic glycogen degradation becomes the major source for ATP resynthesis in maximal exercise tasks lasting approximately 7 s to 1 min. Included in this time frame are events such as the 100, 200, and 400 m runs and the 50 and 100 m swims. Maximal exercise tasks lasting longer than 1 min rely on the aerobic glycogen degradation as their major energy source (figure 10.34). In addition, the aerobic glycogen degradation predominates in hard or moderate-intensity exercises of any duration.

The expansion of the predominance of aerobic catabolism to as short a time as 1 min (compared with the prevalence of anaerobic catabolism extending to 2 or 3 min in older reports) is due to the realization that the methods used in previous decades to estimate the contribution of anaerobic energy sources were positively biased (that is, they overestimated it).

The major anaerobic processes that supply ATP during exercise are the breakdown of carbohydrates into lactate and the reaction catalyzed by creatine kinase. To distinguish them while using shorter terms, authors often refer to the former as the **anaerobic lactic mechanism** and to the latter as the **anaerobic alactic mechanism**.

10.23 Using Lactate

Lactate produced in a hardworking muscle cannot be converted into anything else, other than pyruvate by reversal of reaction 10.18, because it participates

in no other reaction in the human body. However, reaction 10.18 cannot be reversed in the cytosol of active muscle fibers, since there is a shortage of NAD^+. Besides, if NAD^+ were abundant, lactate production would not have increased in the first place. Thus, what lactate does is to leave the muscle fibers. Their plasma membrane is highly permeable to lactate, which exits to the interstitial fluid (down its concentration gradient) and from there to the bloodstream, which disperses it all over the body.

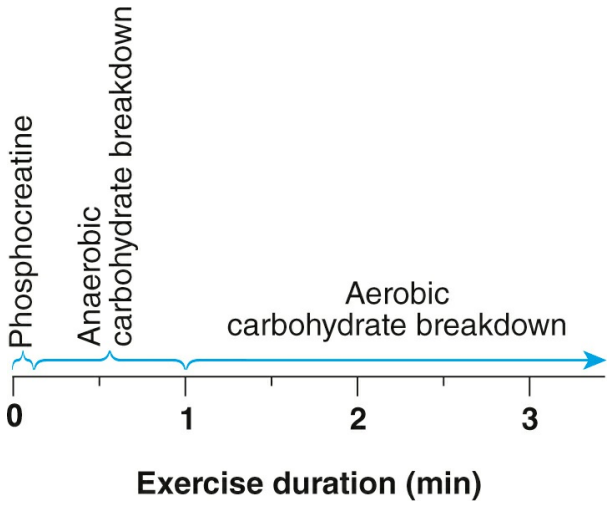


Figure 10.34 Major energy sources in competition. Athletes who "give it their all" in competition rely on different energy sources depending on exercise duration. Events lasting up to about 7 s depend primarily on phosphocreatine for ATP resynthesis, events lasting about 7 s to 1 min depend mainly on anaerobic carbohydrate breakdown, and events lasting over 1 min depend primarily on aerobic carbohydrate breakdown.

The exit of lactate from muscle fibers is facilitated by proteins spanning the sarcolemma and transverse tubule membrane. These proteins are called **monocarboxylate transporters**, or **MCT**, to indicate that, in addition to lactate, they transport other acids with one carboxyl group. An MCT carries one lactate and one H^+ in the same direction, thus contributing to the lowering of the cytosolic $[H^+]$ (figure 10.35). As a result, the plasma $[H^+]$ rises, and the plasma pH may drop by as much as about 0.2 units after exercise. There are several MCT isoforms, two of which, MCT1 and MCT4, are present in human and rat skeletal muscle.

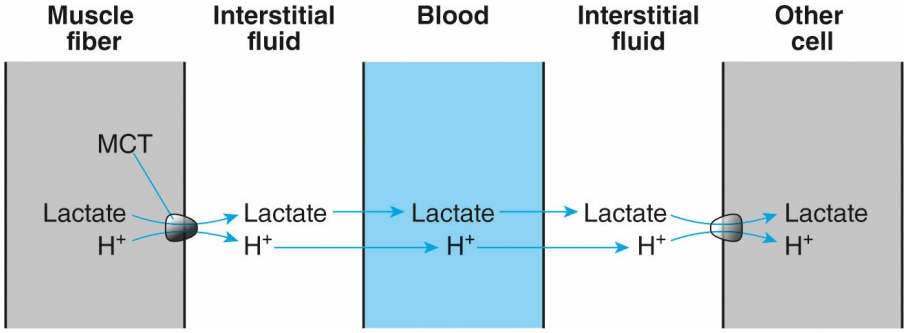


Figure 10.35 **Lactate traffic.** After being produced in a muscle fiber, lactate crosses the plasma membrane toward the interstitial fluid, along with H^+, through a monocarboxylate transporter (MCT). From the interstitial fluid, lactate diffuses to blood and reaches cells that have a lower lactate concentration than does plasma. Lactate enters those cells, again through an MCT.

Because it crosses membranes easily, lactate can enter cells in which its concentration is lower than that in plasma (again, down its concentration gradient). Such cells belong primarily to inactive skeletal muscles, the heart, and the liver. Small quantities of lactate are also taken up by brain and kidney cells. Finally, small quantities of lactate are lost in urine through glomerular filtration.

It is even possible for fibers in a muscle to take up lactate from other fibers of the same muscle. This action can happen because, as discussed in section 10.19, type II fibers produce more lactate than do type I fibers. Thus, type I fibers can absorb part of the lactate exiting type II fibers. Lactate enters muscle fibers through the same MCT it uses to exit. Upon entering a cell, lactate can be oxidized to pyruvate by a reversal of reaction 10.18.

$$\text{Lactate} + \text{NAD}^+ \rightleftharpoons \text{pyruvate} + \text{NADH} + \text{H}^+ \qquad \Delta G^{\circ\prime} = 6.6\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 10.21)}$$

Despite its positive $\Delta G^{\circ\prime}$, the reaction is favored by a low [NADH]/[NAD^+] ratio in the cytosol of the cells that take up lactate (unlike the cytosol of the active muscle fibers). It is also favored by a low [pyruvate]/[lactate] ratio, since the pyruvate produced is continuously removed. Thus the ΔG becomes negative (see section 2.2), and the reaction is shifted to the right.

Once formed, pyruvate follows primarily one of two routes:

1. It is fully oxidized to three CO_2 through the reaction catalyzed by the

pyruvate dehydrogenase complex, followed by the citric acid cycle. Most of the lactate produced during hard exercise follows this route in the heart and skeletal muscles, releasing a substantial amount of energy. How much energy? Consider the following: Each lactate yields one cytosolic NADH upon conversion into pyruvate. This NADH then transfers an electron pair to a mitochondrial NAD^+ or FAD (section 10.14), thus yielding, respectively, 2.5 or 1.5 ATP through the electron-transport chain and oxidative phosphorylation. The conversion of pyruvate into acetyl CoA produces one (mitochondrial) NADH yielding 2.5 ATP. The oxidation of acetyl CoA in the citric acid cycle yields three NADH (7.5 ATP), one $FADH_2$ (1.5 ATP), and one GTP (1 ATP). In all, lactate oxidation produces 15 or 14 ATP. In this way, lactate returns the difference in energy yield between aerobic and anaerobic carbohydrate catabolism. (Remember that one glucose produces two lactates.)

2. Pyruvate can be used to resynthesize glucose. This process happens primarily in the liver, which hosts the metabolic pathway of gluconeogenesis. I will devote the next three sections to this pathway, then return to lactate utilization in section 10.27.

10.24 Gluconeogenesis

Gluconeogenesis is the synthesis of glucose from compounds that are not carbohydrates. Such compounds are pyruvate, lactate, glycerol, and most of the 20 amino acids that form proteins. At present, let's focus on pyruvate and lactate; we will deal with glycerol and the amino acids as sources of glucose in chapters 11 and 12, respectively.

Despite being products of carbohydrate breakdown, pyruvate and lactate belong chemically to carboxylic acids rather than carbohydrates. Lactate, in particular, does not qualify as a carbohydrate, even though its un-ionized form (lactic acid) has the molecular formula $C_3H_6O_3$, or $(CH_2O)_3$. The reason it does not qualify is that it contains only one hydroxyl group and, thus, does not meet the criteria set in section 5.1.

Glucose synthesis from pyruvate (or from lactate that has been converted

into pyruvate, as described in the previous section) involves the reversal of seven of the ten reactions of glycolysis. As expected, the same enzymes catalyze these reactions in gluconeogenesis as in glycolysis. However, three glycolytic reactions—the first, third, and tenth—are irreversible because they are highly exergonic. (Therefore, the reverse reactions are highly endergonic and thus not favored.) To circumvent this problem, gluconeogenesis lines up different reactions, which are exergonic.

Gluconeogenesis takes place largely in the cytosol. However, it starts in the mitochondrial matrix (figure 10.36), where pyruvate is converted into oxaloacetate (a compound of the citric acid cycle) by the addition of one CO_2 and the hydrolysis of one ATP. The enzyme catalyzing this carboxylation reaction, pyruvate carboxylase, contains biotin as coenzyme in its active site (section 6.1). Oxaloacetate is subsequently reduced to malate (another compound of the citric acid cycle), which then passes to the cytosol. Malate is oxidized back to oxaloacetate, which is next converted into phosphoenolpyruvate (the compound preceding pyruvate in glycolysis) at the expense of one GTP and with removal of the CO_2 that had been added to pyruvate earlier.

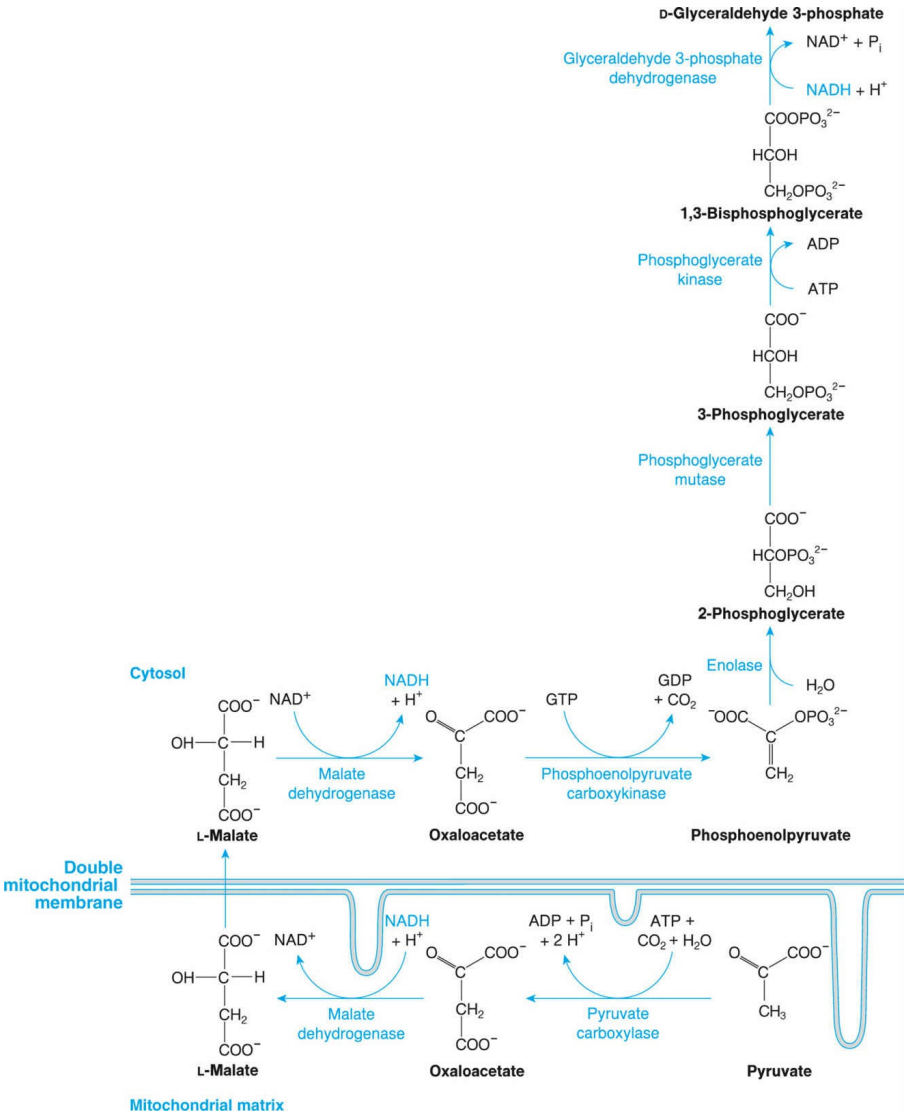


Figure 10.36 **Gluconeogenesis.** Gluconeogenesis produces glucose from pyruvate. The pathway is presented starting from the bottom right of this page and ending at the top of the facing page to help you compare it with glycolysis in figure 10.20. NADH is shown in color in three places to help you follow the discussion in the text.

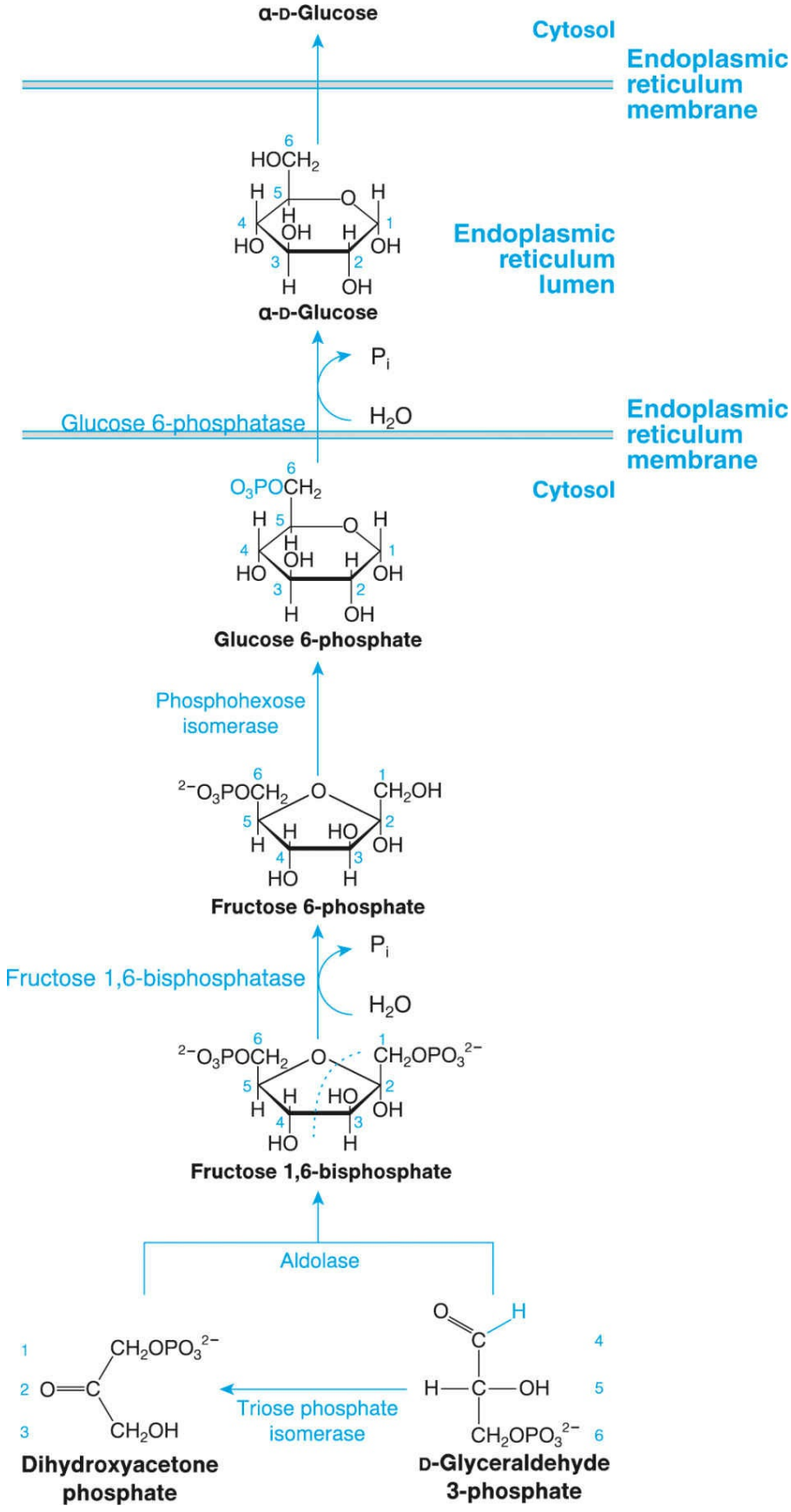

α-D-Glucose
Cytosol
Endoplasmic reticulum membrane
Endoplasmic reticulum lumen
α-D-Glucose
P_i
H_2O
Glucose 6-phosphatase
Endoplasmic reticulum membrane
Cytosol
Glucose 6-phosphate
Phosphohexose isomerase
Fructose 6-phosphate
P_i
Fructose 1,6-bisphosphatase
H_2O
Fructose 1,6-bisphosphate
Aldolase
Triose phosphate isomerase
Dihydroxyacetone phosphate
D-Glyceraldehyde 3-phosphate

Figure 10.36 *(continued)*

Phosphoenolpyruvate is subsequently converted into glyceraldehyde 3-phosphate through four reactions involving the hydrolysis of yet another ATP. The last of these reactions requires NADH as a reductant, which comes from the prior conversion of malate into oxaloacetate in the cytosol. This requirement explains the seemingly futile conversion of oxaloacetate into malate in the mitochondrial matrix and of malate into oxaloacetate in the cytosol: The benefit is the indirect transport of NADH from the mitochondria to the cytosol, which balances its consumption in a subsequent step of gluconeogenesis.

Part of glyceraldehyde 3-phosphate produced in the step just mentioned is isomerized to dihydroxyacetone phosphate, and the two isomers are joined to produce fructose 1,6-bisphosphate. Finally, fructose 1,6-bisphosphate yields glucose through two dephosphorylations and one isomerization. The final reaction in the pathway—that is, the conversion of glucose 6-phosphate into glucose—is catalyzed by **glucose 6-phosphatase**, an enzyme residing in the membrane of the endoplasmic reticulum. The reaction takes place on the side facing the lumen (the interior) of this membranous network. Specific transporters carry the substrate, glucose 6-phosphate, from the cytosol to the endoplasmic reticulum and the products, glucose and P_i, back to the cytosol.

The overall reaction of gluconeogenesis, excluding the accompanying hydrolyses of ATP and GTP, is the reversal of reaction 10.6.

In gluconeogenesis, glucose is synthesized from two pyruvates or other noncarbohydrate precursors.

$$\begin{aligned} &2\ \text{Pyruvate} + 2\ \text{NADH} + 4\ \text{H}^+ \rightleftharpoons \text{glucose} + 2\ \text{NAD}^+ \\ &\Delta G^{\circ\prime} = 31.9\ \text{kcal} \cdot \left(\text{mol glucose}\right)^{-1} \end{aligned} \qquad \text{(equation 10.22)}$$

Being highly endergonic, the reaction requires an input of free energy. Indeed, two ATP and one GTP (in all, three ~P) are hydrolyzed in the conversion of pyruvate into glyceraldehyde 3-phosphate. However, because we need one glyceraldehyde 3-phosphate and one dihydroxyacetone phosphate to synthesize one glucose, we need to start with two pyruvates and

spend six ~P. Thus, the overall reaction of gluconeogenesis, including the accompanying hydrolyses of ATP and GTP, becomes

$$2\ \text{Pyruvate} + 2\ \text{NADH} + 4\ \text{ATP} + 2\ \text{GTP} + 6\ \text{H}_2\text{O} \rightarrow \text{glucose} + 2\ \text{NAD}^+ + 4\ \text{ADP} + 2\ \text{GDP} + 6\ \text{P}_i + 2\ \text{H}^+ \qquad \Delta G^{\circ\prime} = -4.7\ \text{kcal} \cdot \left(\text{mol glucose}\right)^{-1} \qquad \text{(equation 10.23)}$$

The reaction is now exergonic and, hence, favored. Notice that the energy demand of gluconeogenesis is three times the energy yield of glycolysis. This difference confirms the statement, made in section 2.4, that a biosynthetic process is more expensive than the reverse degradation process is lucrative.

The main animal organs performing gluconeogenesis are the liver and kidneys. No glucose synthesis takes place in muscle, because muscle lacks glucose 6-phosphatase. This lack is not, however, a disadvantage, though it might seem so at first glance. To the contrary, the lack of the enzyme prevents glucose 6-phosphate (deriving from glycogen breakdown by way of glucose 1-phosphate) from becoming glucose and diffusing out of the muscle fibers. Glucose 6-phosphate does not cross the plasma membrane readily. Thus, any glucose 6-phosphate appearing in a muscle fiber is fated to be degraded or stored as glycogen.

In contrast to muscle, the liver has a basic mission to provide the rest of the body with glucose when glucose is not available through food. The liver accomplishes this mission partly through gluconeogenesis and partly through glycogenolysis, as we will see in section 10.28.

Although muscle cannot synthesize glucose, it can nevertheless synthesize glucose 6-phosphate from lactate or pyruvate by using all of the reactions of gluconeogenesis except the last one. Glucose 6-phosphate can then isomerize to glucose 1-phosphate, which can be used in glycogen synthesis, as described in section 10.3. Part of the lactate produced during hard exercise follows this route (figure 10.37) in the first minutes of recovery, when the lactate concentration in the cytosol is still high. The energy needed for this process is provided by aerobic ATP regeneration during the recovery period (to be addressed in detail in section 14.26).

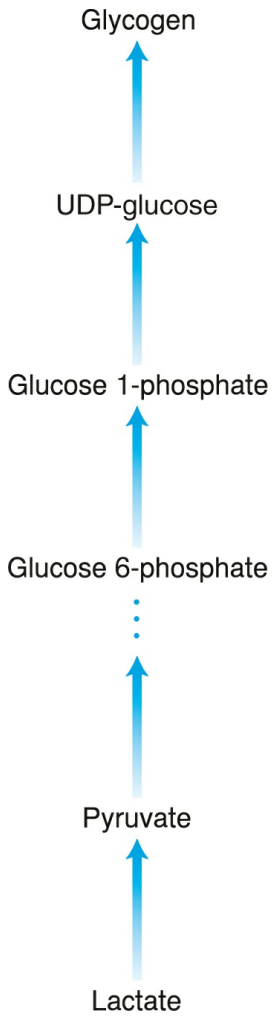


Figure 10.37 **Muscle glycogen from lactate.** Lactate produced in a hardworking muscle can be used to synthesize glycogen during recovery before leaving the muscle.

10.25 A Shortcut in Gluconeogenesis

When gluconeogenesis starts with lactate, rather than with pyruvate, in the liver and kidneys, it may follow a slightly different, and shorter, course than the one described in the previous section. As reviewed by Romana Stark and Richard Kibbey, this course involves the mitochondrial form of **phosphoenolpyruvate carboxykinase**, the enzyme catalyzing the conversion of oxaloacetate into phosphoenolpyruvate, rather than the cytosolic form shown in figure 10.36. Thanks to the mitochondrial enzyme,

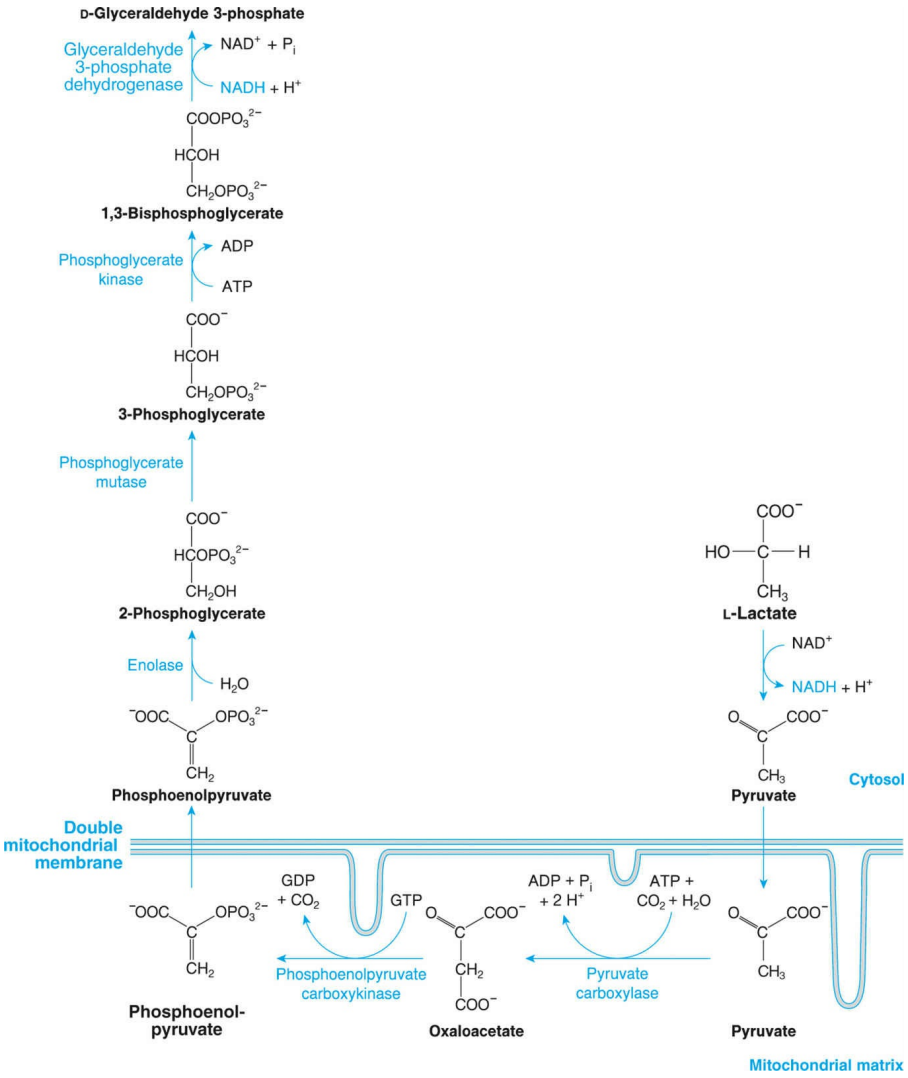


Figure 10.38 **An alternative route of gluconeogenesis.** When lactate serves as the source of glucose in the liver and kidneys, its conversion into pyruvate provides the cytosolic NADH needed for the formation of glyceraldehyde 3-phosphate. This provision, combined with the presence of a mitochondrial form of phosphoenolpyruvate carboxykinase in the human liver and kidneys, allows the synthesis of phosphoenolpyruvate in the mitochondria and its export to the cytosol with no involvement of malate or mitochondrial NADH. Only half of the gluconeogenic pathway is presented here, corresponding to the left part of figure 10.36. The rest of the pathway is identical to the right part of figure 10.36.

phosphoenolpyruvate can be formed in the mitochondrial matrix and then exit to the cytosol in order to continue along the gluconeogenic pathway (figure 10.38).

Why does gluconeogenesis take this shortcut (which obviates the interconversion of oxaloacetate and malate) only when starting from lactate

instead of all the time? Remember that the conversion of oxaloacetate into malate in the mitochondrial matrix and of malate into oxaloacetate in the cytosol ensures cytosolic NADH for the formation of glyceraldehyde 3-phosphate later. However, lactate provides the needed cytosolic NADH when it is converted into pyruvate (reaction 10.21). Thus, there is no need to consume mitochondrial NADH when it can be used in the electron-transport chain to power ATP resynthesis through oxidative phosphorylation.

Once again, one cannot help but notice what a difference is made by lactate. Just as the conversion of pyruvate into lactate eliminates the need for NAD^+ in glycolysis, the conversion of lactate into pyruvate eliminates the need for NADH in gluconeogenesis. At the basis of both observations lies the fact that, as you can see in reaction 10.19, the interconversion of glucose and lactate is "redox-neutral," that is, it does not involve any redox pair (such as NAD^+ and NADH).

10.26 Exercise Speeds Up Gluconeogenesis and Slows Down Glycolysis in the Liver

The rate of gluconeogenesis is controlled by a multitude of factors, several of which relate to exercise. To begin with, exercise increases the supply of gluconeogenic precursors, including lactate, which is produced in vigorously exercising muscles and carried to the liver by blood. In the following two chapters, we will see that the products of the augmented lipid and protein breakdown during exercise can also be channeled to glucose synthesis.

In terms of enzyme activity, the main means of controlling the rate of gluconeogenesis is the allosteric regulation of **fructose 1,6-bisphosphatase**. This enzyme catalyzes the third reaction before the end, which is one of the reactions that are not common to gluconeogenesis and glycolysis. (The corresponding glycolytic reaction is catalyzed by phosphofructokinase.)

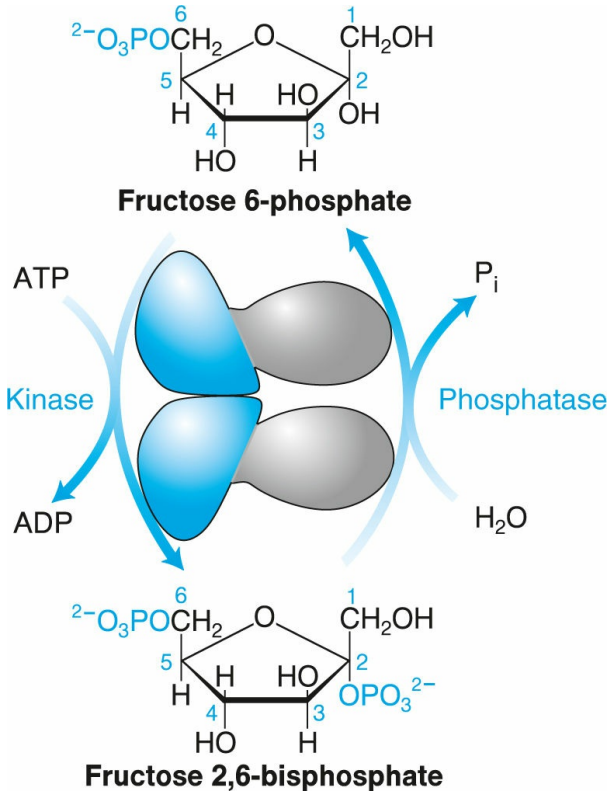


Figure 10.39 **The dimmer of glucose metabolism in the liver.** Fructose 2,6-bisphosphate, the main regulator of glucose metabolism in the liver, is synthesized and degraded through the catalytic actions of a bifunctional enzyme (at the center) possessing both a kinase and a phosphatase activity. The enzyme occurs in the form of a homodimer, with only the kinase domains (in color) of the two identical subunits in contact.

The fact that the main control point of gluconeogenesis is a reaction not shared with glycolysis is not mere chance. If the control point were a common reaction, then an activator of the (common) enzyme catalyzing the reaction would speed up both pathways, whereas an inhibitor would slow both down. This result would be futile, because the two pathways serve conflicting needs: One serves glucose synthesis at the expense of ATP, whereas the other serves ATP synthesis at the expense of glucose. In contrast, controlling reactions that are not common ensures the regulation of one pathway in a manner that is independent of—and usually opposite to—the regulation of the other.

Fructose 1,6-bisphosphatase and phosphofructokinase are controlled in an opposite fashion in the liver: When one is active, the other is inactive. The key to this opposite control is **fructose 2,6-bisphosphate**, a biomolecule

differing from fructose 1,6-bisphosphate (the intermediate compound of glycolysis and gluconeogenesis) in the position of one of the two phosphoryl groups (which is attached to C2 rather than C1). *Fructose 2,6-bisphosphate activates phosphofructokinase and inhibits fructose 1,6-bisphosphatase*.

Fructose 2,6-bisphosphate is synthesized from fructose 6-phosphate and is degraded into it through two different reactions (figure 10.39), just as fructose 1,6-bisphosphate and fructose 6-phosphate are interconverted through different reactions in glycolysis and gluconeogenesis. However, different pairs of enzymes catalyze the two interconversions. What is amazing is that the kinase catalyzing the formation of fructose 2,6-bisphosphate (called **phosphofructokinase 2**) and the phosphatase catalyzing its degradation (**fructose 2,6-bisphosphatase**) reside on the same polypeptide chain, which thus acts as a **bifunctional enzyme**.

The two enzyme activities are controlled by **glucagon**, a peptide hormone consisting of 29 amino acid residues and having a molecular mass of 3.5 kDa. Glucagon is synthesized and secreted by the so-called **α cells** in the pancreas. Its plasma concentration rises when the glucose concentration drops; the glucagon concentration also rises during exercise. The target organ of glucagon is the liver; the hormone does not seem to act on any other human organ.

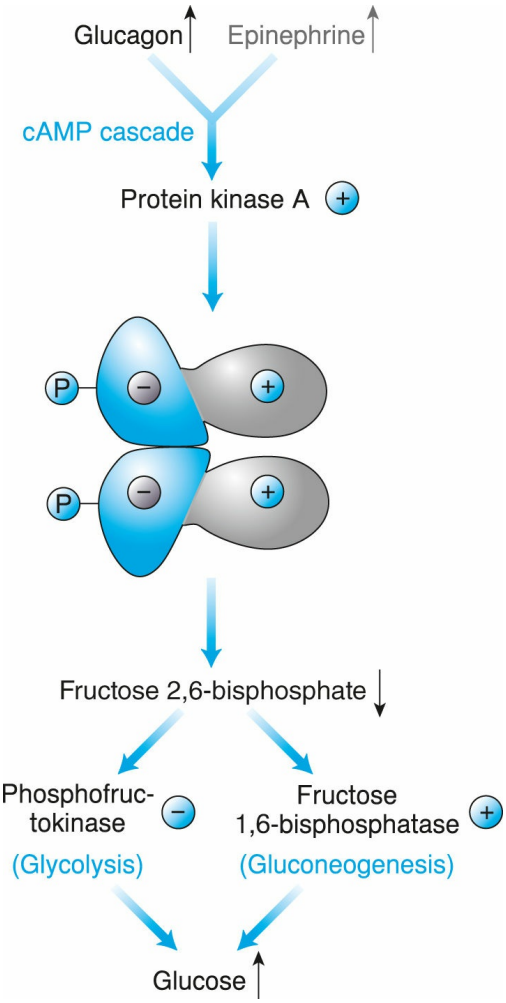


Figure 10.40 Hormonal control of glycolysis and gluconeogenesis in the liver. Glucagon and possibly epinephrine control the rates of glycolysis and gluconeogenesis in the liver through the cAMP cascade. The activated protein kinase A phosphorylates the bifunctional enzyme. Phosphorylation lowers the kinase activity and raises the phosphatase activity. Thus, the fructose 2,6-bisphosphate concentration drops, resulting in deceleration of glycolysis and acceleration of gluconeogenesis. The bottom line is the rise in glucose production in the hepatocytes.

In addition, the two enzyme activities and, in general, carbohydrate metabolism in the liver may by controlled by epinephrine during hard exercise, when its plasma concentration is high. We have considered epinephrine in section 10.5, where we saw that its increased secretion during exercise contributes to the acceleration of glycogenolysis in muscle through the cAMP cascade. However, evidence for an involvement of epinephrine in liver carbohydrate metabolism is controversial, as reviewed by Elijah Trefts

and colleagues.

Glucagon and possibly epinephrine affect liver function by binding to specific receptors at the surface of hepatocytes—the former to the **glucagon receptor** and the latter to the β-adrenergic receptor. After binding to the receptors, the two hormones act through the same signal transduction pathway: They activate adenylate cyclase (figure 10.14), thus eliciting a rise in the cytosolic [cAMP] and activation of PKA (figure 10.15). One of the proteins that the activated kinase phosphorylates is the bifunctional enzyme. Phosphorylation exerts opposite effects on its two activities (figure 10.40): It inhibits phosphofructokinase 2 and activates fructose 2,6-bisphosphatase. Because of this double effect, the concentration of fructose 2,6-bisphosphate drops. Therefore, phosphofructokinase is inhibited (slowing down glycolysis), and fructose 1,6-bisphosphatase is activated (speeding up gluconeogenesis). Glucose production rises, and more glucose exits the hepatocytes toward the bloodstream.

Through this complex mechanism, glucagon and possibly epinephrine ensure the increased supply of glucose from the liver to other tissues (often referred to as extrahepatic tissues) when called for by circumstances such as exercise or a low plasma glucose concentration. The key to this mechanism is fructose 2,6-bisphosphate, which can be viewed metaphorically as *the dimmer of glucose metabolism in the liver*. The stimulatory effect of exercise on gluconeogenesis and its inhibitory effect on glycolysis in the liver exemplify the third principle of exercise metabolism presented in section III.2: Exercise changes the metabolism of other organs in addition to muscle. More effects of exercise on liver metabolism are coming in section 10.28.

10.27 The Cori Cycle

Now that we have examined the effect of exercise on glucose metabolism in the liver, we can return to where we left off regarding lactate utilization in section 10.23. We have seen that, after glucose is anaerobically degraded in a vigorously exercising muscle, the lactate that is produced diffuses to blood, and part of it is taken up by the liver, which uses it to synthesize glucose.

Glucose is then released to blood and can be taken up by muscle, which

catabolizes it to resynthesize ATP. This action completes the **Cori cycle**, which connects muscle, blood, and the liver (figure 10.41). The cycle is named after the Czech American biochemists Carl and Gerty Cori, who proposed it in the 1940s. This route recycles lactate

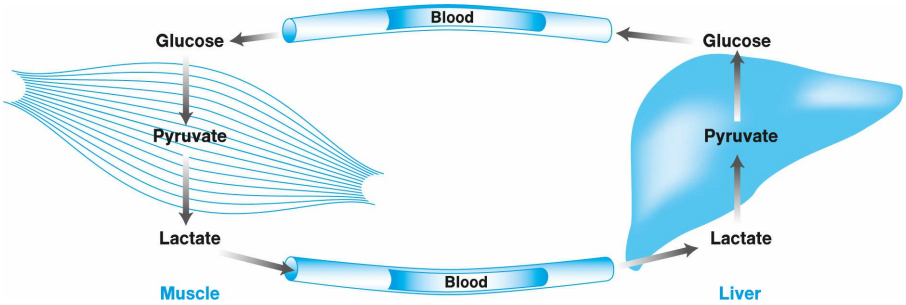


Figure 10.41 The Cori cycle. The cycle provides an exercising muscle with glucose synthesized in the liver from lactate, a product of the anaerobic operation of muscle.

to supply additional energy to muscle. The cycle operates at the expense of energy, since it has a net cost of four ~P (six spent in gluconeogenesis minus two gained in glycolysis) per glucose. However, one organ pays (the liver), and another gains (muscle). In this way, muscle shifts part of its metabolic burden to the liver, which appears to operate as an altruistic organ. In reality, of course, its function obeys the need for survival of the entire body, as does the function of every other organ.

10.28 Exercise Speeds Up Glycogenolysis and Slows Down Glycogenesis in the Liver

Glucose is produced in the liver not only through gluconeogenesis but also through glycogenolysis, since, as we discussed in section 10.2, the liver contains a substantial amount of glycogen (figure 10.4), which varies widely depending on diet and exercise. This wide variation results from the use of liver glycogen as a source of glucose for the rest of the body in case of glucose shortage. Let’s see how this process occurs.

Glycogen is broken down in the liver as described in section 10.4, that is,

into glucose 1-phosphate and glucose through the concerted action of phosphorylase and the debranching enzyme. The rate of the process is controlled by glucagon and possibly (during hard exercise) epinephrine. As we have seen in sections 10.5 and 10.26, the two hormones bind to the glucagon receptor and the β-adrenergic receptor, respectively, thus causing the stimulation of the cAMP cascade and activation of PKA. Through the subsequent phosphorylation of phosphorylase kinase and phosphorylase (figure 10.17), glucagon and possibly epinephrine end up accelerating glycogenolysis.

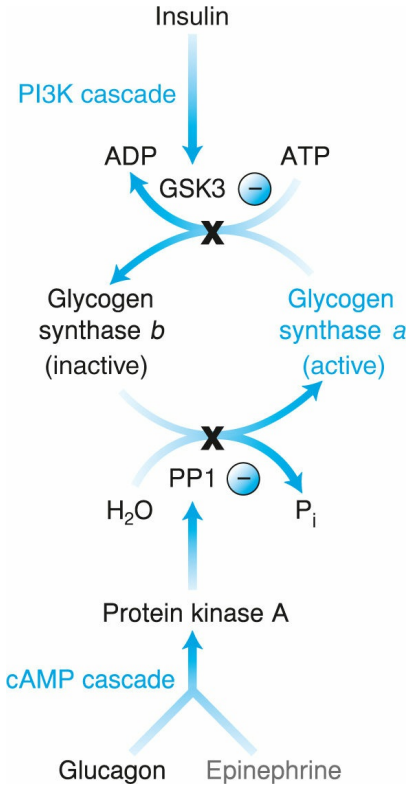


Figure 10.42 **Regulation of glycogen synthase activity in the liver.** Glycogen synthase activity is controlled by reversible phosphorylation thanks to the action of, mainly, GSK3 and PP1. Glucagon and possibly epinephrine slow down glycogenesis by inactivating PP1 through the cAMP cascade, thus blocking the activation of glycogen synthase (X). Insulin, on the other hand, speeds up glycogenesis by inactivating GSK3 and activating PP1 (not shown, for clarity) through the PI3K cascade, thus blocking the inactivation of glycogen synthase. (The PI3K cascade is presented in figure 10.48.)

In addition, glucagon and possibly epinephrine slow down glycogenesis in the liver by deactivating glycogen synthase. As mentioned in section 10.8,

the enzyme can be phosphorylated at multiple sites by multiple kinases. The kinase that plays the major regulatory role is **glycogen synthase kinase 3 (GSK3)**. When GSK3 phosphorylates glycogen synthase at specific serine residues, glycogen synthase assumes a usually inactive form: glycogen synthase *b* (figure 10.42). PP1, the protein phosphatase that dephosphorylates phosphorylase (section 10.5), dephosphorylates glycogen synthase too and converts it into the active *a* form. (Notice that dephosphorylation exerts opposite effects on the activities of phosphorylase and glycogen synthase.) Hormonal stimulation of the cAMP cascade results in inactivation of PP1 by PKA-catalyzed phosphorylation of the enzyme and of regulatory proteins that, when phosphorylated, inhibit PP1. This action prevents glycogen synthase from being in its active *a* form.

Figure 10.43 summarizes the ways in which exercise may affect liver glycogen metabolism.

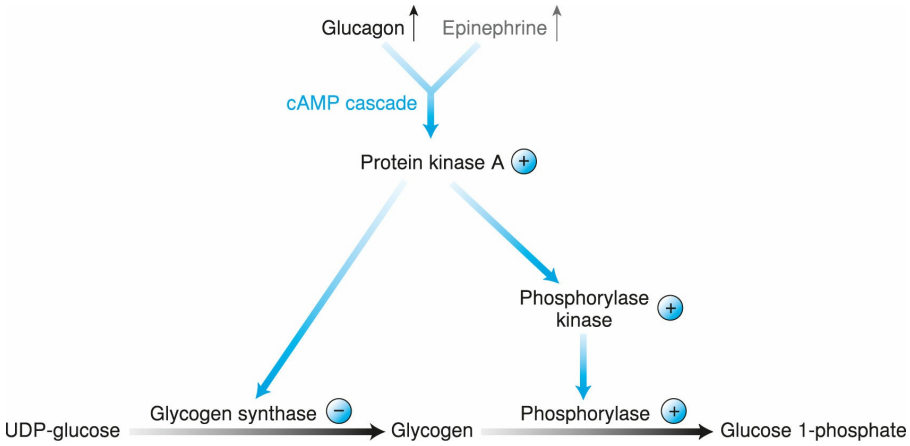


Figure 10.43 Control of liver glycogen metabolism in exercise. Both similarities and differences exist between the liver and muscle regarding the control of glycogen metabolism (cf. figure 10.18). Glucagon and possibly epinephrine, secreted in increased amounts during exercise, speed up glycogenolysis and slow down glycogenesis in the liver by stimulating the cAMP cascade. In contrast to muscle, P_i and AMP do not affect phosphorylase activity in the liver, because their concentrations do not change much.

The stimulatory actions of glucagon and possibly epinephrine on glycogenolysis in the liver cause a rise in the concentration of glucose 1-phosphate and, subsequently, glucose 6-phosphate. Unlike in muscle, degradation of glucose 6-phosphate through glycolysis is not favored in the

liver, because, as we saw in section 10.26, it is inhibited by epinephrine and glucagon. What is favored instead is the conversion of glucose 6-phosphate into glucose through the action of glucose 6-phosphatase (figure 10.44). Thus, the main fate of the glucose residues of liver glycogen consists of conversion into glucose and secretion to the bloodstream for energy supply to extrahepatic tissues.

Because epinephrine and glucagon raise the blood glucose concentration, they are termed **hyperglycemic hormones**.

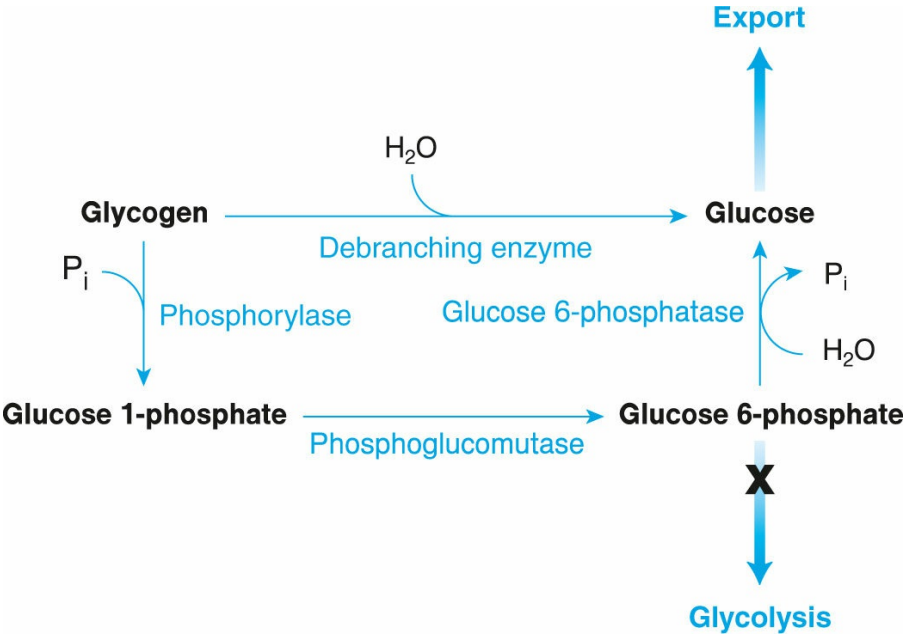


Figure 10.44 **The mission of liver glycogen.** As in muscle, glycogen in the liver is broken down into glucose and glucose 1-phosphate, which isomerizes to glucose 6-phosphate. Contrary to the case with muscle, however, these products do not follow the glycolytic pathway (cf. figure 10.20). Instead, glucose 6-phosphate is converted into glucose and exits the hepatocytes to nourish extrahepatic tissues.

10.29 Control of the Plasma Glucose Concentration

Maintaining a relatively stable plasma glucose concentration is vital to the functioning of the brain—and, hence, the entire body—because this organ uses glucose as its almost exclusive fuel and because it practically lacks

energy reserves (it contains little glycogen). Thus, the brain requires a continuous supply of glucose from blood. If the glucose concentration drops below a critical limit (usually set at 3.9 mmol · L^{-1}), **hypoglycemia** occurs, manifested by a variety of symptoms, including dizziness, confusion, visual disturbances, weakness, hunger, sweating, clumsiness, shakiness, and loss of consciousness. Glucose is also the exclusive energy source for the erythrocytes, which, incidentally, degrade it only anaerobically into lactate, since they lack mitochondria. On the other hand, excess glucose in plasma, or **hyperglycemia**, may cause lesions on the blood vessels in the long run; this result is one of the complications of uncontrolled diabetes, to be discussed in section 15.6.

> It is ironic that the erythrocytes carry a substance (oxygen) that they themselves are unable to exploit.

The body possesses homeostatic mechanisms that protect the plasma glucose concentration against drastic fluctuations. These mechanisms prevent

- an excessive rise in the plasma glucose concentration after a meal, during the so-called **postprandial state** (or fed state), and
- an excessive fall in the plasma glucose concentration after the absorption of nutrients, during the so-called **postabsorptive state** (or fasted state).

In sections 10.26 and 10.28 we explored how glucagon and possibly epinephrine control carbohydrate metabolism in the liver. The picture would be incomplete without **insulin**, the most powerful regulatory hormone of fuel metabolism. Insulin is a peptide hormone consisting of 51 amino acid residues and having a molecular mass of 5.8 kDa. It is synthesized and secreted by the **β cells** in the pancreas.

Insulin secretion depends primarily on the plasma glucose concentration. When the latter increases after a meal due to carbohydrate digestion and absorption, the rate of insulin secretion by the pancreas also increases. The hormone facilitates the entry of glucose and certain other monosaccharides to muscle and adipose tissue cells by promoting the movement of GLUT4 to

their plasma membrane (figure 10.21). When, thanks to this facilitation, the plasma glucose concentration drops, insulin secretion decreases. Thus, both the glucose and insulin concentrations display a biphasic response consisting of an ascending phase and a descending one (figure 10.45).

Because insulin lowers the blood glucose concentration, it is termed a **hypoglycemic hormone**.

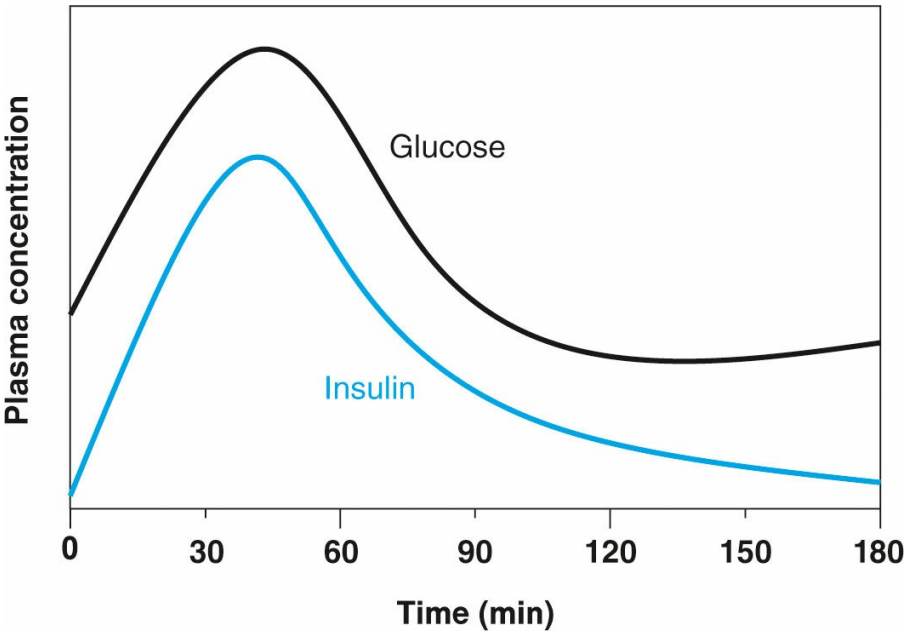


Figure 10.45 Plasma glucose and insulin interrelationship. After a carbohydrate-containing meal, our plasma glucose concentration rises, causing the insulin concentration to rise too. Insulin lowers the glucose concentration by letting glucose enter the muscles and adipose tissue. This, in turn, lowers the insulin concentration by signaling the pancreas to curb insulin secretion. The concentration scale is different for glucose and insulin: The concentration of glucose is much higher than that of insulin.

Apart from facilitating insulin uptake in muscle and adipose tissue, insulin exerts a plethora of effects on carbohydrate metabolism. In particular, insulin

- speeds up glycogenesis in muscle and the liver,
- slows down glycogenolysis in muscle and the liver,
- speeds up glycolysis in the liver, and
- slows down gluconeogenesis in the liver.

The bottom line of these effects is the lowering of the glucose concentration in biological fluids.

The actions of insulin are not confined to carbohydrate metabolism but extend to lipids and proteins. We will consider these actions in subsequent chapters.

Insulin exerts its versatile actions by binding to the **insulin receptor**, an integral protein of the plasma membrane of target cells. The insulin receptor is a tetramer of two α (130 kDa) and two β (95 kDa) subunits (figure 10.46). The α subunits are located extracellularly and bind insulin, whereas the β subunits span the membrane and possess protein kinase activity in their intracellular domains. This activity is dormant in the absence of insulin. When insulin binds to the receptor, a conformational change brings each β subunit inside the active site of the other. The subsequent phosphorylation of three key tyrosine residues (figure 10.47) in each β subunit by the other subunit (an effect termed **autophosphorylation**) activates the receptor, which can now phosphorylate a variety of cytosolic proteins.

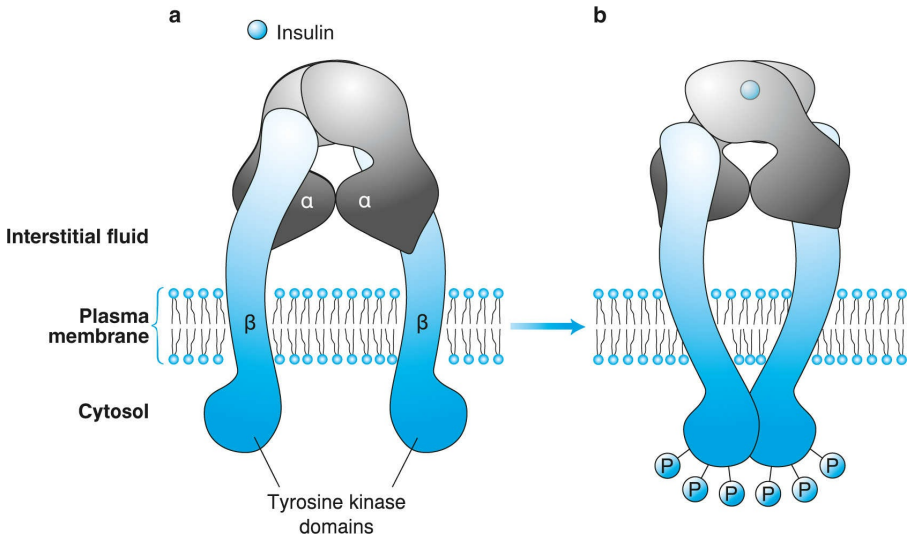


Figure 10.46 **Insulin receptor.** The insulin receptor consists of two α and two β subunits. It features an extracellular binding site for insulin and two intracellular domains possessing tyrosine kinase activity. A putative model of activation is shown. *(a)* When insulin is not bound, the receptor maintains a conformation that keeps the kinase domains apart. *(b)* When insulin binds, the kinase domains come close enough to phosphorylate each other. Phosphorylation of each domain at three tyrosine residues stimulates the protein kinase activity of the receptor.

Adapted by permission from C.C. Yip and P. Ottensmeyer, “Three-Dimensional Structural

Interactions of Insulin And Its Receptor," *Journal of Biological Chemistry* 278 (2003): 27329-27332.

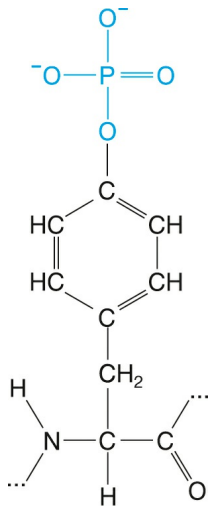
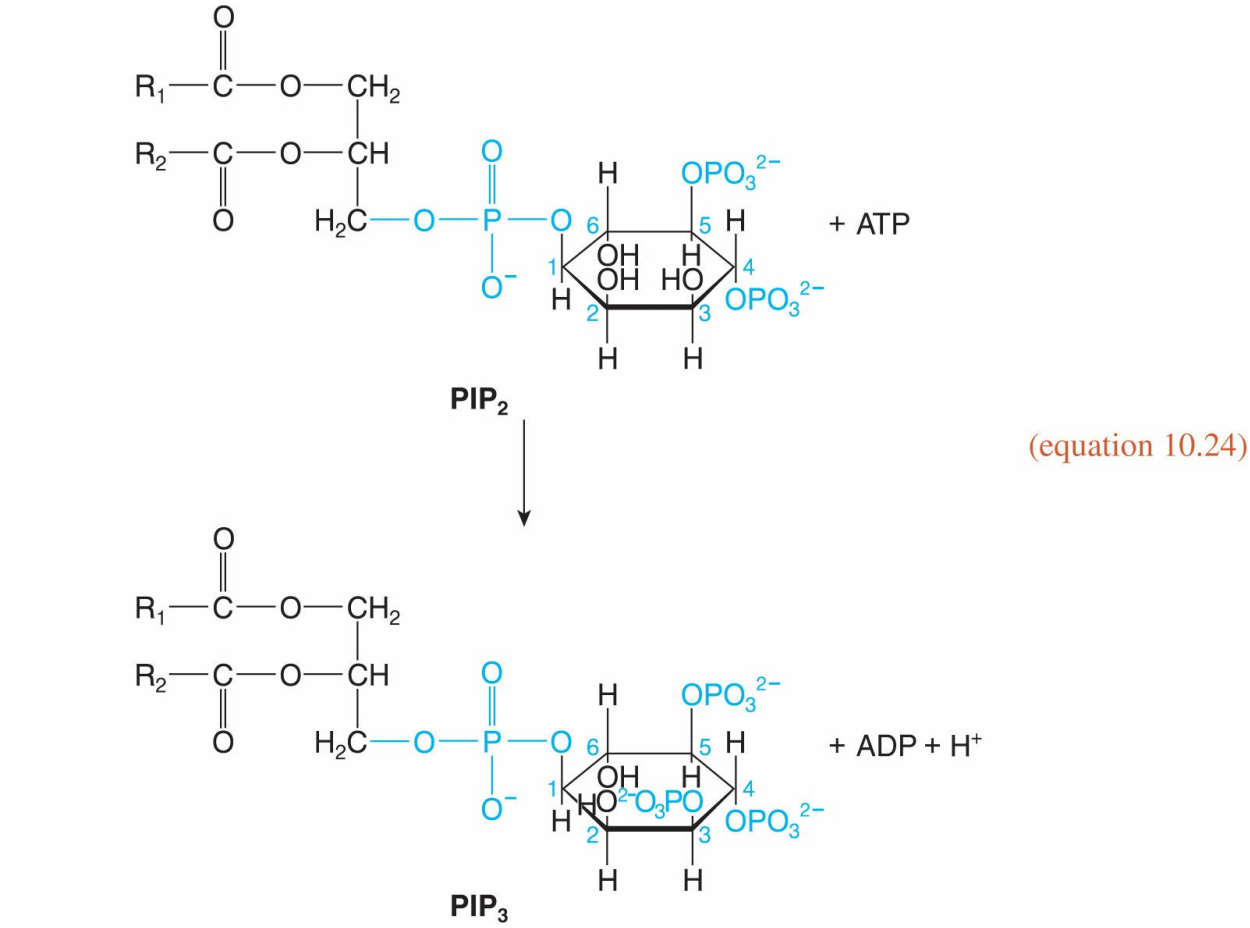


Figure 10.47 **Phosphotyrosine residue in a protein.**

One of the proteins is **insulin receptor substrate 1 (IRS1)**, which, when phosphorylated (also at Tyr residues), activates **phosphatidyl inositol 3 kinase**, or **phosphoinositide 3 kinase**, or **PI3K**. This enzyme initiates the **PI3K cascade** (figure 10.48) by catalyzing the phosphorylation of **phosphatidyl inositol 4,5-bisphosphate (PIP_2)**, a minor phospholipid of the plasma membrane and a derivative of phosphatidyl inositol (the latter introduced in figure 5.15).



(equation 10.24)

The product, **phosphatidyl inositol 3,4,5-trisphosphate (PIP_3)**, activates another protein kinase, **phosphoinositide-dependent kinase 1 (PDK1)**, which, in turn, phosphorylates and activates **protein kinase B (PKB)**, also referred to as **Akt**. PKB phosphorylates several proteins, one of which is GSK3. As we saw in the previous section, GSK3 phosphorylates and inactivates glycogen synthase. However, phosphorylation of GSK3 by PKB inactivates it, thus favoring the dephosphorylated, active, *a* form of glycogen synthase. Through this complex signal transduction pathway, insulin stimulates glycogenesis in muscle and the liver.

Insulin promotes glycogenesis in two additional ways. First, PKB, or Akt, triggers the movement of GLUT4 to the plasma membrane of muscle fibers (figure 10.21) through phosphorylation of a protein known as **Akt substrate of 160 kDa**, or **AS160**, and some additional steps that are not known in full detail. Thus, insulin promotes the entry of glucose, which is the raw material for glycogenesis. Second, insulin activates PP1 indirectly (by promoting the

phosphorylation of a regulatory protein that activates it). As mentioned in the previous section, PP1 dephosphorylates and, hence, activates glycogen synthase.

In closing this discussion of how insulin affects carbohydrate metabolism, let me note that the activated PP1 in the liver also dephosphorylates the bifunctional enzyme discussed in section 10.26. This action reverses the actions that glucagon and epinephrine exert on the enzyme through PKA activation: Now, as a result of insulin secretion and PP1 activation, glycolysis is accelerated, and gluconeogenesis is decelerated. To better appreciate these effects, try to redraw figure 10.40 to show the control of the two pathways by insulin.

Thanks to the opposite effects of glucagon and possibly epinephrine, on one hand, and insulin, on the other hand (figure 10.49), the glucose concentration in the plasma of a healthy person at rest is maintained between 4 and 6 mmol · L^{-1}. This condition is termed **euglycemia** (meaning “good blood glucose” in Greek). Assuming that the average plasma content of the human body is 5% for men and 4% for women, we can calculate that our model man of 75 kg and model woman of 62 kg (introduced in section 10.2), will have about 3.8 and 2.5 L of plasma, respectively. When we multiply these figures by 5 mmol · L^{-1} (the average plasma glucose concentration in euglycemia),

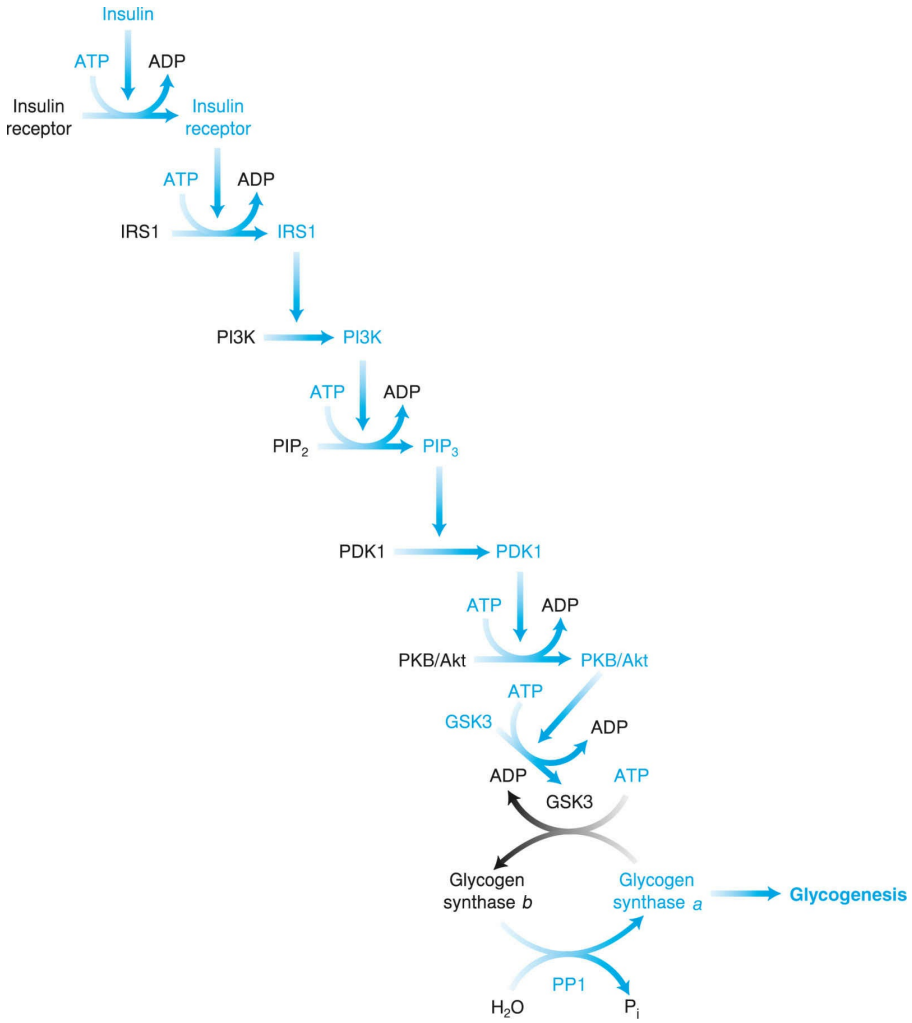


Figure 10.48 **Control of glycogenesis by the PI3K cascade.** Once activated by insulin, the insulin receptor in muscle and the liver phosphorylates IRS1, which activates PI3K, which in turn phosphorylates PIP_2 to form PIP_3. PIP_3 activates PDK1, which phosphorylates and activates PKB, or Akt, which phosphorylates and inactivates GSK3. This inactivation, combined with the activation of PP1 by insulin, promotes the dephosphorylation and activation of glycogen synthase and, hence, the stimulation of glycogenesis. Colored arrows denote activation; gray arrows denote inactivation.

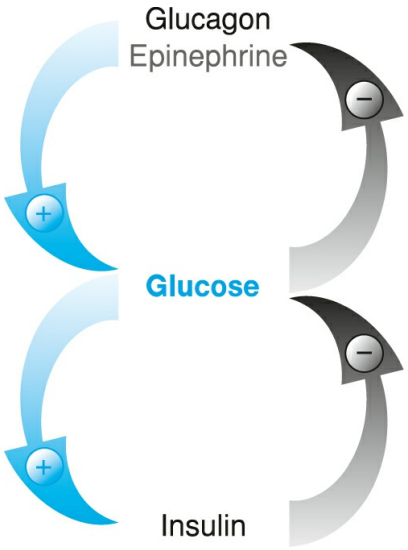


Figure 10.49 Plasma glucose homeostasis. The plasma glucose concentration is maintained within a narrow range mainly thanks to the opposing effects of the hormones insulin, glucagon, and possibly epinephrine. Insulin signals a decrease in plasma glucose, whereas glucagon and epinephrine signal an increase in plasma glucose. Conversely, glucose signals an increase in plasma insulin and a decrease in plasma glucagon and possibly epinephrine.

we get 19 and 12.5 mmol of glucose, respectively. Finally, when we multiply the new figures by the molecular mass of glucose (180 Da), we get 3,420 and 2,250 mg, or 3.42 and 2.25 g, respectively. These values are much lower than the glycogen content of the human body (375 for the man and 233 g for the woman, as calculated in section 10.2).

In addition to hormonal control, an important euglycemic mechanism is found in the direct effect of glucose on liver phosphorylase (but not muscle phosphorylase): When glucose binds to an allosteric site on phosphorylase *a* (the phosphorylated and usually active form), the enzyme is deactivated. In addition, glucose binding to phosphorylase *a* exposes the phosphorylation site to PP1, which then dephosphorylates and deactivates phosphorylase. Thus, *glucose controls the rate of glycogenolysis in the liver by exerting feedback inhibition on its own production.*

10.30 Control of the Plasma Glucose Concentration in Exercise

Exercise poses a serious threat to euglycemia: As already discussed (section 10.9), it boosts the rate of glucose uptake by muscle. To use a technical term appearing in research papers, exercise increases the **rate of disappearance** of glucose from plasma. However, this threat is not left unanswered. The lowering of the plasma glucose concentration is reflected in the cytosol of the hepatocytes, thus lifting the deactivation of phosphorylase according to the discussion at the end of the previous section. This action speeds up glycogenolysis in the liver.

In addition, glycogenolysis in the liver is accelerated by the increased secretion of glucagon and possibly epinephrine (sparked both by exercise and by the drop in the plasma glucose concentration), which also speeds up gluconeogenesis. Thus, more glucose enters the bloodstream to be taken up primarily (about 80%) by the exercising muscles. In technical terms, then, exercise increases the **rate of appearance**, as well as the rate of disappearance, of glucose in plasma.

So, what happens to the plasma glucose concentration during exercise? The answer depends on the difference between the rates of appearance and disappearance of glucose. If the difference is positive (that is, the rate of appearance is higher than the rate of disappearance), then plasma glucose concentration increases; if the difference is negative, then plasma glucose concentration decreases. The sign of the difference, in turn, depends largely on the exercise parameters. In general, light exercise does not affect plasma glucose considerably because it augments the rates of appearance and disappearance similarly, as summarized by Anirban Roy and Robert Parker.

Moderate-intensity or hard exercise tends to increase plasma glucose initially (due to a higher hormonal stimulation of release from the liver than stimulation of uptake by the active muscles) and then decrease it even to levels below baseline (that is, the value at rest) if exercise is very prolonged, since liver glycogen is gradually depleted. Near-maximal or maximal exercise, which cannot be sustained for more than a few minutes, increases plasma glucose, again due to a higher release than uptake. Glucose uptake may be further decreased, and plasma glucose thus increased, by the inhibition of hexokinase in muscle at high exercise intensities, as discussed in section 10.9.

A large liver glycogen depot is ensured—and the rate of appearance of glucose augmented—by carbohydrate intake before exercise that tends to lower the plasma glucose concentration, such as prolonged moderate-intensity or hard exercise. Carbohydrate intake during such exercise (often in the form of solutions) exerts a similar effect. We will return to this effect when we discuss the broader issue of how diet affects the selection of energy sources during exercise in section 14.12.

Control of the plasma glucose concentration during exercise does not appear to include a major role for the plasma insulin concentration. During moderate-intensity exercise, plasma insulin usually drops due to suppression of its secretion by epinephrine, as reviewed by Errol Marliss and Mladen Vranic. During hard or maximal exercise, plasma insulin has been reported to decrease, increase, or not change.

Because of the multitude of factors fighting over it during exercise, the plasma glucose concentration can reach extreme values. On one end, it can drop to as low as 2.5 mmol · L^{-1} after exhaustive exercise lasting a few hours, which depletes most of the liver glycogen. On the other end, it can exceed 10 mmol · L^{-1} after hard exercise of short duration.

10.31 Blood Lactate Accumulation

The blood lactate concentration is determined by its rates of appearance and disappearance (terms introduced in the previous section). The blood lactate concentration at rest is approximately 1 mmol · L^{-1} and reflects a so-called **steady state**, in which the rate of appearance (mainly from muscle and the erythrocytes) equals the rate of disappearance toward other tissues.

When we begin to exercise, the steady state of lactate is disturbed because its rate of appearance in blood rises. The rate of appearance relates to the concentration gradient between the cytosol of the active muscle fibers and plasma (see figure 10.35). It also relates to the blood flow through the exercising muscles. Both parameters are increased by exercise. As a result, lactate begins to accumulate in blood. One can measure a rise in its concentration as early as one minute after the onset of hard exercise. Let’s follow, then, the kinetics of blood lactate, that is, the progress of its

concentrations over time.

If we continue to exercise at a steady pace, the lactate concentration gradient between the cytosol of the active muscle fibers and plasma may diminish gradually as lactate begins to accumulate in plasma. The gradient diminishes further when exercise stops, because the rate of lactate production in muscle returns to baseline. In effect, the rate of lactate appearance in blood gradually declines.

Let’s consider now the rate of lactate disappearance from blood. As with the rate of appearance, the rate of disappearance relates to the concentration gradient between plasma and the cytosol of the cells that absorb lactate, such as the cells in nonexercising muscles, the heart, and the liver. It also relates to the blood flow through these organs. The gradient increases progressively during exercise as the blood lactate concentration rises; the blood flow as a whole does not change appreciably. In effect, then, the rate of lactate disappearance from blood gradually rises during exercise.

When the gradually declining rate of lactate appearance becomes equal to its gradually rising rate of disappearance, the blood lactate concentration stops rising, and the plot of the lactate concentration versus exercise time, or **lactate–time plot** for short (figure 10.50), peaks. A peak is usually measured between 2 and 10 min after the onset of exercise.

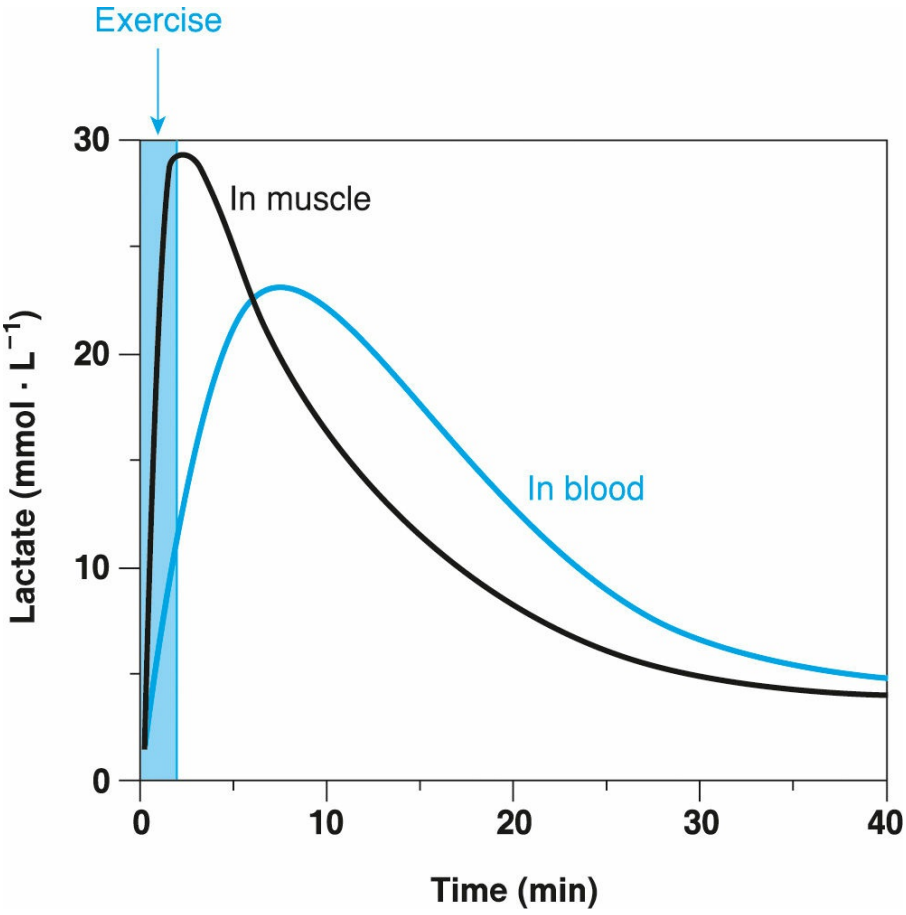


Figure 10.50 **Lactate–time plot.** A typical curve of the lactate concentration in an athlete's blood during and following short maximal exercise exhibits a peak within a few minutes after the start of exercise and then slowly returns to baseline. The lactate concentration in the exercising muscle peaks at the end of exercise, reaching a higher value than in blood.

The peak lactate concentration in blood is at best equal to, but usually lower than, the peak lactate concentration in the cytosol of the contracting muscle fibers. This is the case because by the time lactate peaks in blood, other organs have absorbed a considerable amount of it. Thus, it is not right to consider that, by measuring the peak lactate concentration in blood, we determine the peak concentration in the exercising muscles. Nevertheless, the peak concentration in blood relates to the peak concentration in muscle; and since it is easier to sample a little blood than a little muscle, the former is used widely as a simple marker of anaerobic carbohydrate catabolism. (Chapter 17 provides more detail on the utility of measuring blood lactate.)

10.32 Blood Lactate Decline

When we stop exercising, the muscle lactate concentration begins to fall (figure 10.50). The blood lactate concentration follows suit a few minutes later. Now the rate of lactate disappearance from blood begins to decline, as the concentration gradient between blood and the cytosol of the cells that absorb lactate gradually decreases. The lactate–time plot then converges slowly to the lactate concentration at rest. The **half-life** of blood lactate—that is, the time it takes for a given concentration to halve—is at least 12 min.

The rapid removal of lactate from the muscles after the end of exercise is often considered desirable in order for the muscles to regain full contractile capacity. In reality, lactate removal should not be a concern, because lactate is not a probable cause of fatigue, as discussed in section 10.20. On the contrary, keeping lactate in a muscle after the end of exercise might be beneficial, since lactate can serve as a source of glycogen (section 10.24 and figure 10.37; see also problem 13). What might be a justifiable concern is proton removal to reduce the acidity in muscle. However, since the MCT transports H^+ along with lactate (section 10.23 and figure 10.35), the possibility exists that anything speeding up lactate removal might speed up proton removal at the same time.

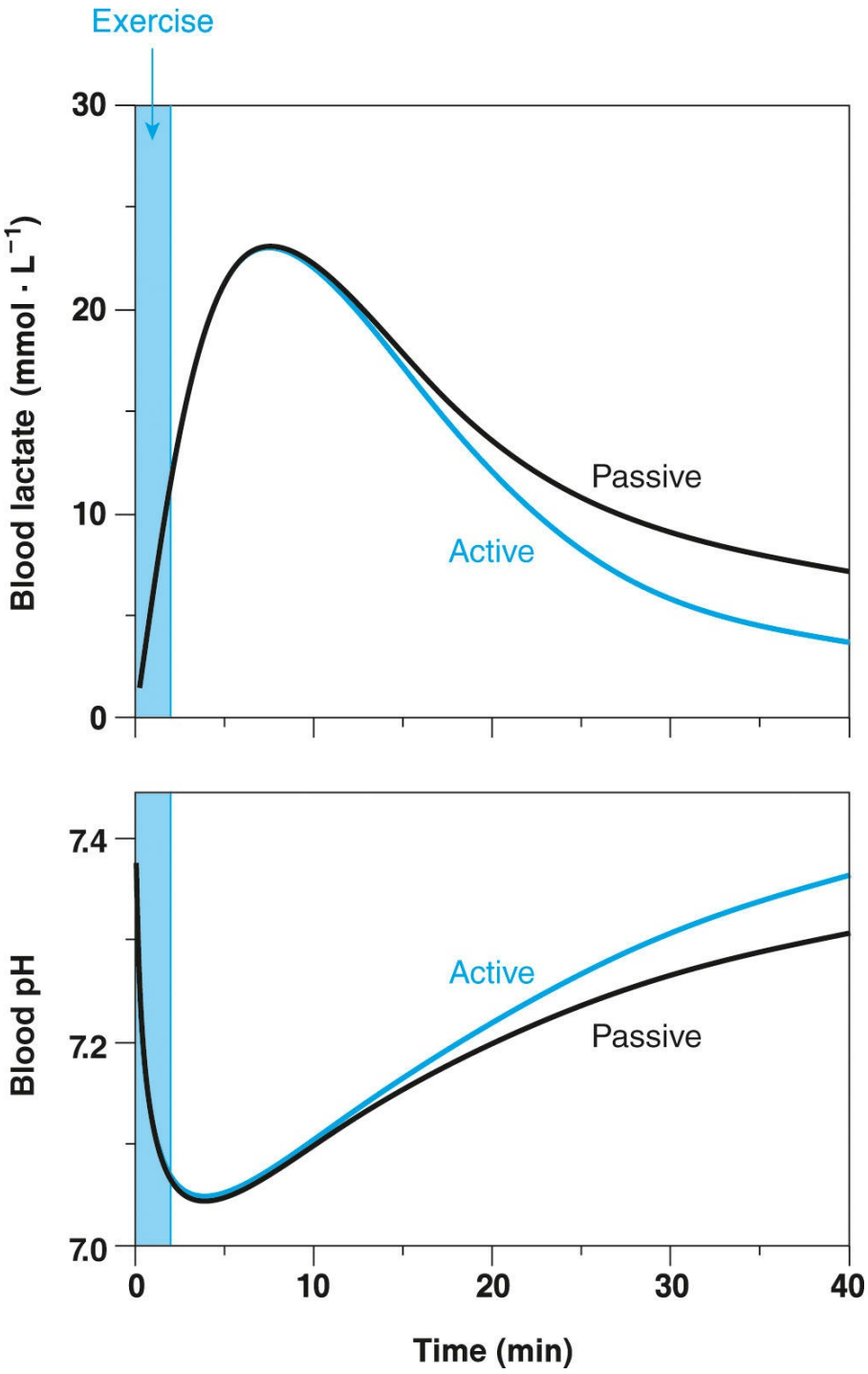


Figure 10.51 **Active versus passive recovery.** As compared with resting, performing light exercise in the recovery period after hard or maximal exercise speeds up the clearance of lactate from blood and the return of blood pH to its resting value. It is unclear, however, whether these effects provide any benefit for subsequent exercise.

To speed up lactate removal, athletes often continue to exercise lightly (at

around 20% to 40% of their $\dot{V}O_2max$), rather than rest, after hard or maximal exercise. This approach constitutes **active recovery** as opposed to **passive recovery**. Research has shown that active recovery speeds up the return of blood lactate and pH to baseline (figure 10.51). To explain this effect, researchers hypothesize that light exercise (powered mostly by energy from aerobic catabolism) causes the muscles to oxidize part of the lactate they have produced (as described in section 10.23), rather than release it into blood.

Another plausible explanation is that light exercise maintains increased blood flow through the active muscles, which expedites lactate removal. However, there is no agreement among studies as to whether active recovery speeds up the disappearance of *muscle* lactate or the restoration of resting muscle pH. Equally questionable is whether the mode of recovery (that is, active versus passive) influences performance during subsequent exercise bouts. Thus, it remains up to the athlete and coach to decide which recovery mode to apply.

10.33 “Thresholds”

In the previous two sections, we examined the blood lactate concentration as a function of time elapsed from the onset of exercise. Let’s consider now the blood lactate concentration as a function of exercise intensity by performing an experiment. Suppose we produce a certain amount of work (for example, we run 400 m) at a steady slow pace. At the end, we determine the peak lactate concentration in our blood through sequential sampling over a period of some minutes (see section 17.3 for details). We note this value, along with the value of exercise intensity, measured in any of the ways listed in section III.3 (such as running speed, heart rate, oxygen uptake, MET, RPE).

After resting for a while, we repeat the same task over and over at ever-increasing intensity. Again, at the end of each bout, we determine our peak blood lactate concentration. Finally, we plot the peak values that we obtained against exercise intensity. Doing so will give us a plot of lactate intensity versus exercise time (**lactate–intensity plot** for short), which is increasing and concave (figure 10.52).

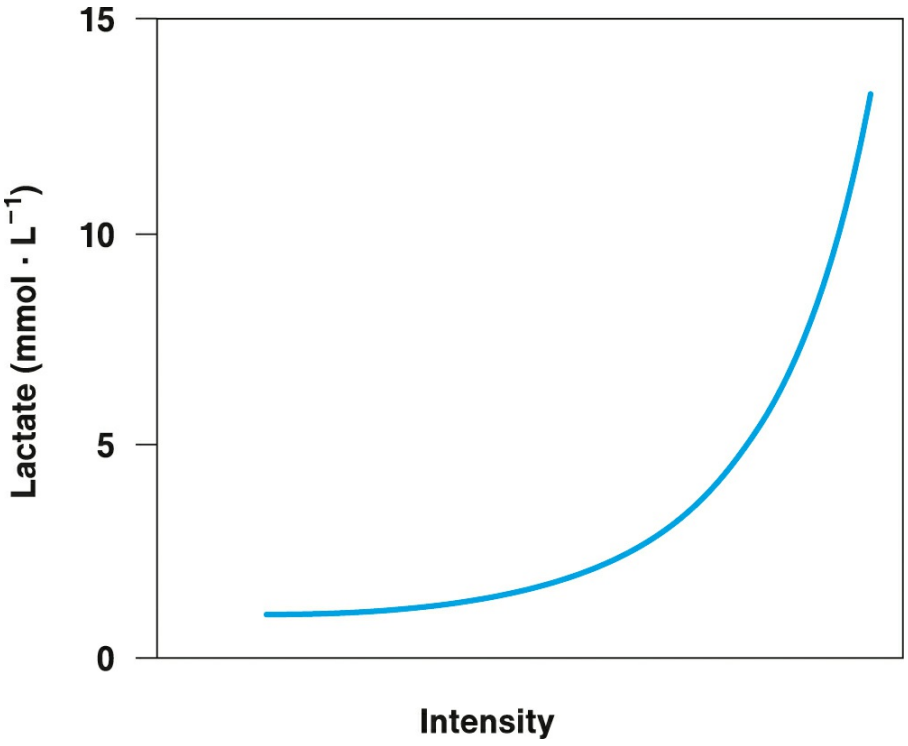


Figure 10.52 Lactate–intensity plot. The blood lactate concentration increases exponentially with exercise intensity.

Exercise scientists and coaches have striven over the past several decades to draw information on exercise metabolism and sport performance from lactate–intensity plots. Many efforts have focused on identifying points on the plot having the most useful coordinates (that is, exercise intensity and blood lactate concentration). To this end, several thresholds have been proposed, including, but not limited to, the following:

- The **lactate threshold** is defined as the exercise intensity above which blood lactate begins to rise, or the exercise intensity corresponding to a blood lactate concentration of 1 mmol · L^{-1} above baseline, or the exercise intensity corresponding to a blood lactate concentration of 2.5 mmol · L^{-1}.
- The **aerobic threshold** is defined as the exercise intensity above which blood lactate begins to rise (same as the first definition of the lactate threshold).
- The **anaerobic threshold** is defined as the exercise intensity above

which blood lactate begins to rise (same as the aerobic threshold and first definition of the lactate threshold) or the exercise intensity corresponding to a blood lactate concentration of 4 mmol · L^{-1}.

- The **onset of blood lactate accumulation (OBLA)** is defined as the exercise intensity corresponding to a blood lactate concentration of 4 mmol · L^{-1} (same as the second definition of the anaerobic threshold).
- The **individual anaerobic threshold** is defined as the maximal exercise intensity that can be maintained for some time (usually 30 to 40 min) without a continuous rise in the blood lactate concentration (also called the **maximal lactate steady state**). Alternatively, it is determined by drawing tangents to the slowly rising parts of the lactate-intensity plot, the fast-rising parts, or both, or by drawing other kinds of lines on the lactate–intensity plot.
- The **lactate inflection point** is defined as the exercise intensity at which the blood lactate concentration begins to rise exponentially. It is determined by drawing tangents as described for the individual anaerobic threshold.

For a more extensive discussion of the matter, see the review by Oliver Faude and associates, who identified as many as 25 lactate threshold concepts.

This is a rather confusing situation: A term is defined in different ways, and different terms mean the same thing. The reason for this confusion may be the questionable relevance of the terms listed. Take, for example, the anaerobic threshold, by far the most popular of the terms. The anaerobic threshold is often thought of as the point of transit from aerobic to anaerobic catabolism for energy production. However, such a thing does not exist: There is no state of exclusively aerobic or exclusively anaerobic muscle function. Even in the resting state, lactate is constantly produced anaerobically in the muscles at a slow rate. At the other extreme, the kinds of exercise that elicit maximal lactate concentrations in muscle and blood are accompanied by a burgeoning oxygen consumption to meet the increased demand for aerobic ATP resynthesis.

Thus, it is wrong to seek a point of passage from aerobic to anaerobic muscle function. Unfortunately, this misunderstanding is encouraged by the

term *threshold*, which denotes the border between two states. It is even wrong to think that the "anaerobic threshold" is the point of passage from *mostly* aerobic to *mostly* anaerobic muscle function: As I will show in section 14.7, aerobic catabolism continues to dominate over anaerobic catabolism at exercise intensities well above the "anaerobic threshold."

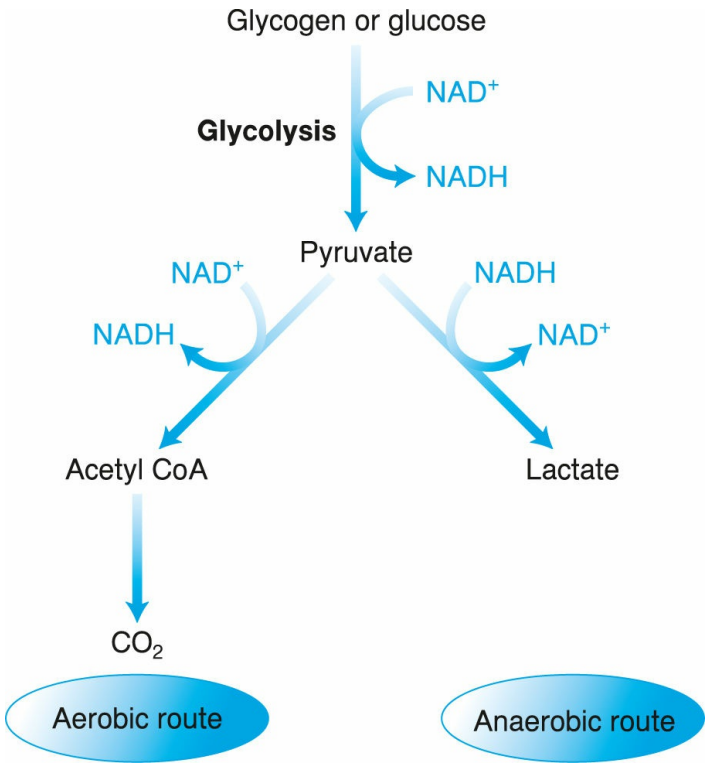


Figure 10.53 **Pyruvate's "dilemma."** Pyruvate can follow either an aerobic or an anaerobic metabolic route in muscle. The anaerobic route is gradually favored as exercise intensity increases, because it recycles NAD^+ needed in glycolysis, whereas the aerobic route requires additional NAD^+. However, there is no threshold of lactate production or accumulation.

How then should we interpret a lactate–intensity plot? As exercise intensity rises, a gradual increase occurs in the flow toward lactate and a gradual decrease in the flow toward acetyl CoA at the "pyruvate crossroads" in the exercising muscles (figure 10.53). These changes occur because of the enhanced conversion of NAD^+ into NADH in glycolysis and because the conversion of pyruvate into lactate absorbs NADH, whereas the conversion of pyruvate into acetyl CoA requires additional NAD^+. Since, as mentioned in section 10.31, the peak lactate concentration in muscle relates to the peak

lactate concentration in blood, the latter increases with increasing exercise intensity.

However, the gradual nature of the shift from aerobic to anaerobic function does not justify any *threshold* terminology, and this is even more the case because the blood lactate concentration reflects lactate removal as well as production. Even the OBLA, as defined earlier, is not justified as a term, because lactate begins to accumulate in blood as soon as it departs from the resting value of about 1 mmol · L^{-1}, that is, at intensities considerably lower than the one corresponding to 4 mmol · L^{-1}.

Is then the lactate–intensity plot useless? No! As I analyze in chapter 17, it can be used to program training by setting training intensities, evaluate aerobic endurance, and assess the effectiveness of endurance training programs. Certain intensities determined on the basis of the plot (such as the one corresponding to blood lactate of 4 mmol · L^{-1}) can provide helpful reference points, or landmarks, in training. It's just that it would be preferable to call things by their—admittedly lengthier—real names, such as "intensity corresponding to a blood lactate concentration of 4 mmol · L^{-1}," or to use abbreviations, such as "V4" (speed corresponding to 4 mmol · L^{-1}), rather than terms lacking biochemical or physiological relevance.

Summary

The carbohydrates that serve as energy sources during exercise include muscle and liver glycogen, as well as muscle, liver, and plasma glucose. Glycogen is synthesized from dietary glucose entering the muscle fibers and hepatocytes after a meal. Exercise speeds up glycogen breakdown, or glycogenolysis, in muscle thanks to the increased cytosolic P_i, Ca^{2+}, and AMP concentrations, as well as increased epinephrine secretion. In addition, increased glucagon secretion speeds up glycogenolysis and slows down glycogen synthesis, or glycogenesis, in the liver during exercise. In the liver, glucose 6-phosphate deriving from glycogenolysis is converted into glucose, which is exported to blood and enters extrahepatic tissues, including muscle. In

muscle, glucose 6-phosphate from glycogen and glucose from blood are broken down into pyruvate through the anaerobic pathway of glycolysis. Continuing on an aerobic route of degradation, pyruvate enters the mitochondria to become acetyl CoA and to end up in carbon dioxide through the citric acid cycle. This process yields a sum of about 30 ATP per glucose and 31 ATP per glucose residue of glycogen. Of these 30 or 31 ATP, 26 derive from oxidative phosphorylation, which is driven by the flow of electrons from NADH and $FADH_2$ to oxygen in the electron-transport chain.

Despite yielding high amounts of ATP, the aerobic breakdown of carbohydrates does so at a rate that lags behind the rate of ATP consumption during hard exercise. Part of pyruvate is then converted into lactate anaerobically (more so in type II than in type I muscle fibers), thus regenerating NAD^+ needed for glycolysis. The harder the exercise is, the more lactate is produced. The anaerobic breakdown of carbo hydrates yields only two ATP per glucose and three ATP per glucose residue of glycogen. Nevertheless, both are produced very fast, thus rendering the anaerobic route the major energy source in maximal efforts lasting about 7 s to 1 min. The aerobic route predominates in maximal exercise tasks lasting more than 1 min and in hard or moderate-intensity exercise tasks of any duration. However, there is never a state of exclusively aerobic or exclusively anaerobic energy production. Hence, there is hardly any relevance for the term *anaerobic threshold*, which is often meant to mark the transition from aerobic to anaerobic catabolism. Likewise, there is little evidence that lactate is a fatigue-causing substance or that lactate production during exercise is due to a lack of oxygen.

Lactate, produced in high amounts in hardworking muscles, can either be used for glycogen resynthesis in the early recovery period or exit to the bloodstream and enter other tissues and organs. These tissues and organs either oxidize lactate to

regenerate ATP (a process taking place in the heart and resting muscles) or use it to synthesize glucose through the pathway of gluconeogenesis (taking place in the liver and kidneys). Glucose synthesis requires 6 ~P and is accelerated during exercise thanks to the increased secretion of glucagon and possibly epinephrine. This acceleration, along with the acceleration of glycogenolysis in the liver and—if applied—carbohydrate intake during exercise, usually maintains euglycemia despite increased glucose uptake by the exercising muscles. Insulin, on the other hand, maintains euglycemia in the postprandial state by lowering plasma glucose and promoting glycogenesis.

Problems and Critical Thinking Questions

1. Which substances can speed up glycogenolysis in muscle during exercise through an increase in their concentrations?
2. Can glycolysis begin in vitro if there is no ATP present?
3. (Integrative problem) The fact that 1,3-bisphosphoglycerate and phosphoenolpyruvate synthesize ATP in the 7th and 10th reactions of glycolysis by transferring their phosphoryl groups to ADP renders them compounds of high phosphoryl-transfer potential. Indeed, the $\Delta G^{\circ\prime}$ of their hydrolysis are –10.7 and –12.9 kcal · mol^{-1}, respectively. Where do these values place them in the list of problem 10 in chapter 9?
4. Which substances can speed up glycolysis in muscle during exercise through an increase in their concentrations, and which through a decrease in their concentrations?
5. (Integrative problem) Is the flow of protons from the mitochondrial matrix to the intermembrane space through the complexes of the electron-transport chain a case of passive or active transport? What about the flow of protons from the intermembrane space to the mitochondrial matrix through ATP synthase?

6. Baker's yeast contains the aerobic microorganism *Saccharomyces cerevisiae.* How does the dough rise?
7. Calculate the $\Delta G^{\circ\prime}$ of the anaerobic glucose catabolism (reaction 10.19).
8. Compare the aerobic to the anaerobic carbohydrate breakdown in muscle in terms of amount of ATP produced, rate of ATP resynthesis, and exercise tasks in which each of the two predominates.
9. Suppose you run 400 m at such a speed that 50% of the energy from the breakdown of muscle glycogen is produced aerobically and 50% is produced anaerobically. Now suppose you run the same distance at a higher speed, resulting in 48% of the energy from glycogen being produced aerobically and 52% anaerobically. Assuming you need the same total amount of ATP from glycogen for both runs, answer the following questions:
    a. What is the proportion of glycogen being broken down aerobically to glycogen being broken down anaerobically in the first run?
    b. What is the proportion of glycogen being broken down aerobically to glycogen being broken down anaerobically in the second run?
    c. Is the difference between the answers to questions *a* and *b* proportional to the change in the energy mixture? Explain!
    (*Hint:* You may find it easy to use an arbitrary amount of ATP, say 1 mol, as the total amount of ATP from glycogen needed for each run, although this will not affect your results.)
10. In the movie *A Few Good Men,* starring Tom Cruise, a marine dies of "lactic acidosis," and two fellow soldiers are accused of murder. Asked before the court what lactic acidosis is, a physician says, "If the muscles and all the cells

in the body burn sugar instead of oxygen, lactic acid is produced." Correct him!

11. Which branch of glucose metabolism and which branch of glycogen metabolism are stimulated in the liver during exercise? Through which mechanisms?
12. Which events tend to decrease and which events tend to increase the plasma glucose concentration during exercise?
13. In a study by Daihyuk Choi and coworkers, the participants performed cycling exercise followed by either active or passive recovery. Needle biopsy samples from the vastus lateralis muscle—performed before exercise, immediately after exercise, and at the end of the recovery period—revealed that, on average, the muscle glycogen content decreased by 49 mmol glucosyl units per kilogram during exercise. During active recovery, it decreased by another 6 $mmol \cdot kg^{-1}$, whereas, during one hour of passive recovery, it increased by 15 $mmol \cdot kg^{-1}$. Answer the following questions:
    a. On the basis of this finding, which of the two—active or passive recovery—is preferable?
    b. Why do you think muscle glycogen decreased during active recovery?
    c. Why do you think muscle glycogen increased during passive recovery?
    d. If the sole source for glycogen resynthesis during passive recovery was lactate produced during exercise, how much of that lactate was used?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

carbohydrate

carbohydrate digestion

starch
α-amylase
amylose
amylopectin
maltose
maltotriose
α-dextrin, limit dextrin
α-D-glucose
maltase
α-dextrinase, limit dextrinase
microvillus
enterocyte
intestinal lumen
villus
sucrase
lactase
carbohydrate absorption
glycogen
carbohydrate distribution
cellulose
cellulase
hepatocyte
glycogenesis
uridine triphosphate, UTP
α-D-glucose 1-phosphate
sugar phosphate
uridine diphosphate glucose, UDP-glucose
UDP-glucose pyrophosphorylase
pyrophosphatase
glycogen synthase

glycogenin
glycogen-branching enzyme, branching enzyme
glycogenolysis
phosphorolysis
glycogen phosphorylase, phosphorylase
glycogen-debranching enzyme, debranching enzyme
homodimer
phosphorylase kinase
phosphoserine
dephosphorylation
protein phosphatase 1, PP1
phosphatase
calmodulin
epinephrine, adrenaline
catecholamine
norepinephrine, noradrenaline
adrenal medulla
sympathoadrenergic system
adrenergic receptor
β-adrenergic receptor
adenylate cyclase
cyclic adenylate, cyclic AMP, cAMP
G protein
protein kinase A, PKA
cAMP cascade
intermyofibrillar
intramyofibrillar
subsarcolemmal
glycolysis
pyruvate

glucose 6-phosphate
fructose 6-phosphate
fructose 1,6-bisphosphate
dihydroxyacetone phosphate
D-glyceraldehyde 3-phosphate
1,3-bisphosphoglycerate
3-phosphoglycerate
2-phosphoglycerate
phosphoenolpyruvate
hexokinase
glucose transporter, GLUT
GLUT4
phosphofructokinase
pyruvate kinase
mitochondrial matrix
mitochondrial crista
mitochondrial reticulum
coenzyme A, CoA
acetyl coenzyme A
acetyl group
acyl group
pyruvate dehydrogenase complex
pyruvate dehydrogenase
citric acid cycle, Krebs cycle, tricarboxylic acid cycle
oxaloacetate
citrate
dehydration
hydration
isocitrate
α-ketoglutarate, 2-oxoglutarate

succinyl CoA
succinate
fumarate
L-malate
citrate synthase
isocitrate dehydrogenase
α-ketoglutarate dehydrogenase
α-ketoglutarate dehydrogenase complex
NADH-Q oxidoreductase, NADH dehydrogenase, complex I
succinate dehydrogenase, complex II
Q-cytochrome *c* oxidoreductase, cytochrome reductase, complex III
cytochrome *c* oxidase, complex IV
ubiquinone, coenzyme Q, Q
cytochrome *c*
electron-transport chain, respiratory chain
molecular shuttle
oxidative phosphorylation
ATP synthase, F_0F_1 ATPase
ATP–ADP translocase
phosphate carrier
chemiosmotic hypothesis
L-lactate
lactate dehydrogenase
anaerobic carbohydrate catabolism
lactate tolerance
anaerobic lactic mechanism
anaerobic alactic mechanism
monocarboxylate transporter, MCT
gluconeogenesis
glucose 6-phosphatase

phosphoenolpyruvate carboxykinase
fructose 1,6-bisphosphatase
fructose 2,6-bisphosphate
phosphofructokinase 2
fructose 2,6-bisphosphatase
bifunctional enzyme
glucagon
α cell
glucagon receptor
Cori cycle
glycogen synthase kinase 3, GSK3
hyperglycemic hormone
hypoglycemia
hyperglycemia
postprandial state
postabsorptive state
insulin
β cell
hypoglycemic hormone
insulin receptor
autophosphorylation
insulin receptor substrate 1, IRS1
phosphatidyl inositol 3 kinase, phosphoinositide 3 kinase, PI3K
PI3K cascade
phosphatidyl inositol 4,5-bisphosphate, PIP_2
phosphatidyl inositol 3,4,5-trisphosphate, PIP_3
phosphoinositide-dependent kinase 1, PDK1
protein kinase B, PKB, Akt
Akt substrate of 160 kDa, AS160
euglycemia

rate of disappearance
rate of appearance
steady state
lactate–time plot
half-life
active recovery
passive recovery
lactate–intensity plot
lactate threshold
aerobic threshold
anaerobic threshold
onset of blood lactate accumulation, OBLA
individual anaerobic threshold
maximal lactate steady state
lactate inflection point

References and Suggested Readings

Areta JL, Hopkins WG (2018). Skeletal muscle glycogen content at rest and during endurance exercise in humans: A meta-analysis. *Sports Medicine* 48:2091-2102.

Choi D, Cole KJ, Goodpaster BH, Fink WJ, Costill DL (1994). Effect of passive and active recovery on the resynthesis of muscle glycogen. *Medicine and Science in Sports and Exercise* 26: 992-996 (cited in problem 13).

Faude O, Kindermann W, Meyer T (2009). Lactate threshold concepts: How valid are they? *Sports Medicine* 39: 469-490.

Ferguson BS, Rogatzki MJ, Goodwin ML, Kane DA, Rightmire Z, Gladden LB (2018). Lactate metabolism: Historical context, prior misinterpretations, and current understanding. *European Journal of Applied Physiology* 118: 691-728.

He F, Li J, Liu Z, Chuang CC, Yang W, Zuo L (2016). Redox

mechanism of reactive oxygen species in exercise. *Frontiers in Physiology* 7: 486.

Marchand I, Chorneyko K, Tarnopolsky M, Hamilton S, Shearer J, Potvin J, Graham TE (2002). Quantification of subcellular glycogen in resting human muscle: Granule size, number, and location. *Journal of Applied Physiology* 93: 1598-1607.

Marchand I, Tarnopolsky M, Adamo KB, Bourgeois JM, Chorneyko K, Graham TE (2007). Quantitative assessment of human muscle glycogen granules size and number in subcellular locations during recovery from prolonged exercise. *Journal of Physiology* 580.2: 617-628.

Marliss EB, Vranic M (2002). Intense exercise has unique effects on both insulin release and its roles in glucoregulation: Implications for diabetes. *Diabetes* 51: S271-S283.

Mul JD, Stanford KI, Hirshman MF, Goodyear LJ (2015). Exercise and regulation of carbohydrate metabolism. *Progress in Molecular Biology and Translational Science* 135: 17-37.

Nielsen J, Wojtaszewski J (2004). Regulation of glycogen synthase activity and phosphorylation by exercise. *Proceedings of the Nutrition Society* 63: 233-237.

Ørtenblad N, Nielsen J (2015). Muscle glycogen and cell function—Location, location, location. *Scandinavian Journal of Medicine and Science in Sports* 25 (Suppl. 4): 34-40.

Parker GJ, Koay A, Gilbert-Wilson R, Waddington LJ, Stapleton D (2007). AMP-activated protein kinase does not associate with glycogen α-particles from rat liver. *Biochemical and Biophysical Research Communications* 362: 811-815.

Picard M, White K, Turnbull DM (2013). Mitochondrial morphology, topology, and membrane interactions in skeletal muscle: A quantitative three-dimensional electron microscopy study. *Journal of Applied Physiology* 114: 161-171.

Richardson RS, Noyszewski EA, Leigh JS, Wagner PD (1998). Lactate efflux from exercising human skeletal muscle: Role of

intracellular pO_2. *Journal of Applied Physiology* 85: 627-634.

Richardson RS, Wary C, Wray DW, Hoff J, Rossiter HB, Layec G, Carlier PG (2015). MRS evidence of adequate O_2 supply in human skeletal muscle at the onset of exercise. *Medicine and Science in Sports and Exercise* 47: 2299-2307.

Richter EA, Hargreaves M (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews* 93: 993-1017.

Roy A, Parker RS (2007). Dynamic modeling of exercise effects on plasma glucose and insulin levels. *Journal of Diabetes Science and Technology* 1: 338-347.

Schiaffino S, Reggiani C (2011). Fiber types in mammalian skeletal muscles. *Physiological Reviews* 91: 1447-1531.

Stark R, Kibbey RG (2014). The mitochondrial isoform of phosphoenolpyruvate carboxykinase (PEPCK-M) and glucose homeostasis: Has it been overlooked? *Biochimica et Biophysica Acta* 1840: 1313-1330.

Trefts E, Williams AS, Wasserman DH (2015). Exercise and the regulation of hepatic metabolism. *Progress in Molecular Biology and Translational Science* 135: 203-225.

Wilson DF (2017). Oxidative phosphorylation: Regulation and role in cellular and tissue metabolism. *Journal of Physiology* 595: 7023-7038.

Zeqiraj E, Sicheri F (2015). Getting a handle on glycogen synthase—Its interaction with glycogenin. *Molecular Aspects of Medicine* 46: 63-69.

CHAPTER 11

Lipid Metabolism in Exercise

Learning Objectives

After reading this chapter, you should be able to do the following:

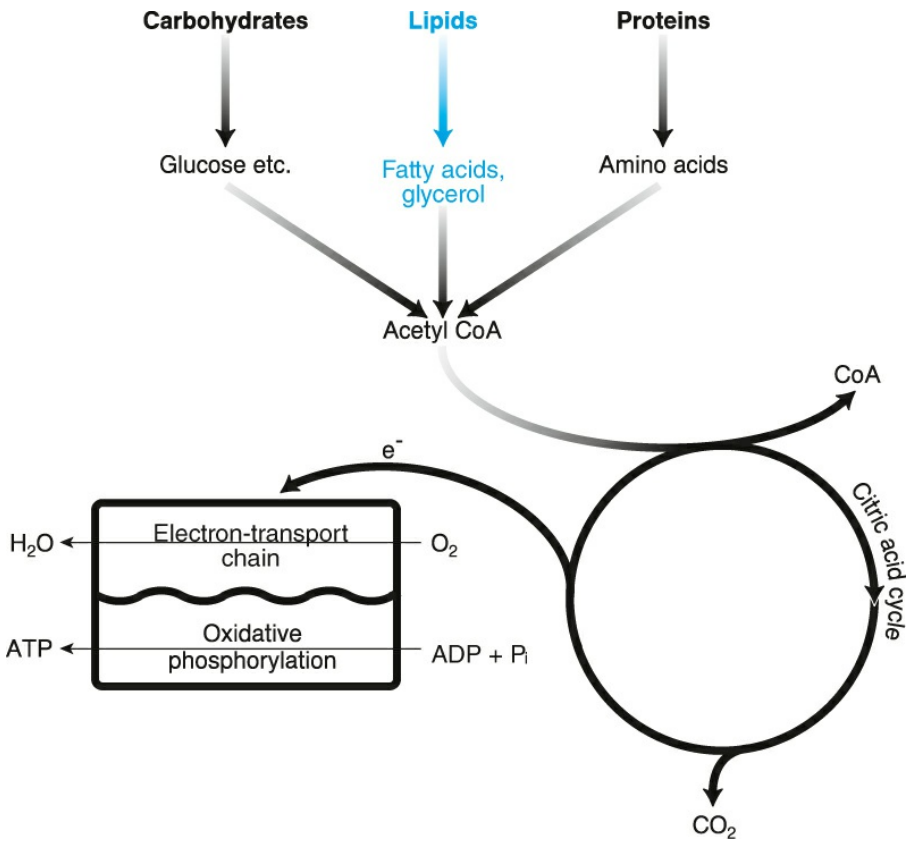
- Discuss how the body processes dietary fat and identify common characteristics in the processing of the different lipid classes.
- Describe the fat content of the human body and compare it with the carbohydrate content.
- Describe the process of lipolysis and the factors that speed it up during exercise.
- Explain the steps in fatty acid degradation and how exercise speeds it up.
- Describe the energy yield of fatty acid degradation, the rate of ATP resynthesis from it, and the activities in which it predominates.
- Calculate lipid use in exercise and contrast it with carbohydrate use.
- Explain how fatty acids are synthesized.
- List the major classes of lipoproteins, describe their constituents, and discuss the biological functions they serve.
- Describe how exercise affects the plasma concentration of the

lipids present in the various lipoproteins.
- Discuss how ketone bodies are produced and how exercise affects their plasma concentration.

In chapter 5, we saw that lipids constitute a diverse class of biological compounds characterized by low solubility in water. Three of the lipid categories that we examined are of interest in exercise metabolism: fatty acids, triacylglycerols, and steroids. Fatty acids and triacylglycerols serve as important energy sources during exercise. As we will see, they yield the highest amounts of ATP among energy sources, albeit at low rates. In addition, we will deal with triacylglycerols and cholesterol (the parent steroid) as compounds whose concentrations in blood relate to cardiovascular disease and are modified by exercise.

11.1 Triacylglycerol Digestion, Absorption, and Distribution

Most of the **lipids** in our bodies derive from dietary fat, the overwhelming majority of which (90% to 95%) is in the form of **triacylglycerols**. The rest is mainly **phospholipids**, and less than 0.5% is accounted for by **cholesterol** (in either free or esterified form; figure 5.17). **Fatty acids**, in their free, nonesterified form, are present in negligible amounts in our diet. The fate of dietary triacylglycerols is the topic of this section.



You are here: fat digestion, absorption, and distribution.

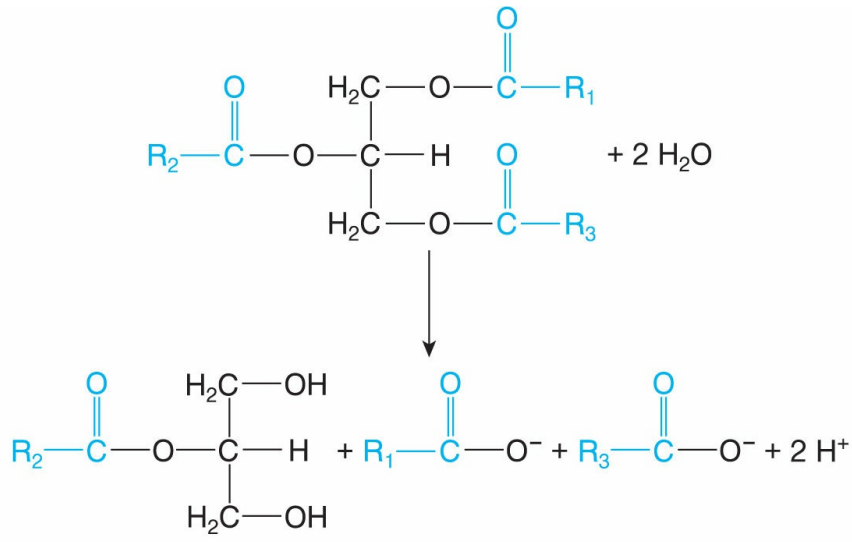
Triacylglycerol Digestion

Dietary triacylglycerols cannot cross the plasma membrane of the cells lining the gastrointestinal tract and, therefore, cannot be absorbed. Rather, they are broken down primarily in the small intestine, and their degradation products get absorbed (figure 11.1). **Triacylglycerol digestion** is hampered by the fact that they are insoluble in water, whereas the enzyme degrading them is water soluble. The solution to this conundrum is **bile acids** (figure 11.2), a group of amphipathic compounds synthesized in the liver from cholesterol. Bile acids are transported to the gallbladder through the hepatic ducts and form part of bile, the liquid stored in the gallbladder.

Dietary fat leaves the stomach in the form of an **emulsion**, that is, a suspension of lipid droplets in water, created mainly by the contractions of the smooth muscle forming the stomach wall. Upon entering the duodenum, the fat causes the duodenal wall to release the hormone **cholecystokinin**,

which directs the gallbladder to secrete bile to the duodenum through the bile duct. Being amphipathic, the bile acids reconcile triacylglycerols and water, in effect reducing the size of the lipid droplets, much as the egg does in mayonnaise (see problem 8 in chapter 5).

Triacylglycerols in the fine lipid droplets produced with the aid of bile acids are hydrolyzed by **pancreatic lipase**, an enzyme secreted from the pancreas to the duodenum in response to cholecystokinin secretion. Pancreatic lipase catalyzes the hydrolysis of the ester linkages at positions 1 and 3 of the glycerol unit to yield **2-monoacylglycerol** and two fatty acids.



2-Monoacylglycerol

As in chapter 5, R_1, R_2, and R_3 in the reactions and figures of this chapter represent the aliphatic chains of the fatty acids, which usually differ from each other.

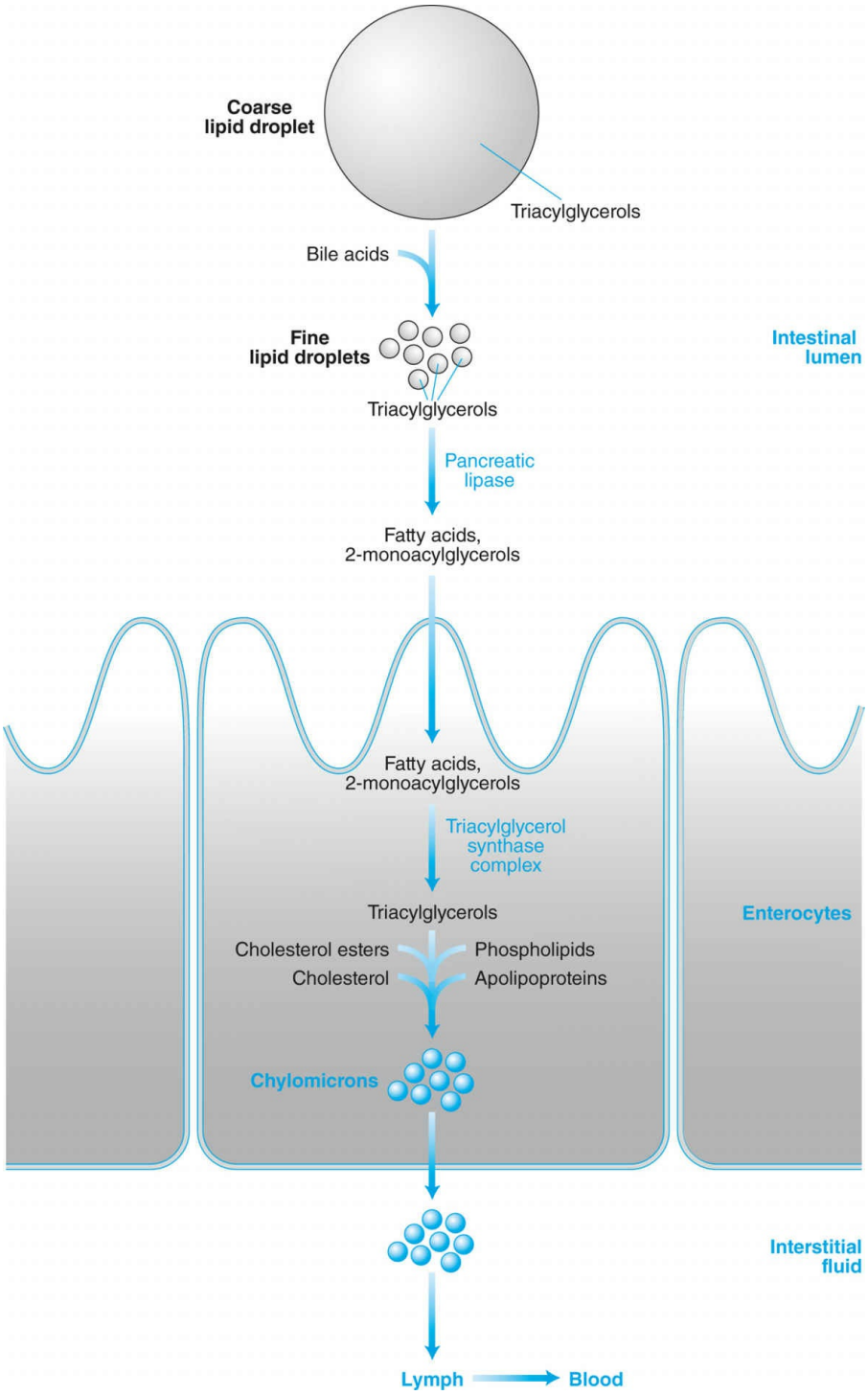


Figure 11.1 Triacylglycerol digestion, absorption, and distribution. Dietary fat, consisting primarily of triacylglycerols, enters the intestinal lumen in the form of coarse lipid droplets, which become much smaller through the emulsifying action of bile acids. Fine lipid droplets are then attacked by pancreatic lipase, which catalyzes the hydrolysis of triacylglycerols to fatty acids and 2-monoacylglycerols. These products pass from the intestinal lumen into the enterocytes (cf. figure 10.3), where they are converted back into

triacylglycerols through the action of the triacylglycerol synthase complex. Triacylglycerols, along with cholesterol esters, cholesterol, phospholipids, and apolipoproteins, are packaged in chylomicrons, which are exported to the interstitial fluid, from there to the lymph, and from there to blood.

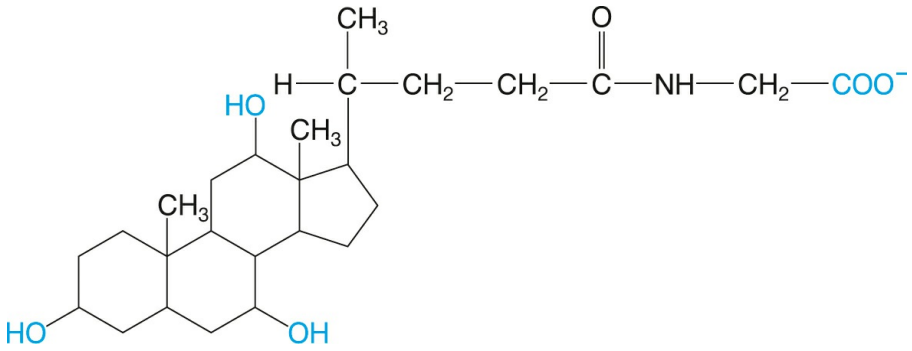


Figure 11.2 **A bile acid.** Glycocholate is a major bile acid that is used to emulsify dietary fat in the small intestine thanks to its amphipathic character. Its hydrophilic ends are shown in color, whereas the hydrophobic parts are shown in black. Notice the structural similarity to cholesterol, from which it is synthesized in the liver (cf. figure 5.17).

Triacylglycerol Absorption

Although triacylglycerols cannot cross the plasma membrane of enterocytes, 2-monoacylglycerols and fatty acids can and do. Once inside the enterocytes, they are reunited to form triacylglycerols in the process of **triacylglycerol absorption**. However, the reverse of reaction 11.1 is not favored. To make triacylglycerol synthesis favored, a fatty acid is first activated by being converted into **acyl coenzyme A**, or **acyl CoA**, through the joining of an acyl group to CoA. For example, the joining of a **palmitoyl group** to CoA yields **palmitoyl CoA**. The synthesis of acyl CoA requires an input of energy from the hydrolysis of ATP to AMP and PP_i.

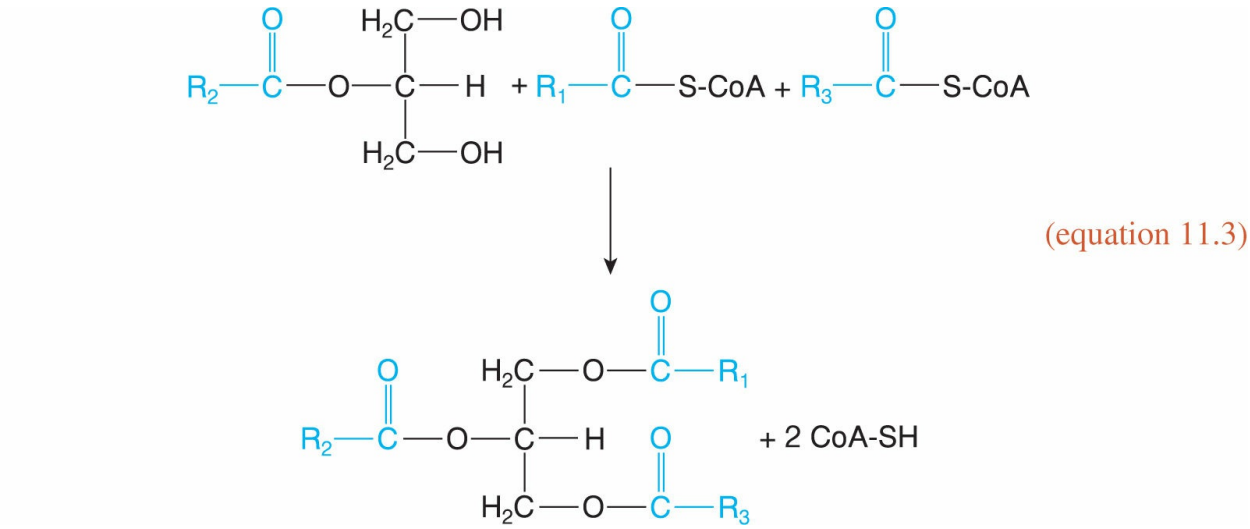
(equation 11.2)

Acyl CoA

Reaction 11.2 is shifted to the right by the subsequent exergonic hydrolysis of PP_i to two P_i according to reaction 2.7. Acyl CoA synthesis is catalyzed by **acyl CoA synthetase**, an enzyme located in the plasma membrane, the endoplasmic reticulum membrane, and the outer

mitochondrial membrane.

Two acyl groups are subsequently added to 2-monoacylglycerol through the successive action of **monoacylglycerol acyltransferase** and **diacylglycerol acyltransferase**.



(equation 11.3)

Acyl CoA synthetase and the two acyltransferases are organized in a multienzyme system—the **triacylglycerol synthetase complex—**which is bound to the endoplasmic reticulum membrane. Once triacylglycerols are synthesized, they are ready to be distributed to the body.

Triacylglycerol Distribution

Because triacylglycerols are insoluble in water, they do not circulate in body fluids in free form. **Triacylglycerol distribution** is accomplished through incorporation of triacylglycerols in **lipoproteins**, that is, globular aggregates of lipids and proteins, as the name indicates. There are several classes of lipoproteins, which we will explore in section 11.19. For now, let's concentrate on the class that carries dietary triacylglycerols: **chylomicrons**.

Chylomicrons form inside the enterocytes. Triacylglycerols are sequestered in a hydrophobic core (along with cholesterol esters), surrounded by a thin shell of phospholipids, cholesterol, and special kinds of proteins termed **apolipoproteins**, or **apoproteins** for brevity (figure 11.3). Unlike cell membranes, which consist of lipid bilayers, the shell of chylomicrons (and all other lipoproteins) features a single layer, in which the hydrophobic parts of

phospholipids and cholesterol are in contact with the hydrophobic core, while the polar head groups face the cytosol.

The prefix *apo*-, taken from Greek, indicates what is left of a lipoprotein when it is experimentally stripped of its lipids.

Once formed, chylomicrons exit the enterocytes to the extracellular space by exocytosis (figure 11.1) and enter the lymphatic vessels of the villi (consult figure 10.3), which pour their contents into the left subclavian and left jugular veins through the thoracic duct. This process goes on for several hours after a meal.

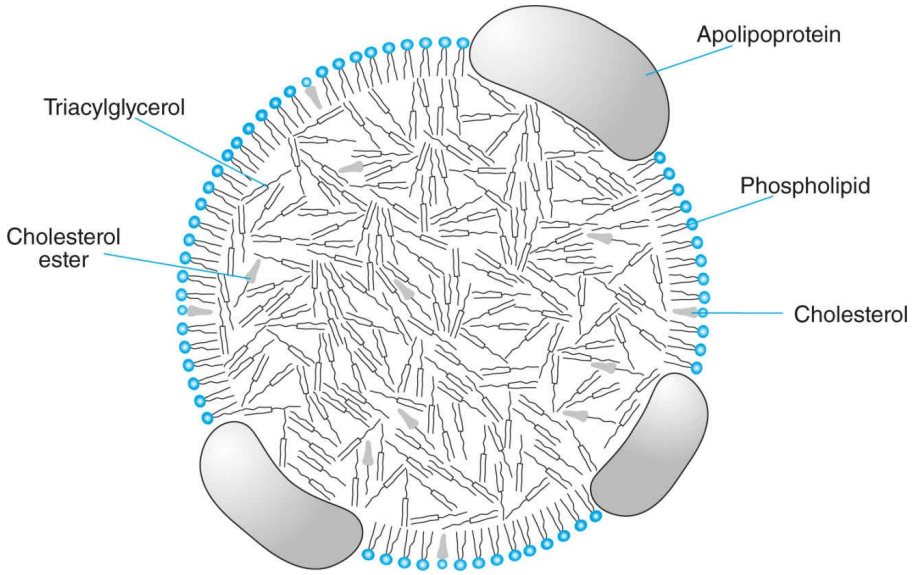
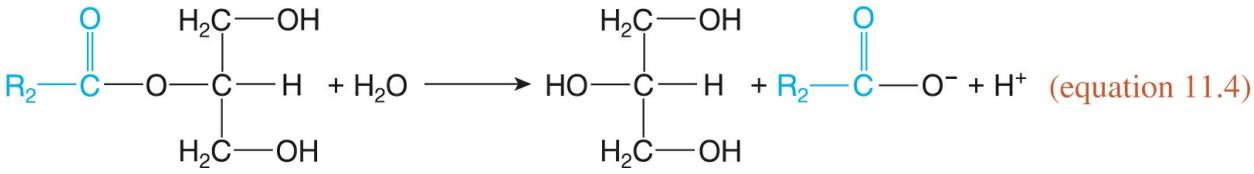


Figure 11.3 **Anatomy of a chylomicron.** A chylomicron is a globular formation containing thousands of molecules of dietary fat. Hydrophobic triacylglycerols (constituting most of a chylomicron's mass) and cholesterol esters are sequestered in the core, whereas cholesterol, phospholipids, and apolipoproteins form an amphipathic shell.

Within a few minutes of entering the bloodstream, a chylomicron encounters **lipoprotein lipase** on the surface of the capillaries in muscle, adipose tissue, and other extrahepatic tissues (figure 11.4). Lipoprotein lipase hydrolyzes triacylglycerols to 2-monoacylglycerols and fatty acids, just as pancreatic lipase does. Lipoprotein lipase is synthesized in muscle fibers, adipocytes, and other cells, which export it to their capillaries.

The reason that triacylglycerols in chylomicrons need to be broken down is that, again, they cannot cross cell membranes, whereas 2-monoacylglycerols and fatty acids can. Thus, thanks to lipoprotein lipase, tissues take up the components of dietary triacylglycerols. 2-Monoacylglycerols are then hydrolyzed to **glycerol** and fatty acids by **monoacylglycerol lipase (MGL)**.



(equation 11.4)

Glycerol usually exits to the bloodstream to be taken up by the liver in a way that I will describe in section 11.8. Fatty acids are either oxidized (as I will describe in section 11.9) or used for the synthesis of triacylglycerols, phospholipids, cholesterol esters, and other biomolecules.

The triacylglycerols in certain foods—notably, milk and dairy products—contain small amounts of acyl groups that are shorter (four to ten C) than the usual ones presented in section 5.7 and table 5.2 (12 C and up). Short acyl groups can be detached from triacylglycerols by lipases in the mouth and stomach (as well as by pancreatic lipase in the small intestine). The resulting fatty acids pass through the stomach and intestinal walls directly to the portal vein and are taken up by the liver.

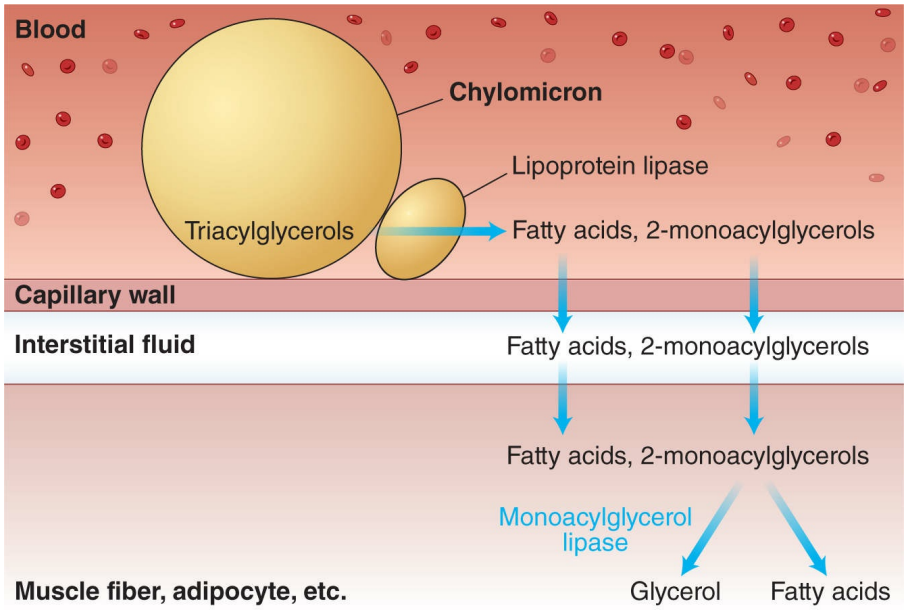
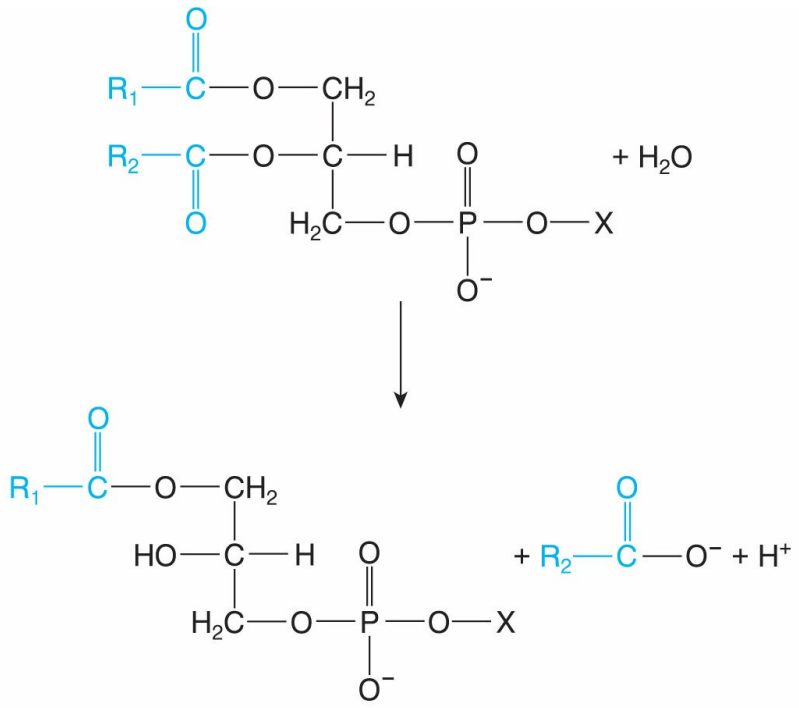


Figure 11.4 **Triacylglycerol delivery.** A chylomicron travels to the depths of a tissue to meet its demise in the face of lipoprotein lipase. Molecules of the enzyme stick out from the walls of the capillaries, facing blood, and hydrolyze the triacylglycerols in the chylomicron to fatty acids and 2-monoacylglycerols. These products then pass to the interstitial fluid and from there to a variety of cells, including muscle and fat cells. Once inside these cells, 2-monoacylglycerols are further hydrolyzed to glycerol and fatty acids.

11.2 Digestion, Absorption, and Distribution of Other Lipids

Glycerophospholipids, the major group of phospholipids (section 5.9), are digested in the small intestine by **pancreatic phospholipase A_2**, an enzyme present in the pancreatic juice along with pancreatic lipase. Phospholipase A_2 hydrolyzes the ester linkage at position 2 of glycerol in glycerophospholipids, producing **2-lysophospholipids** and fatty acids.



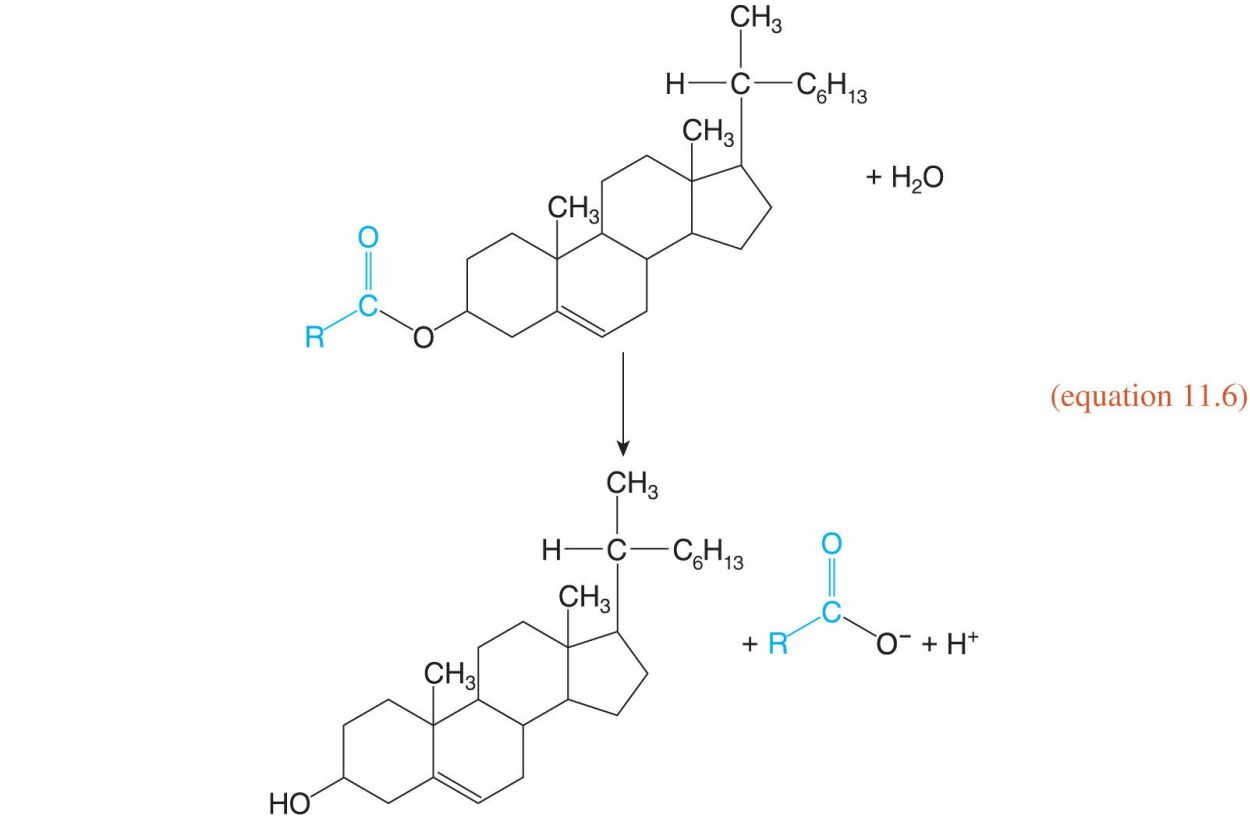
(equation 11.5)

2-Lysophospholipid

Phospholipase A_2 is found in the venom of several snakes, such as the Indian cobra. When injected into the victim's body by a bite, the enzyme catalyzes the hydrolysis of phospholipids in the membranes of a variety of tissues, resulting in extended tissue damage that may lead to death.

X represents one of the alcohols introduced in figure 5.15. 2-Lysophospholipids follow a course similar to that followed by the products of triacylglycerol digestion: They enter the enterocytes, where glycerophospholipids are resynthesized through two alternative routes that we will not consider. The newly formed phospholipids become part of the shell of chylomicrons, which carry them to the rest of the body.

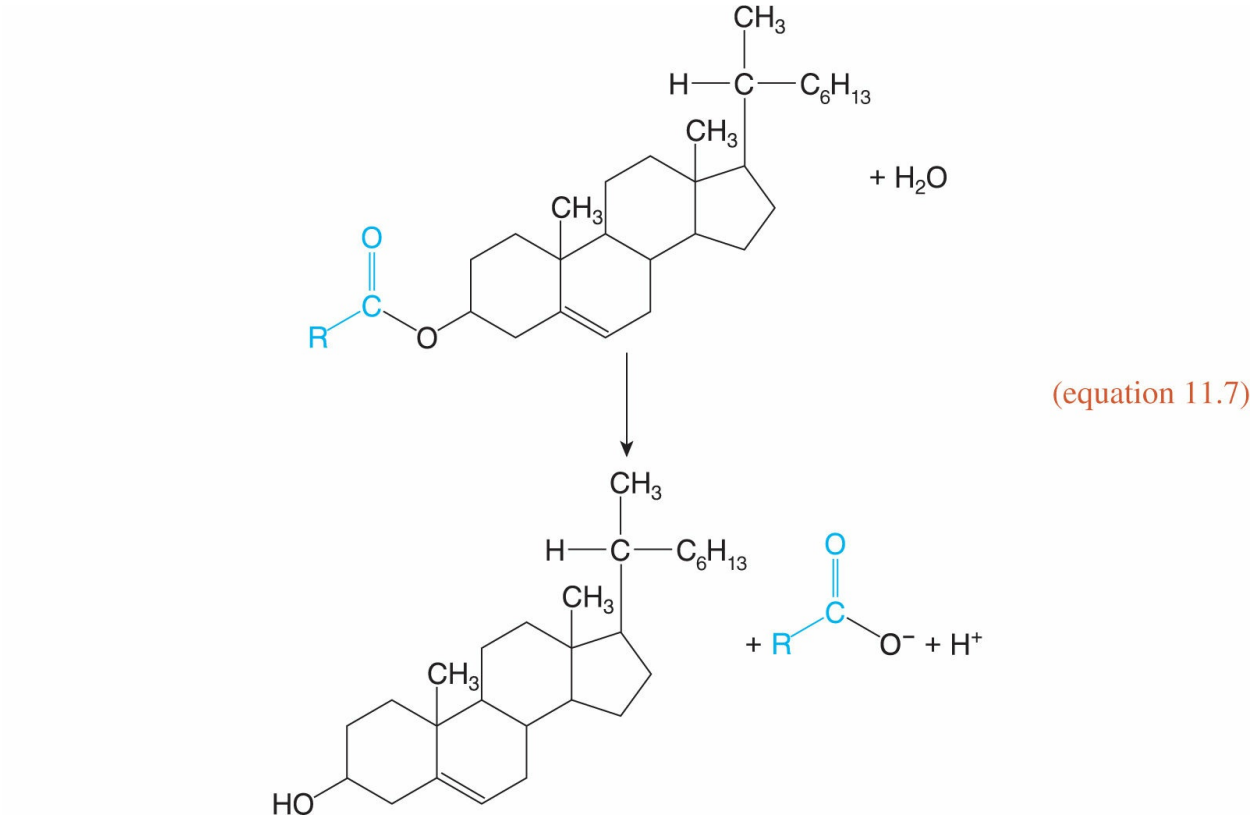
Dietary cholesterol can pass into the enterocytes in its free form, but cholesterol esters, which constitute 10% to 15% of total cholesterol, need to be hydrolyzed first. Being hydrophobic, cholesterol esters require bile acids for complete emulsification, just as triacylglycerols do. **Pancreatic cholesterol esterase** then hydrolyzes a cholesterol ester to cholesterol and fatty acid.



(equation 11.6)

Only about half of the total cholesterol in our food is taken up by enterocytes; the remainder is excreted in the feces.

In the enterocytes, most of the cholesterol (about 70% to 80%) is converted into cholesterol esters by the catalytic action of **acyl CoA:cholesterol acyltransferase**.



(equation 11.7)

Cholesterol esters then find their way to the chylomicron core, whereas free cholesterol is incorporated in the chylomicron shell. Thus, the two forms of cholesterol are delivered to the tissues in the body.

11.3 Fat Content of the Human Body

Fat is the largest energy depot in the body. It is also the energy depot that exhibits the highest variability between individuals, depending on how lean, overweight, or obese we are. Women generally have more fat than men do as a percentage of body mass. An average (and healthy) body fat content for a lean man is 15%, whereas the corresponding value for a woman is 23%. Thus, the model man weighing 75 kg (introduced in section 10.2) has 11.25 kg of fat, and the model woman of 62 kg has 14.26 kg of fat. Most of this fat consists of triacylglycerols; thus, I will use the round figures of 11 and 14 kg for the triacylglycerol stores in men and women, respectively, in future calculations. Of course, these figures change with changing body weight and fat percentage. Athletes usually have lower body fat content than do nonathletes of the same, sex, age, and body mass index, or BMI.

BMI is calculated as the ratio of body mass to the square of body height and is usually measured in $kg \cdot m^{-2}$. It is considered a handy index of adiposity, though it makes no distinction between lean and fat mass, given that it uses total body mass in the numerator.

Most of our body fat is gathered in adipose tissue—a loose, yellowish tissue distributed all over the body. Its two main locations are under the skin (subcutaneous adipose tissue) and around internal organs (abdominal, or visceral, adipose tissue). Subcutaneous adipose tissue is usually larger than abdominal adipose tissue. About 80% of the adipose tissue mass consists of triacylglycerols, making it the most amazing accumulation of a substance in a tissue.

Researchers sometimes refer to the adipose tissue that I just described as **white adipose tissue**, or **white fat**, to distinguish it from **brown adipose tissue**, or **brown fat**. Brown adipose tissue has a darker appearance, and in adults it is segregated mainly in the upper chest and neck areas, amassing just about 50 g. Brown adipose tissue is more prevalent in infancy (accounting for about 5% of their body mass) and gradually shrinks as we grow up. We will examine the special role of brown adipose tissue in section 11.18. Apart from that discussion, whenever I refer to adipose tissue I will mean white adipose tissue.

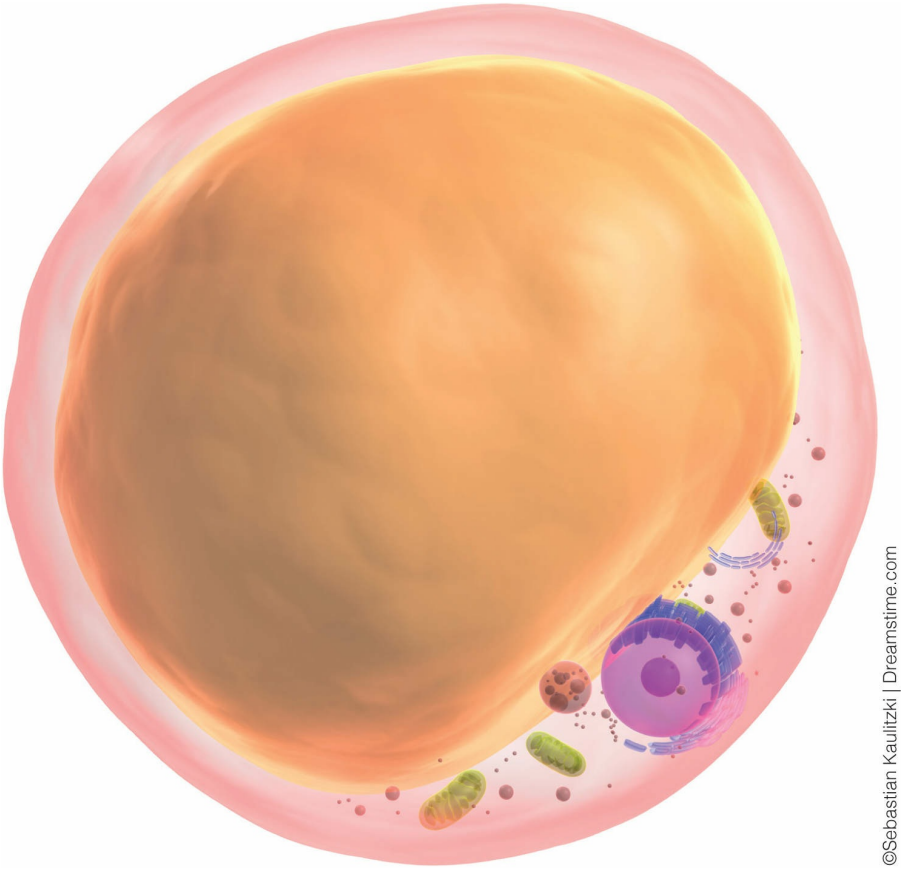


Figure 11.5 **Adipocyte.** A diagrammatic cross-section of an adipocyte reveals that it is almost filled by a lipid droplet, which contains mainly triacylglycerols. Apart from the lipid droplet, you can see the nucleus and some mitochondria at the bottom right.

The main cells in adipose tissue are the **adipocytes**, or fat cells (figure 11.5). These round cells normally measure 25 to 50 µm in diameter. Most of an adipocyte's cytoplasm is occupied by a droplet filled with triacylglycerols and cholesterol esters. The droplet is surrounded by a single layer of phospholipids and cholesterol, which are oriented in the same way as in lipoproteins, that is, with their hydrophobic parts facing inward and their polar head groups facing outward. In addition, the droplet is coated with **perilipins** (from the Greek words *perí*, meaning "around," and *lípos*, meaning "fat"), a family of proteins regulating access to the droplet, as we will see in section 11.6. The major perilipin isoform in adipocytes is termed **perilipin 1**.

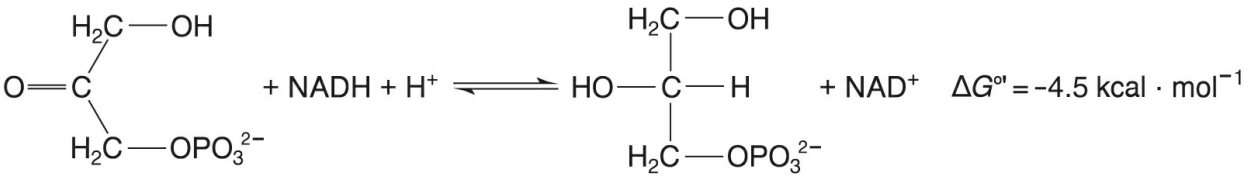
A lipid droplet in an adipocyte is like a fuel tank: Its triacylglycerols are the fuel, the

phospholipid monolayer surrounding it forms the wall of the tank, and perilipins act as guards restricting access to the tank.

The main metabolic activities of adipocytes are triacylglycerol synthesis and triacylglycerol breakdown. Synthesis prevails during the postprandial (fed) state, whereas breakdown occurs mainly during the postabsorptive (fasted) state and during exercise. If the two processes are balanced over the day, then the fat mass in the body remains constant. If synthesis outweighs breakdown, then we gain fat; if breakdown outweighs synthesis, then we lose fat. The net amount of fat deposited or lost in a day (if the two processes are not balanced) is minuscule—usually a few grams or, at the most, tens of grams. However, if the imbalance remains over weeks, months, or years, then a measurable fat—and weight—gain or loss will ensue. Of course, we are more concerned about fat and weight gain, which may result in obesity, a health-threatening condition that has become increasingly prevalent in recent decades. Because exercise promotes the breakdown of fat, it is an essential weapon in the fight against obesity, as we will see in chapter 15.

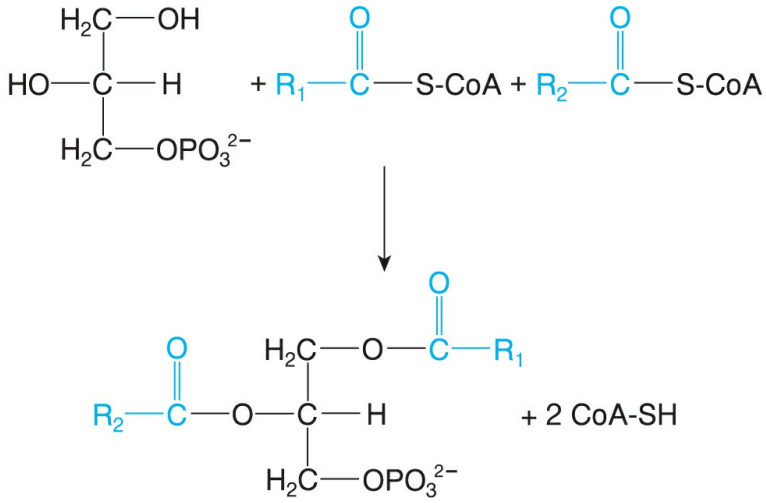
11.4 Triacylglycerol Synthesis in Adipose Tissue

Triacylglycerols are synthesized in adipocytes through a pathway that differs from the one taking place in enterocytes and described in section 11.1. Here, a triacylglycerol is made from three acyl CoA (synthesized according to reaction 11.2) and **L-glycerol 3-phosphate**. The latter derives mainly from dietary glucose entering the adipocytes. Through the four first reactions of glycolysis, glucose is converted into dihydroxyacetone phosphate (section 10.8 and figure 10.20), which is then reduced to glycerol 3-phosphate through a reaction catalyzed by **glycerol phosphate dehydrogenase**.



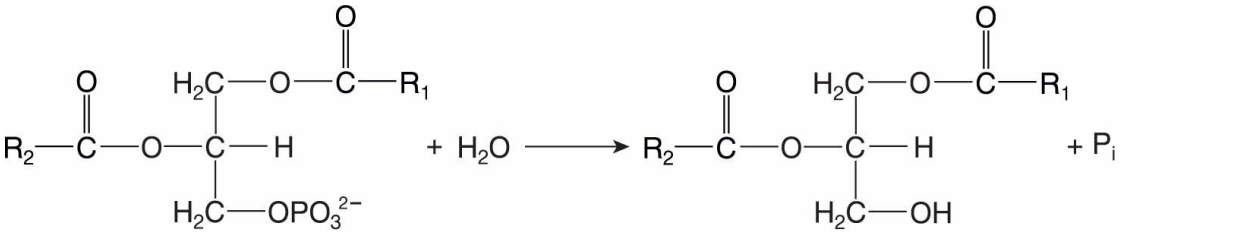
Dihydroxyacetone phosphate **L-Glycerol 3-phosphate** (equation 11.8)

Next, two acyl groups, borne by two acyl CoA, are attached to positions 1 and 2 of glycerol 3-phosphate.

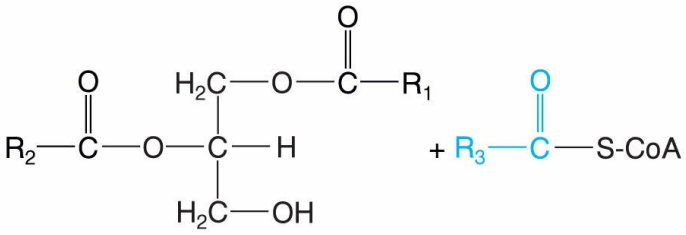


(equation 11.9)

The reaction is catalyzed by **glycerol phosphate acyltransferase**. The product is **phosphatidate** (familiar from section 5.9 and figure 5.14), which is finally converted into triacylglycerol after losing its phosphoryl group by hydrolysis according to reaction 11.10 and accepting a third acyl group from another acyl CoA according to reaction 11.11.

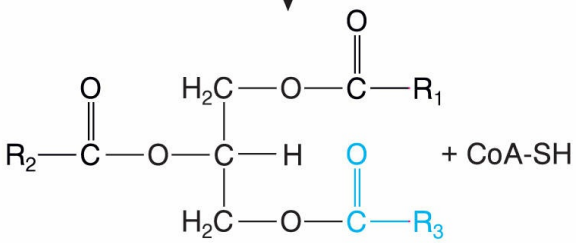


Phosphatidate **1,2-Diacylglycerol** (equation 11.10)



1,2-Diacylglycerol

(equation 11.11)

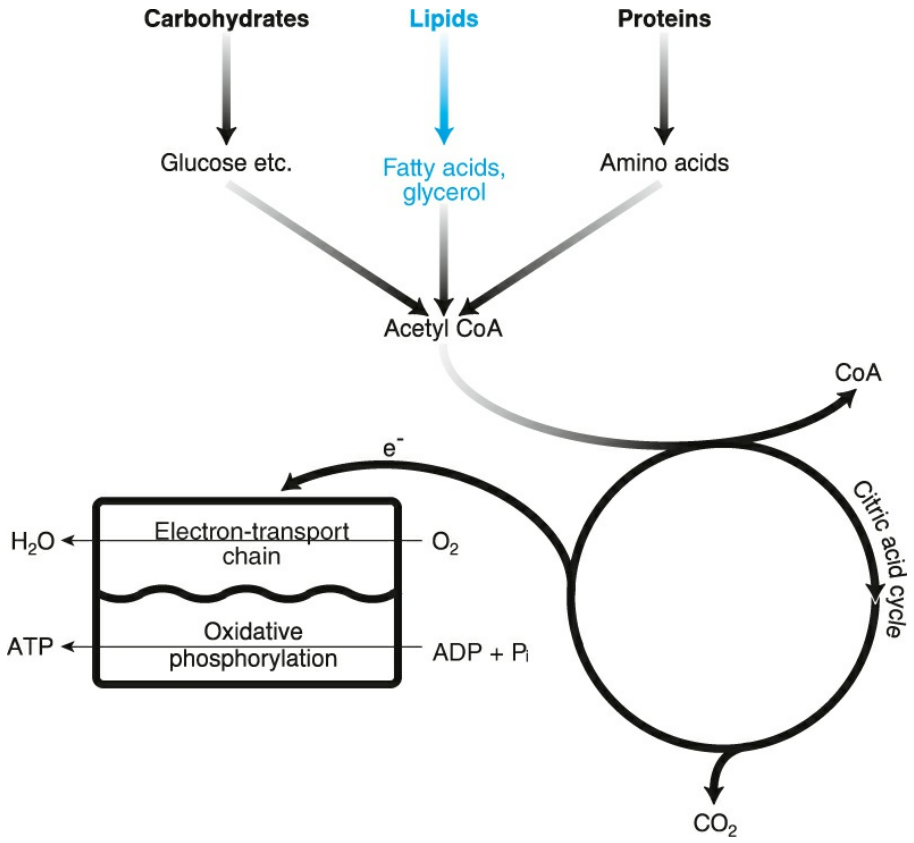


Triacylglycerol

The two reactions are catalyzed by another triacylglycerol synthetase complex (different from the one presented in section 11.1), which is also bound to the endoplasmic reticulum membrane.

Triacylglycerol synthesis prevails over breakdown in the postprandial state thanks to the presence of increased amounts of the substrates—glucose and fatty acids—entering the adipocytes after a meal. An important role is played in this process by the rise in the plasma insulin concentration resulting from the rise in the plasma glucose concentration after eating. Insulin promotes the entry of glucose into adipocytes by enhancing the translocation of GLUT4 from intracellular vesicles to the plasma membrane, just as it does in muscle fibers (figure 10.21). In addition, insulin increases the amount of lipoprotein lipase in the capillaries of adipose tissue, thus enhancing the hydrolysis of the

triacylglycerols in chylomicrons and the delivery of fatty acids to adipocytes.



You are here: lipolysis.

11.5 Lipolysis

Triacylglycerol breakdown, referred to as **lipolysis**, takes place in the cytosol through the sequential action of three enzymes: **adipose triacylglycerol lipase (ATGL)**, **hormone-sensitive lipase (HSL)**, and MGL (presented in section 11.1; figure 11.6). The former catalyzes the hydrolysis of the ester linkage at, primarily, position 2 or, secondarily, position

In lipolysis, a triacylglycerol is hydrolyzed to three fatty acids and glycerol.

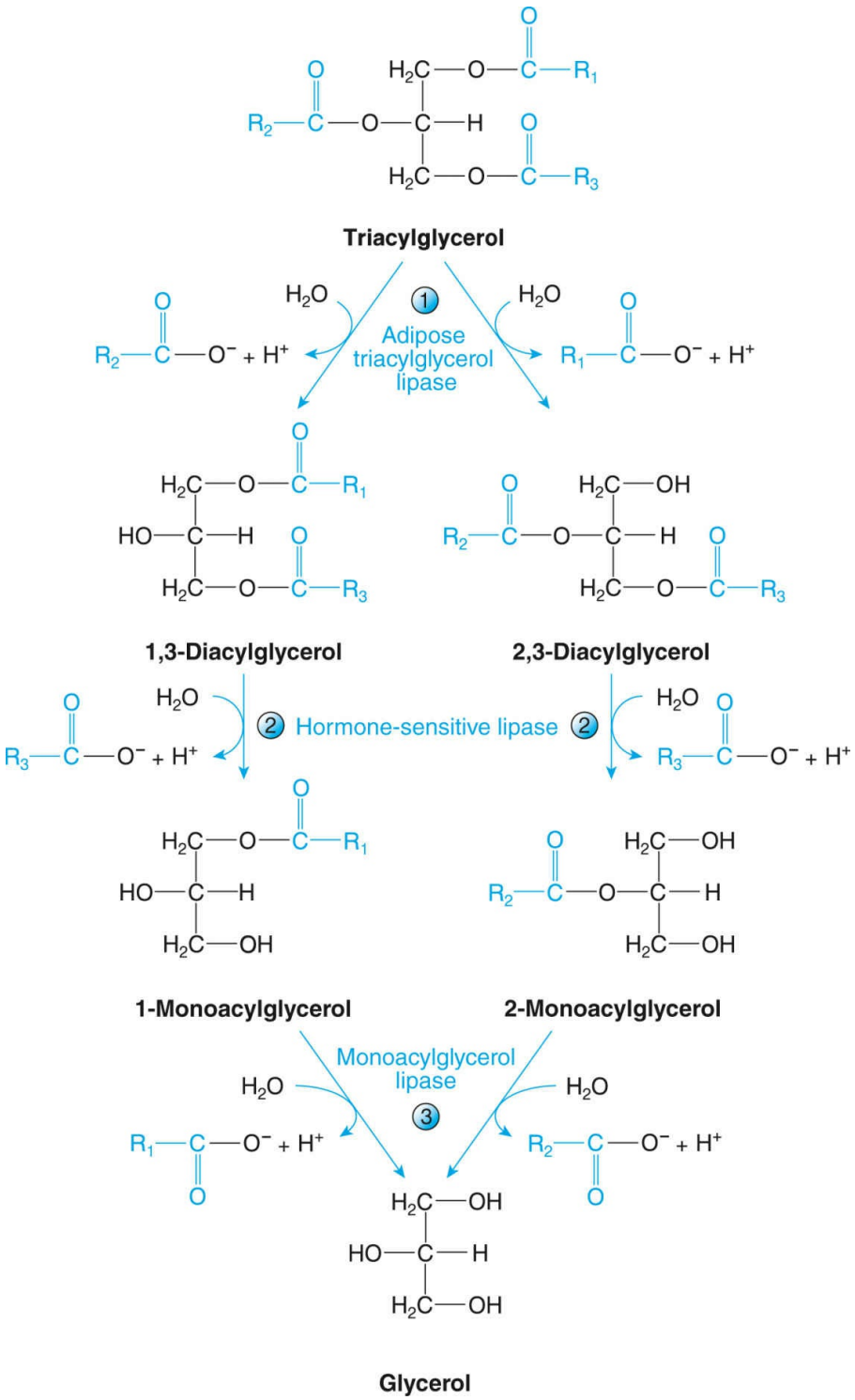


Figure 11.6 Lipolysis. The successive hydrolysis of the three ester linkages in a triacylglycerol, catalyzed by three different lipases, results in its degradation into three fatty acids and glycerol.

1 of the glycerol unit to yield **1,3-diacylglycerol** or **2,3-diacylglycerol**,

respectively, as Thomas Eichmann and coworkers have shown. Hydrolysis also yields a fatty acid.

Next, HSL (whose name I will explain in the next section) catalyzes the removal of a second fatty acid from position 3 of the glycerol unit, thus producing **1-monoacylglycerol** or 2-monoacylglycerol. Finally, MGL completes the dismantling of triacylglycerol by hydrolyzing the last remaining ester linkage. The end products of lipolysis are three fatty acids and one glycerol per triacylglycerol.

$$\text{Triacylglycerol} + 3\ H_2O \rightarrow 3\ \text{fatty acids} + \text{glycerol} + 3\ H^+ \quad \text{(equation 11.12)}$$

11.6 Exercise Speeds Up Lipolysis in Adipose Tissue

Exercise speeds up lipolysis in adipose tissue primarily through the augmented release of **epinephrine** from the **adrenal medulla** into the bloodstream, an effect we first considered in section 10.6. Epinephrine molecules bind to molecules of the **β-adrenergic receptor** located in the plasma membrane of adipocytes and stimulate the **cAMP cascade** (figure 11.7), resulting in the activation of **protein kinase A (PKA)** in a way identical to that described in section 10.6 and figures 10.14 and 10.15. PKA then phosphorylates three proteins that are instrumental in the acceleration of lipolysis. Two, perilipin 1 and HSL, have already been presented, in sections 11.3 and 11.5, respectively. The third one is known by the unusual name **comparative gene identification 58 (CGI–58)** and is associated with perilipin 1 at the surface of the lipid droplet. Let’s see how phosphorylation of these proteins speeds up lipolysis.

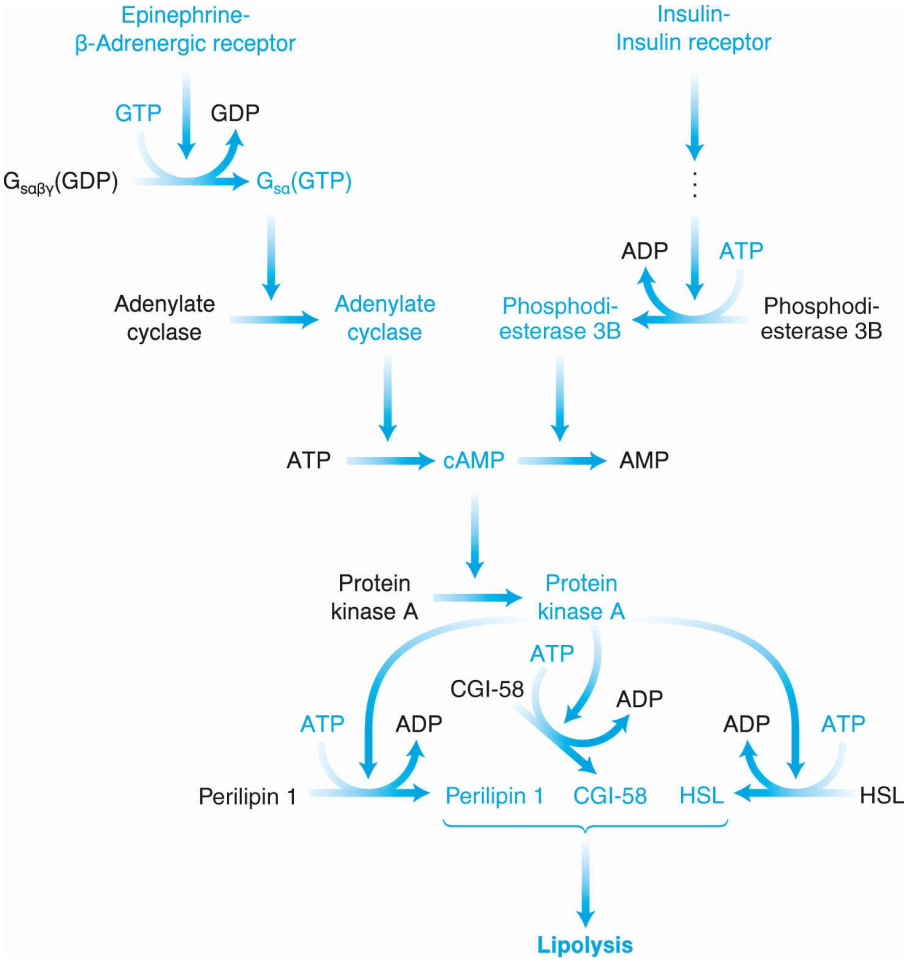


Figure 11.7 Control of lipolysis by the cAMP cascade. Epinephrine binding to the β-adrenergic receptor in the plasma membrane of adipocytes activates $G_{s\alpha}$, which in turn activates adenylate cyclase, resulting in a rise of the [cAMP]. This rise activates protein kinase A, which phosphorylates perilipin 1, CGI–58, and HSL, thus speeding up lipolysis. In contrast, insulin binding to its own receptor in the plasma membrane leads to activation of phosphodiesterase 3B, which hydrolyzes cAMP to AMP, thus slowing down lipolysis. As with the signal transduction pathways outlined in chapter 10, the more active forms of proteins are depicted in color.

At rest, ATGL and HSL are located primarily in the cytosol, barred from their substrates (that is, triacylglycerols and diacylglycerols) by unphosphorylated perilipin 1 and CGI–58 (figure 11.8*a*). As reviewed by Alan Kimmel and Carole Sztalryd, phosphorylation of perilipin 1 and CGI–58 by PKA disrupts their interaction, causing the release of CGI–58 from the lipid droplet. The liberated CGI–58 binds to ATGL and activates it (figure 11.8*b*), resulting in its recruitment to the lipid droplet and acceleration of

triacylglycerol hydrolysis to diacylglycerol and fatty acid (figure 11.8*c*).

Phosphorylation of the third partner in lipolytic activation, HSL, produces two effects. First, it causes the enzyme to move from the cytosol to the surface of the lipid droplet, where it binds to phosphorylated perilipin 1. Second, phosphorylation activates HSL (as it does phosphorylase kinase and phosphorylase; section 10.6). As a result, the rate of diacylglycerol hydrolysis to monoacylglycerol and fatty acid rises. Finally, MGL, which is abundantly present in the cytosol and on the lipid droplet, rapidly hydrolyzes monoacylglycerol to glycerol and fatty acid.

In parallel to its lipolytic action, epinephrine exerts an opposite one (that is, an antilipolytic action) in adipose tissue by binding to a different type of receptor, the **α_2-adrenergic receptor**. This one differs from the β-adrenergic receptor in that it associates with an inhibitory (rather than stimulatory) form of G protein, denoted by G_i (rather than G_s; section 10.6). Like G_s, G_i lies at the cytosolic side of the plasma membrane and consists of three subunits, α, β, and γ, of which α features a binding site for GTP and GDP.

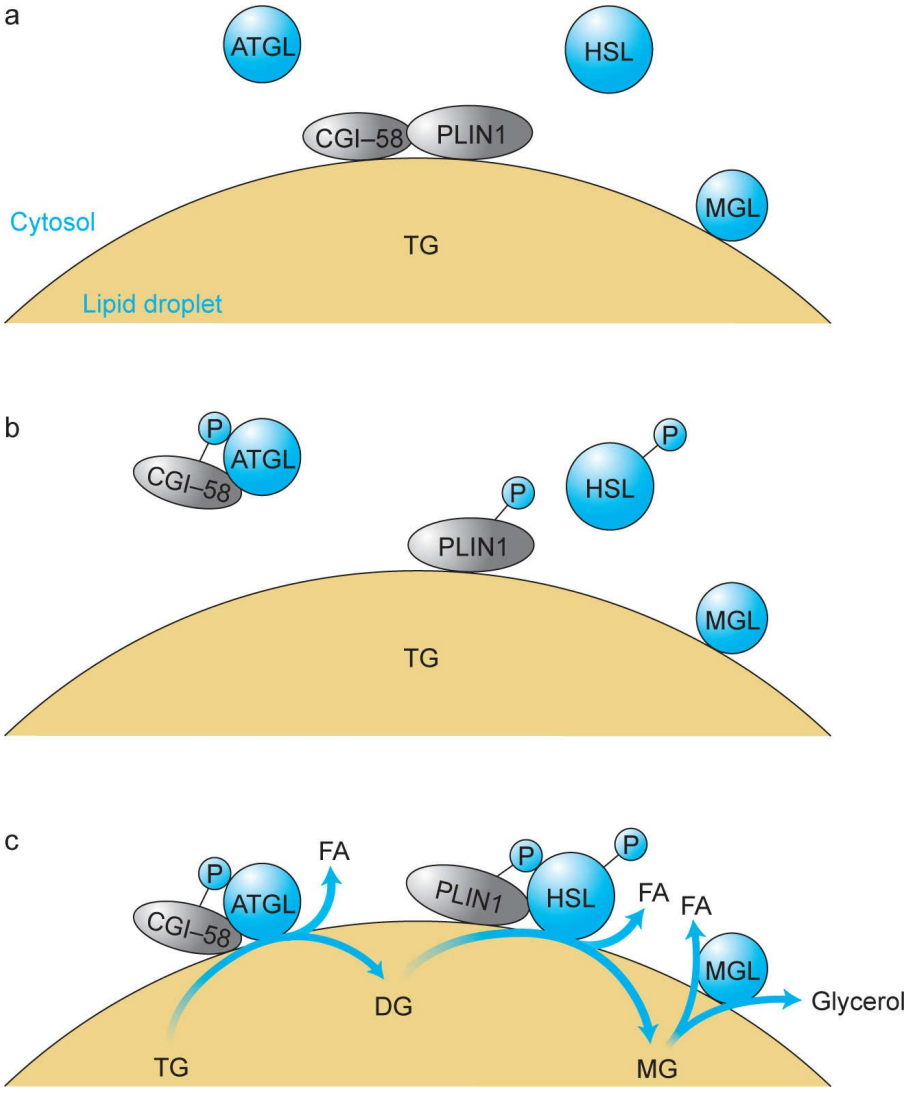


Figure 11.8 **Stimulation of lipolysis in the lipid droplet by exercise.** Exercise shifts metabolism in an adipocyte from a low to a high lipolytic rate mainly through β-adrenergic stimulation of the cAMP cascade. *(a)* At rest, triacylglycerol (TG) hydrolysis is slow because perilipin 1 (PLIN1) and CGI–58 are bound together, thus preventing most of the ATGL and HSL molecules from contacting the lipid droplet. MGL is available but cannot work before ATGL and HSL do so. (For clarity, one molecule of each protein is shown.) *(b)* β-Adrenergic stimulation causes the phosphorylation of PLIN1, CGI–58, and HSL. As a result, CGI–58 dissociates from PLIN1, binds to ATGL, and activates it. In addition, phosphorylation activates HSL directly. *(c)* The phosphorylated CGI–58 recruits ATGL to the lipid droplet, where ATGL hydrolyzes TG to diacylglycerol (DG), releasing a fatty acid (FA) into the cytosol. Similarly, the phosphorylated PLIN1 attracts the phosphorylated HSL to the lipid droplet, where HSL hydrolyzes DG to monoacylglycerol (MG), releasing another FA. Finally, MGL hydrolyzes MG, releasing FA and glycerol into the cytosol.

When the α_2-adrenergic receptor is vacant, $G_{i\alpha}$ is occupied mainly by GDP

and is inactive. However, epinephrine binding causes the attachment of the receptor to G_i (figure 11.9), the substitution of GTP for GDP in $G_{i\alpha}$, and the separation of $G_{i\alpha}$ from the β and γ subunits. The liberated $G_{i\alpha}$ diffuses across the membrane, binds to adenylate cyclase, and inhibits it. This action blocks the production of cAMP and, hence, the cAMP cascade, resulting in inhibition of lipolysis.

Opposing actions of a single agent, such as epinephrine, on a single cell are unusual and may serve to moderate the agent's action. In general, the lipolytic effect of epinephrine on adipose tissue overpowers its antilipolytic effect. Thus, the net effect of exercise is a rise in lipolytic rate, which is another example of the third principle of exercise metabolism presented in section III.2: Exercise changes the metabolism of other tissues in addition to muscle tissue. Nevertheless, differences in the proportion of β- to α_2-adrenergic receptors may cause a mismatch between the epinephrine concentration and the lipolytic response among fat depots or individuals.

This result appears to be the case in the comparison between sexes with regard to exercise-induced lipolysis: Moderate-intensity exercise at a given percentage of $\dot{V}O_2$max elicits higher plasma epinephrine concentration in men but higher lipolytic rate in women matched for percent body fat. Bettina Mittendorfer and collaborators have suggested that this difference results from women having lower activity than men of the antilipolytic α_2-adrenergic receptor in their adipose tissue.

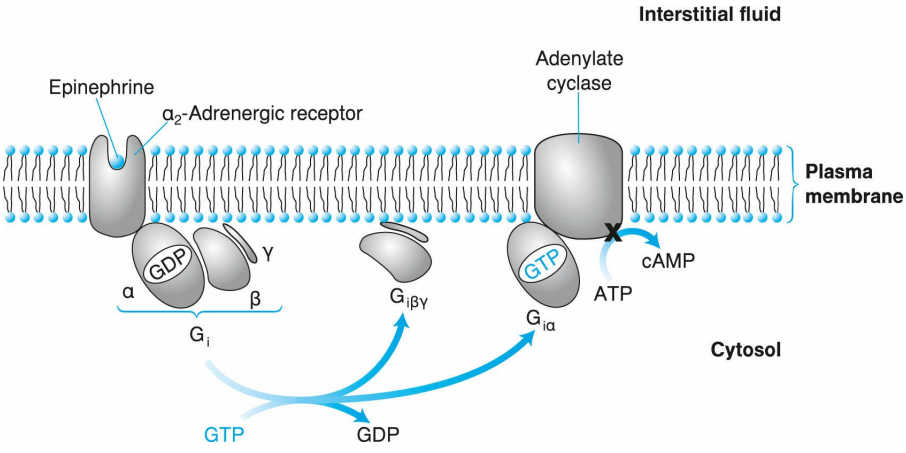
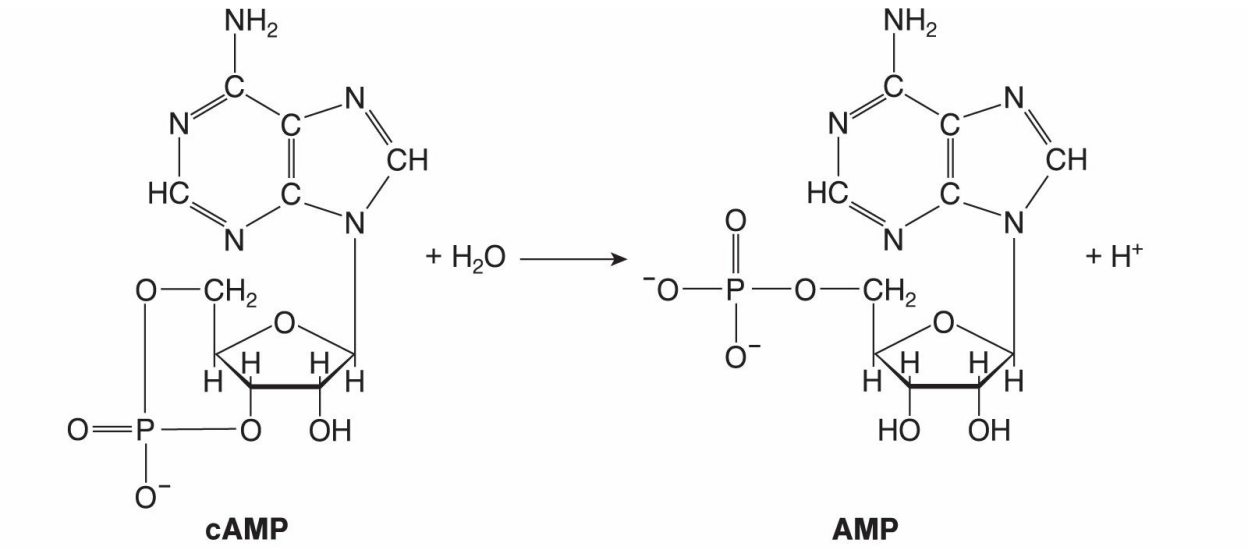


Figure 11.9 Inhibition of lipolysis through α_2-adrenergic stimulation. Epinephrine binding to the α_2-adrenergic receptor in the plasma membrane of an adipocyte causes the

replacement of GDP by GTP in the α subunit of G_i protein and the detachment of the α from the β and γ subunits. Next, $G_{i\alpha}$ binds to adenylate cyclase and inhibits it, thus slowing down cAMP production, the cAMP cascade, and lipolysis.

Lipolysis is under the influence of another hormone, namely insulin. Through its signal transduction pathway, described in section 10.29 and figure 10.48, insulin activates PKB, which phosphorylates and activates **phosphodiesterase 3B**. This enzyme catalyzes the hydrolysis of cAMP to AMP.



$\Delta G^{\circ\prime} = -9.9\ \text{kcal} \cdot \text{mol}^{-1}$ (equation 11.13)

Cyclic AMP degradation deactivates the cAMP cascade and prevents the phosphorylation of the proteins that stimulate lipolysis. In this way, insulin inhibits the lipolytic action of epinephrine. Now we can justify HSL's name: Because its phosphorylation state and, hence, activity depend on epinephrine and insulin, it is a hormone-sensitive lipase.

As we have seen in section 10.30, insulin secretion usually decreases during moderate-intensity exercise, which favors lipolysis over triacylglycerol synthesis. During hard or maximal exercise, when plasma insulin does not change in a consistent manner, its influence on the balance between lipolysis and triacylglycerol synthesis becomes small.

Because the stimulation of lipolysis in adipose tissue during exercise is primarily due to increased epinephrine secretion and activation of the cAMP cascade, it is not as fast as the stimulation of carbohydrate breakdown in

muscle. Nevertheless, it is faster than usually thought: Studies with exercising humans (for example, by Anatoli Petridou and colleagues) have shown maximal lipase activity in adipose tissue and increased exit of the lipolytic products from adipocytes within five to ten minutes of the onset of exercise.

What effect does exercise intensity have on the lipolytic rate? Apparently, little. Investigators have measured the lipolytic rate in adipose tissue or in the whole body during exercise tasks ranging in intensity from 25% to 85% of $\dot{V}O_2max$. The results show that even light exercise can elicit maximal lipolytic rate, probably because the hormonal changes that take place are sufficient for that. However, similar lipolytic rates at different exercise intensities do not mean similar utilization of fat for energy production. Fat utilization does depend on exercise intensity, as we will see in section 14.5.

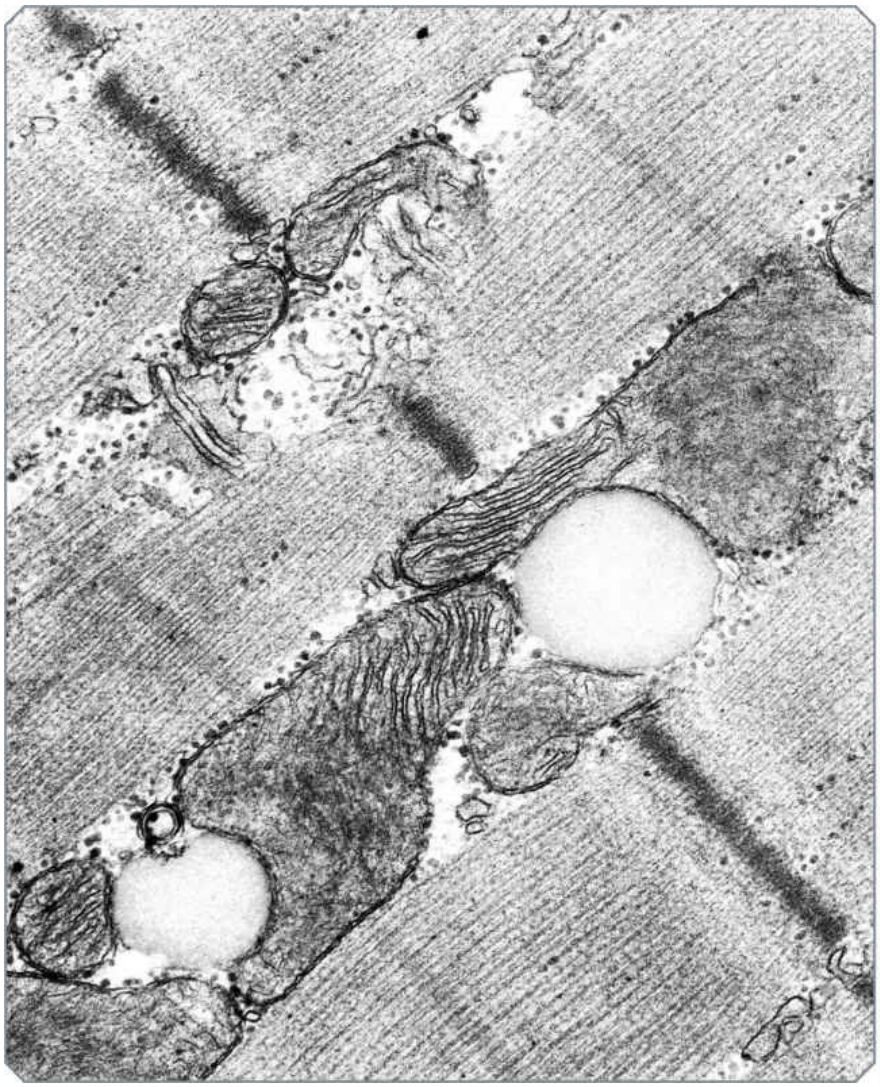


Figure 11.10 Triacylglycerols in a muscle fiber. Two lipid droplets lie in touch with mitochondria and myofibrils in this electron micrograph of part of a muscle fiber. The droplets measure 0.5 to 0.6 µm in diameter, that is, much smaller than the lipid droplet in an adipocyte (figure 11.5). The proximity of the droplets with mitochondria minimizes the distance that the fatty acids produced from lipolysis need to cross for degradation and energy production (see section 11.9). As in figure 10.5, you can see glycogen granules scattered throughout the sarcoplasm.

Finally, is it possible to cause local fat loss (also referred to as spot reduction) by exercising a specific part of the body? For example, can we reduce abdominal fat by performing sit-ups? Since adipose tissue itself does not exercise and since the signals to speed up lipolysis are hormonal (that is, global rather than local), there is no theoretical ground for targeted fat reduction through local exercises. In addition, studies that tested this

hypothesis have rejected it. If one performs sufficient exercise to achieve fat loss, it will come from all over the body, regardless of which parts of the body are involved in the exercise.

11.7 Exercise Speeds Up Lipolysis in Muscle

Skeletal muscles, as well as adipose tissue, contain triacylglycerols, which are located either in adipocytes scattered among muscle fibers or inside the muscle fibers themselves. We call the latter **myocellular triacylglycerols**. The triacylglycerol content of muscle fibers is about 0.5%, generally ranges from 0.2% to 0.8%, is higher in type I fibers than in type II fibers, and is higher in women than in men. Myocellular triacylglycerols are gathered in lipid droplets (figure 11.10), which are much smaller than the lipid droplets in adipocytes and are covered in perilipin isoforms other than perilipin 1. Lipid droplets are mostly found near the mitochondria, in both their intermyofibrillar and subsarcolemmal locations described in section 10.10. Most of the myocellular triacylglycerols (more than 85%) are stored in the intermyofibrillar lipid droplets, similarly to glycogen granules (section 10.7).

In addition to perilipins, muscle fibers contain the other protagonists of lipolysis, that is, ATGL, HSL, MGL, and CGI–58, as reviewed by Małgorzata Knapp and Jan Górski. The breakdown of myocellular triacylglycerols is usually accelerated during exercise, especially in type I fibers, which are the only ones that contain ATGL. However, we know less about the control of lipolysis in muscle fibers as compared with adipocytes.

Although the term ATGL hints at adipose tissue, the enzyme is found in many tissues, including muscle. It just happens that it was discovered in adipose tissue, where it is most abundant.

11.8 Fate of the Lipolytic Products During Exercise

Lipolysis results in the breakdown of approximately 100 to 300 g of triacylglycerols in the body daily, depending on the amount of food ingested

and on how physically active or inactive we are. However, not all of this quantity is lost, since adipocytes use part of the fatty acids produced to resynthesize triacylglycerols in the manner described in section 11.4. This recycling of fatty acids, often termed **reesterification** (that is, remaking of the ester linkages between glycerol and fatty acids), may seem futile, but it is inevitable once the enzymes and substrates for triacylglycerol synthesis are there. Nevertheless, the rate of triacylglycerol resynthesis is lower than the rate of lipolysis in the postabsorptive state and during exercise, mainly because part of the fatty acids produced escape from the adipocytes before they are reesterified.

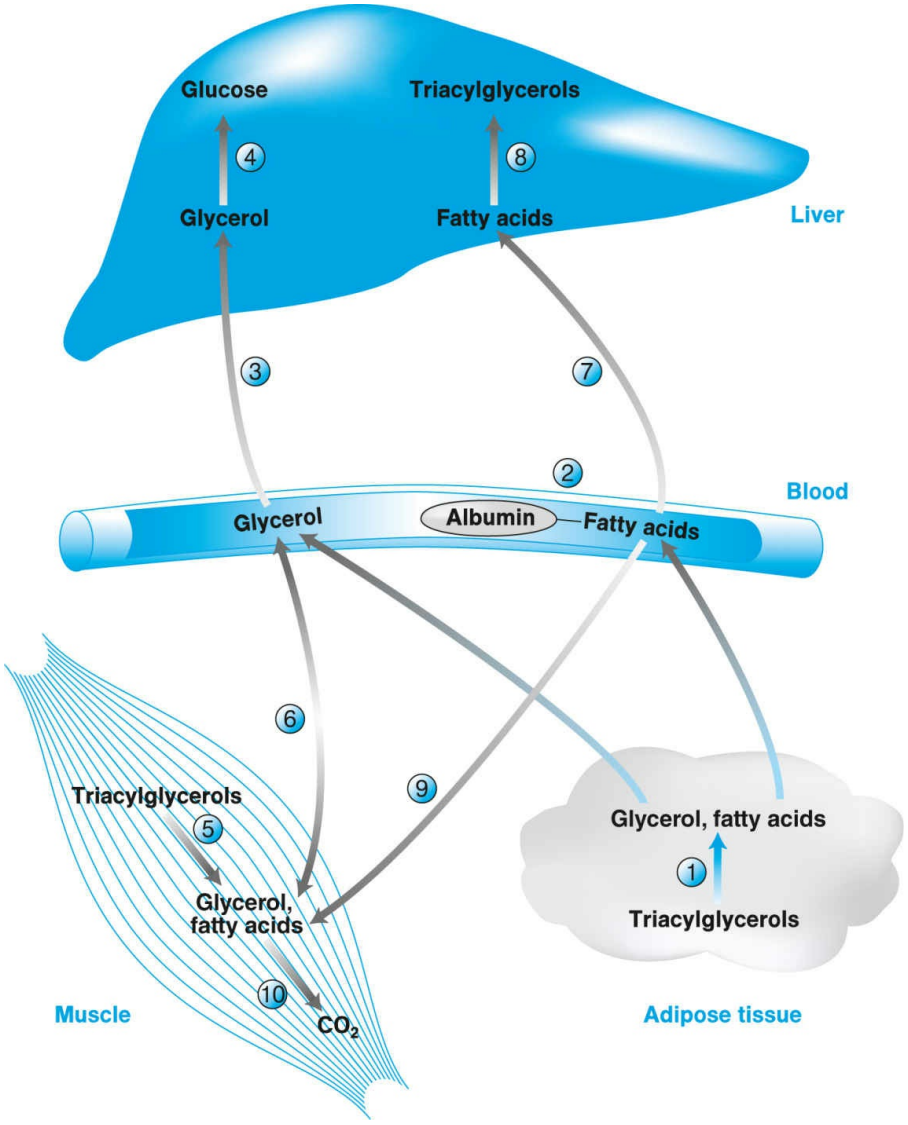


Figure 11.11 **Fate of the lipolytic products during exercise.** Triacylglycerols in adipose tissue are broken down to fatty acids and glycerol *(1)*, which are released to the bloodstream and are taken up by other tissues. Fatty acids circulate bound to albumin *(2)*. Glycerol enters the liver *(3)*, which uses it to make glucose *(4)*. Muscle glycerol, deriving from the hydrolysis of myocellular triacylglycerols *(5)*, has the same fate, although the reverse movement (that is, of plasma glycerol into the muscle) also takes place *(6)*. Blood-borne fatty acids can either enter the liver *(7)*, which uses them to produce triacylglycerols *(8)*, or enter the muscles *(9)*, where they join the domestic fatty acids and get oxidized to CO_2 *(10)*.

Indeed, a large portion of the fatty acids and glycerol produced by lipolysis in adipocytes exits to the interstitial space and then to the circulation. Thus, the acceleration of lipolysis during exercise leads to an increase in the rate of appearance of fatty acids and glycerol in plasma (figure 11.11). Glycerol is water soluble and circulates easily, but fatty acids, being amphipathic (section 5.7), are poorly soluble. For this reason, they are carried by **albumin**, the most abundant plasma protein (at a concentration of about 40 $g \cdot L^{-1}$). Albumin binds up to 10 fatty acids; it also carries other substances of low water solubility.

Plasma glycerol is taken up mainly by the liver and is converted into glycerol 3-phosphate through the catalytic action of **glycerol kinase**.

$$\text{Glycerol} + \text{ATP} \rightleftharpoons \text{L-glycerol 3-phosphate} + \text{ADP} + \text{H}^+$$
$$\Delta G^{\circ\prime} = -1.9 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 11.14)}$$

Glycerol 3-phosphate is then converted into dihydroxyacetone phosphate by reversal of reaction 11.8. Being an intermediate compound of both glycolysis and gluconeogenesis, dihydroxyacetone phosphate can go either way. However, most of it goes in the direction of gluconeogenesis, because this pathway is accelerated, whereas glycolysis is decelerated (in the liver) during exercise (section 10.26). Thus, triacylglycerols offer a small part of their structure to support gluconeogenesis during exercise. Another considerable source of plasma glycerol is the hydrolysis of myocellular triacylglycerols, although a similar amount of glycerol to the one leaving the muscle moves in the opposite direction, that is, from plasma to muscle.

Plasma fatty acids stemming from adipose tissue can be taken up by the liver and used for triacylglycerol synthesis. However, most of them are taken up by the active muscles and are degraded to regenerate ATP, as we will see in the next section. The entry of fatty acids into the sarcoplasm is facilitated

by three membrane proteins working either independently or in concert:

- **Fatty acid-binding protein at the plasma membrane (FABP-PM)**
- **Fatty acid translocase (FAT)**, or **cluster of differentiation 36 (CD36)**
- **Fatty acid transport protein (FATP)**

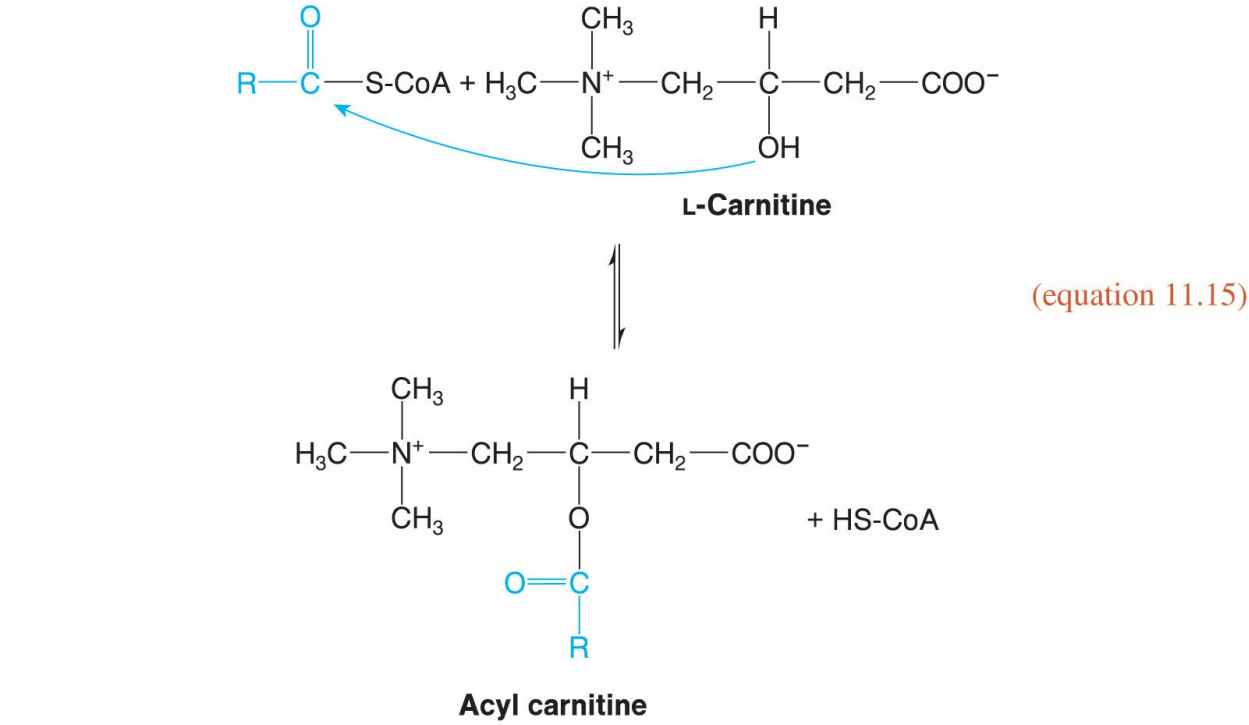
These proteins are more abundant in the sarcolemma of type I muscle fibers than of type II muscle fibers. Once inside the muscle fibers, fatty acids bind to a **cytosolic fatty acid-binding protein (FABP-C)**.

ATP is yielded from the degradation not only of fatty acids entering the muscles from plasma but also of fatty acids deriving from the hydrolysis of myocellular triacylglycerols. These domestic fatty acids are thought to contribute less than or, at most, as much as the imported ones to meeting the energy demands of moderate-intensity exercise.

11.9 Fatty Acid Degradation

Fatty acids are degraded through the pathway of **β oxidation**, which occurs in the mitochondria. To get there, fatty acids in the cytosol need to be activated and then cross the two mitochondrial membranes.

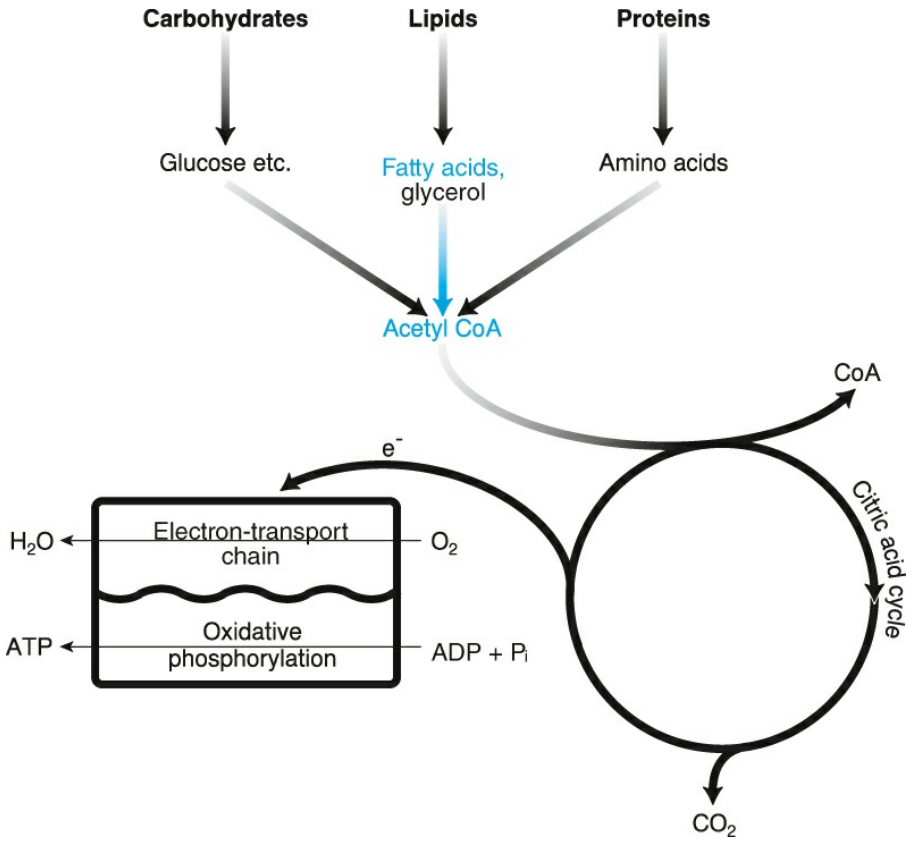
Fatty acids are activated in the manner discussed in section 11.1, that is, by conversion into acyl CoA (reaction 11.2). Acyl CoA readily crosses the outer mitochondrial membrane but not the inner one when the acyl group carries 14 carbons or more (which is the case for most acyl groups deriving from dietary or stored fat). We characterize such fatty acids as long-chain ones. These acyl groups are carried into the mitochondria by **L-carnitine**, a compound synthesized from the amino acid lysine. Carnitine detaches the acyl group from acyl CoA, producing CoA and **acylcarnitine**.



(equation 11.15)

The reaction is catalyzed by **carnitine acyltransferase I**, an integral protein of the outer mitochondrial membrane. The acylcarnitine produced crosses the inner mitochondrial membrane with the help of a transmembrane protein called **carnitine–acylcarnitine translocase** (figure 11.12). In the mitochondrial matrix, reaction 11.15 is reversed, and the acyl group is linked back to CoA. This reversal is catalyzed by **carnitine acyltransferase II**, an enzyme attached to the matrix side of the membrane in complex with the translocase. Thus, acyl CoA emerges in the mitochondrial matrix. Finally, carnitine returns to the intermembrane space through the translocase in exchange for the entry of an acylcarnitine.

Carnitine acyltransferase (either I or II) is also called **carnitine palmitoyltransferase** (**I** or **II**, respectively) because researchers measure its activity by using palmitoyl CoA as substrate.



You are here: β oxidation.

Once in the mitochondrial matrix, acyl CoA enters the pathway of β oxidation. This series of four reactions ends in the detachment of two carbons, in the form of acetyl CoA, from the carboxyl end of the acyl group (figure 11.13). In the first reaction, acyl CoA is oxidized to **enoyl CoA** by losing two H to FAD, which is converted into $FADH_2$. In the second reaction, enoyl CoA is hydrated to **L-3-hydroxyacyl CoA**, which, in the third reaction, is oxidized to **3-ketoacyl CoA**. NAD^+ serves as the hydrogen acceptor here to become NADH. Finally, in the fourth reaction, coenzyme A splits 3-ketoacyl CoA into acetyl CoA and an acyl CoA that is shorter than the initial one by two C. This reaction concludes one round of β oxidation; the overall reaction of the round is

$$\begin{aligned} &C_n\text{-acyl CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + H_2O \rightarrow \\ &C_{n-2}\text{-acyl CoA} + \text{acetyl CoA} + FADH_2 + \text{NADH} + H^+ \end{aligned} \qquad \text{(equation 11.16)}$$

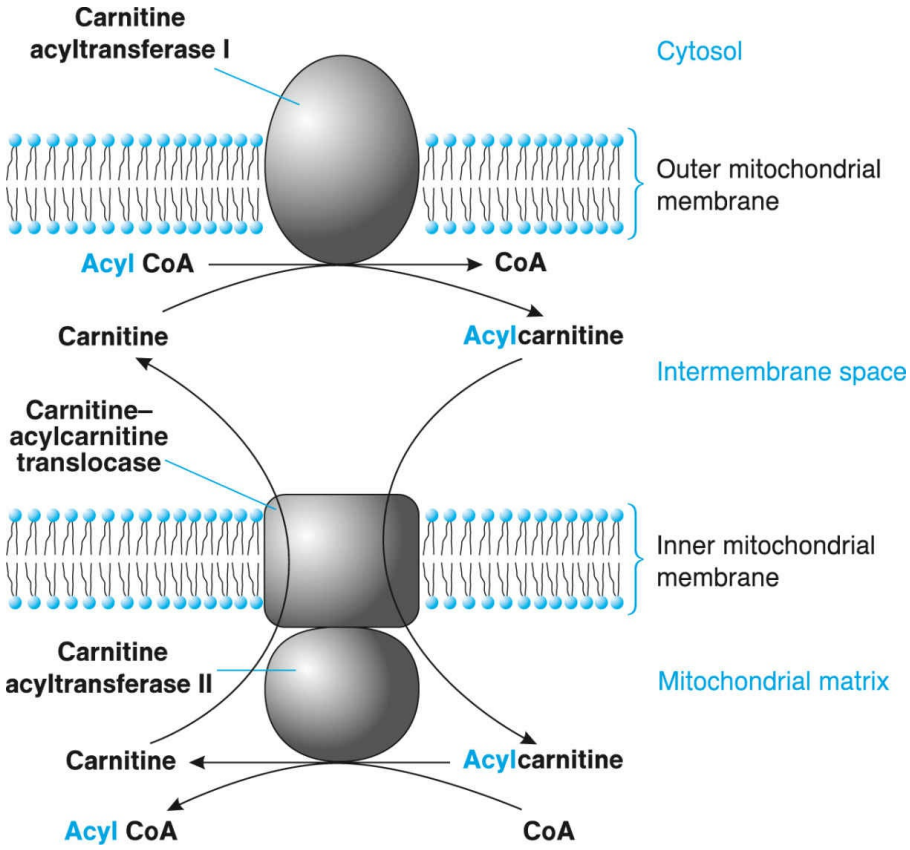


Figure 11.12 **Fatty acid transport into the mitochondria.** Acyl CoA, the activated form of a fatty acid, is transported to the mitochondrial matrix "disguised" as acylcarnitine. Carnitine acyltransferases I and II catalyze the interconversion of acyl CoA and acylcarnitine, and carnitine–acylcarnitine translocase transports acylcarnitine and carnitine in opposite directions across the inner mitochondrial membrane. The acyl group is rendered in color to help you follow its fate.

A new round of β oxidation follows, resulting in the shortening of acyl CoA by another two C. This sequence is repeated as many times as it takes to fully degrade a fatty acid. As an example, follow the fate of palmitate in figure 11.14. The vast majority of fatty acids have an even number of carbons; thus, a fatty acid having *n* C is degraded into *n*/2 acetyl CoA.

In β oxidation, a fatty acid is degraded into a number of acetyl CoA equaling half the number of its carbon atoms.

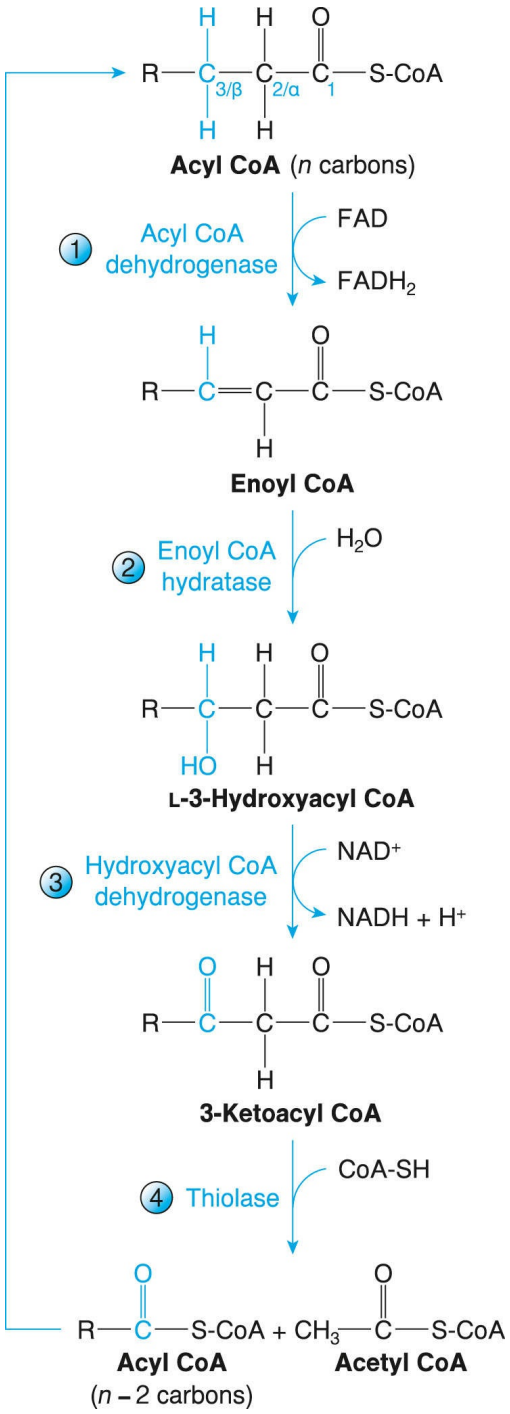


Figure 11.13 **β Oxidation of fatty acids.** Through a series of four reactions, an acyl CoA is broken down into another acyl CoA lacking two C and into acetyl CoA. Only the three terminal carbons of the acyl group on the side of its carboxyl group are presented, since only these take part in the reactions. The shorter acyl CoA produced follows the fate of the initial one through a new round of β oxidation (upward arrow at left). β Oxidation owes its name to the fact that it results in the oxidation of C3 (in color), which, according to older nomenclature, is the β carbon. (The α carbon is C2, next to the carboxyl group.) Indeed, compare the original acyl CoA with the product of the third reaction, 3-ketoacyl CoA, and see that the two H of C3/β

have been replaced by an O.

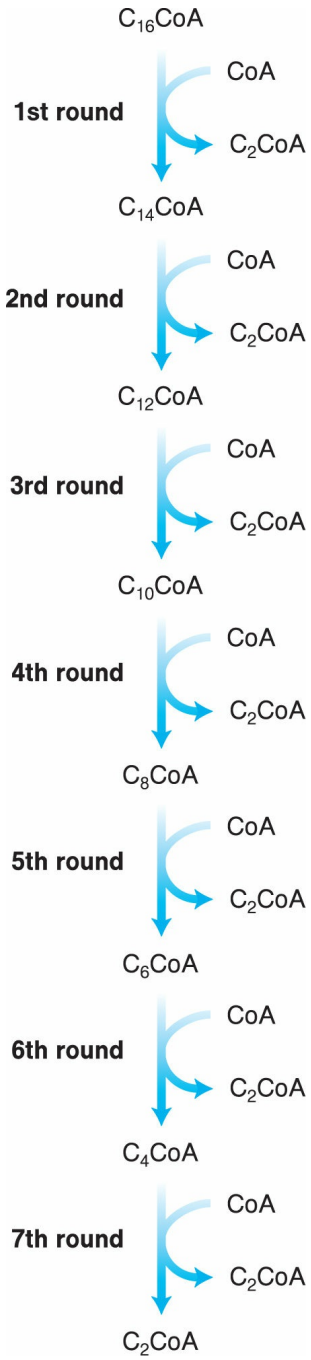


Figure 11.14 **Palmitate degradation.** Starting with the activated form of palmitate (palmitoyl CoA, symbolized as $C_{16}CoA$), seven rounds of β oxidation detach acetyl CoA (C_2CoA) molecules one after the other until nothing but C_2CoA is left. Each pair of arrows (one straight and one curved) in this diagram is equivalent to all the downward arrows in the previous figure.

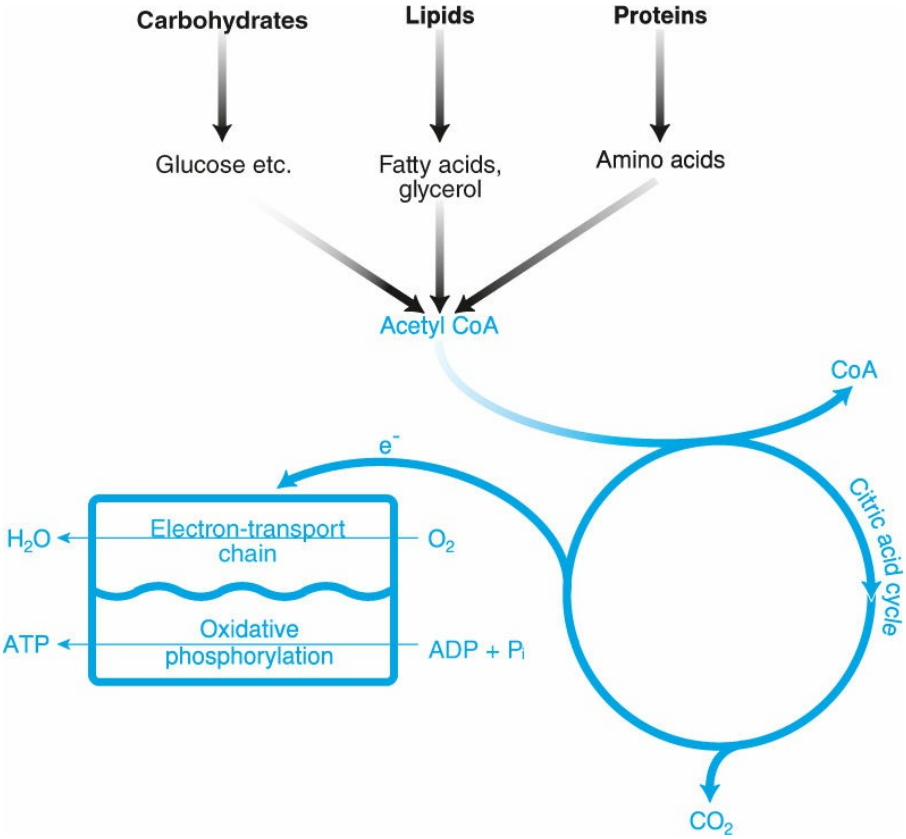
These are produced in $n/2 - 1$ rounds of β oxidation because the final round yields two acetyl CoA. Thus, the overall reaction of palmitate degradation through β oxidation is

$$\text{Palmitoyl CoA} + 7\ \text{CoA} + 7\ \text{FAD} + 7\ \text{NAD}^+ + 7\ \text{H}_2\text{O} \rightarrow 8\ \text{acetyl CoA} + 7\ \text{FADH}_2 + 7\ \text{NADH} + 7\ \text{H}^+ \qquad \Delta G^{\circ\prime} = 55.3\ \text{kcal} \cdot \text{mol}^{-1}$$

(equation 11.17)

You may be surprised to see that although reaction 11.17 is highly endergonic, I have used a unidirectional, rightward-pointing arrow. As with the oxidation of acetyl CoA in the citric acid cycle (section 10.12 and reaction 10.10), β oxidation is coupled to the highly exergonic electron-transport chain, which removes the $FADH_2$ and NADH produced, as we will see below. Thus, the electron-transport chain pulls β oxidation as well as the citric acid cycle.

The products of reaction 11.17, without leaving the mitochondria, are funneled to pathways that are known to you from carbohydrate oxidation: Acetyl CoA enters the citric acid cycle, where it is oxidized to two CO_2 while yielding three NADH, one $FADH_2$, and one GTP (section 10.12). NADH and $FADH_2$ (from β oxidation and from the citric acid cycle) are oxidized in the electron-transport chain to produce large amounts of ATP through oxidative phosphorylation (sections 10.14 through 10.16).



You are here: citric acid cycle, electron-transport chain, and oxidative phosphorylation.

As in the citric acid cycle, oxygen is not directly involved in β oxidation. However, the FAD and NAD^+ that are consumed in the process can be regenerated in the mitochondria only through the electron-transport chain. Thus, β oxidation requires O_2 to operate and must be considered an aerobic process. In addition, the processes through which the products of β oxidation yield energy (that is, the citric acid cycle, electron-transport chain, and oxidative phosphorylation) are also aerobic. Therefore, fatty acid degradation as a whole is aerobic. In fact, there is no anaerobic alternative for energy production from fatty acids (as there is with carbohydrates).

11.10 Energy Yield of Fatty Acid Oxidation

Let's wrap up fatty acid oxidation by sticking with the example of palmitate initially and then generalizing. As you can deduce from figure 11.14 and from the discussion in the preceding section, 8 acetyl CoA are produced after

seven rounds of β oxidation. In the citric acid cycle, these acetyl CoA yield 16 (that is, 8 · 2) CO_2, 24 (that is, 8 · 3) NADH, 8 $FADH_2$, and 8 GTP. Add 7 NADH and 7 $FADH_2$ produced in the seven rounds of β oxidation, and you are ready to count ATP.

- 24 + 7, that is, 31 NADH yield approximately 31 · 2.5, that is, 77.5 ATP.
- 8 + 7, that is, 15 $FADH_2$ yield approximately 15 · 1.5, that is, 22.5 ATP.
- 8 GTP are equivalent to 8 ATP.

The result is a sum of about 108 ATP. This value should be lowered by two ATP that are consumed to activate a fatty acid, although only one ATP seems to be consumed in reaction 11.2. The reason we must count two ATP is that ATP is hydrolyzed to AMP and PP_i, which is further hydrolyzed to two P_i. Thus, two phosphoanhydride linkages are broken down, which is equivalent to the loss of two ATP. Therefore, the net yield of palmitate oxidation is approximately 106 (that is, 108 – 2) ATP.

Remember that glucose also requires the expenditure of two ATP before it starts yielding more ATP through its breakdown (section 10.8). This requirement resembles what usually happens in our personal, social, and professional lives: We need to invest considerable energy and effort into something before we get a generous reward.

The overall reaction of the complete oxidation (or combustion) of palmitate, excluding the accompanying synthesis of ATP, is

$$C_{16}H_{31}O_2^- + 23\ O_2 + H^+ \rightarrow 16\ CO_2 + 16\ H_2O \qquad \Delta G^{\circ\prime} = -2426\ \text{kcal}\cdot\text{mol}^{-1} \quad \text{(equation 11.18)}$$

Reaction 11.18 represents the most exergonic process you will encounter in this book. The amount of free energy released is so high that it can fuel the synthesis of about 106 ATP (having a $\Delta G^{\circ\prime}$ of 668 kcal for 106 moles according to reaction 10.14) while remaining extremely exergonic:

$$\text{Palmitate} + 23\ O_2 + 106\ ADP + 106\ P_i + 107\ H^+ \rightarrow 16\ CO_2 + 106\ ATP + 16\ H_2O \qquad \Delta G^{\circ\prime} = -1758\ \text{kcal}\cdot\text{mol}^{-1} \quad \text{(equation 11.19)}$$

Now let's calculate the ATP yield from the oxidation of any saturated fatty acid with an even number of carbons, such as palmitate. If you substitute $n/2$ for 8 and $n/2 - 1$ for 7 in the calculations at the start of this section, then you

will be able to show that, in general, a saturated fatty acid of *n* carbons yields 7 *n* – 6 ATP.

11.11 Degradation of Unsaturated Fatty Acids

If a fatty acid is unsaturated, it follows a slightly different route of degradation in as many rounds of β oxidation as it has double bonds. Initially, it will be converted into acyl CoA in the cytosol and transported into the mitochondria with the help of carnitine, just like a saturated fatty acid. Then acyl CoA enters the β-oxidation pathway and is degraded as described in section 11.9 until a double bond reaches position 3 or 4 (depending on whether it lies, respectively, after an odd- or even-number carbon in the original fatty acid). Because the enzymes of β oxidation cannot handle an acyl CoA with a double bond so close to coenzyme A, two additional enzymes, an isomerase and a reductase, come into play.

Without going into the details of the reactions that the two enzymes catalyze, I will say that the former is needed to handle a double bond at position 3, and both are needed to handle a double bond at position 4. Thus, oleate, a monounsaturated fatty acid with the double bond at C9 (figure 5.10 and table 5.2) requires only the isomerase, whereas linoleate, a polyunsaturated fatty acid with double bonds at C9 and C12 (figure 5.11 and table 5.2) requires the isomerase for the first double bond and both the reductase and isomerase (in that order) for the second double bond. Which enzymes do the other polyunsaturated fatty acids in table 5.2 require?

The reductase, the isomerase, or both modify the remainder of an unsaturated fatty acid so that it acquires the structure of enoyl CoA, the first intermediate compound of the β-oxidation pathway. Degradation then continues through the pathway until the fatty acid is fully degraded or the next double bond gets in position 3 or 4, in which case the two enzymes resume action. In the end, and one way or another, the entire unsaturated fatty acid is degraded.

What about the energy yield of the oxidation of an unsaturated fatty acid? Because it lacks two hydrogens per double bond, as compared with a saturated fatty acid of the same chain length, an unsaturated fatty acid may

yield slightly fewer ATP molecules (up to 1.5 ATP per double bond, depending on its position). Because this makes little difference, and because of the uncertainty in the ATP yield of NADH and $FADH_2$ oxidation (section 10.16), we can say that the energy yield of the oxidation of an unsaturated fatty acid is similar to that of the corresponding saturated fatty acid.

11.12 Degradation of Odd-Number Fatty Acids

A small fraction of fatty acids in our diet and in our cells bear an odd number of carbon atoms. Like the even-number ones, odd-number fatty acids enter the β-oxidation pathway following conversion into acyl CoA and transport into the mitochondria. After successive rounds of β oxidation, a five-carbon acyl CoA is produced, which, in the next round, splits into acetyl CoA and **propionyl CoA**, the three-carbon acyl CoA (figure 11.15).

To be used in cellular metabolism, propionyl CoA enters a short pathway of three reactions, which ends in the formation of succinyl CoA, the product of the fifth reaction of the citric acid cycle (section 10.12 and figure 10.27). Propionyl CoA can thus be fully oxidized and yield about 16.5 ATP.

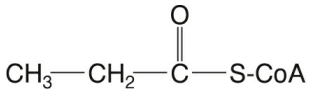


Figure 11.15 Propionyl CoA. This three-carbon acyl CoA is produced in the final round of β oxidation of odd-number fatty acids.

It is remarkable that two of the three enzymes catalyzing the conversion of propionyl CoA into succinyl CoA contain biotin and a derivative of vitamin B_{12} as coenzymes. As mentioned in section 6.1, biotin deficiency is rare, but vitamin B_{12} deficiency is more common, and one consequence of it is nerve damage. Researchers have suggested that the lack of vitamin B_{12} prevents the processing of propionyl CoA, which may hence be used in the synthesis of abnormally high amounts of unusual fatty acids, thus damaging the nervous tissue.

11.13 Fatty Acid Synthesis

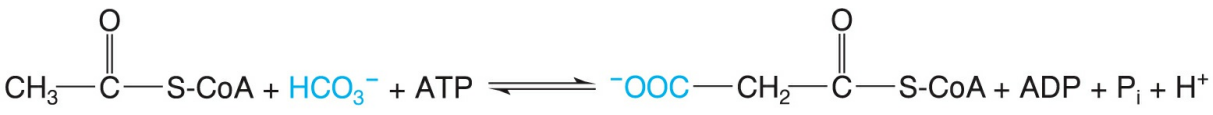
Let's consider now how our cells synthesize fatty acids. Their starting material is acetyl CoA. Fatty acids grow in two-carbon steps, just as they wither, although the path to synthesis is not the reverse of the path to degradation. We have seen the same with the other anabolic pathways that we have encountered so far (glycogenesis, gluconeogenesis, and triacylglycerol synthesis). In fact, the differences between fatty acid synthesis and degradation are more pronounced: The two processes take place in different intracellular compartments, and all reactions and enzymes in the anabolic pathway are different from those in the catabolic. Let's examine, then, the pathway of fatty acid synthesis.

Fatty acids are synthesized in the cytosol, but acetyl CoA is produced in the mitochondrial matrix. This is true whether acetyl CoA derives from carbohydrates through pyruvate oxidation (section 10.10), from fatty acids through β oxidation (section 11.9), or from amino acids through pathways that we will consider in the next chapter. Because acetyl CoA cannot cross the mitochondrial membranes, citrate, formed in the mitochondria from acetyl CoA and oxaloacetate in the first reaction of the citric acid cycle (section 10.12 and figure 10.27), is transported to the cytosol, where it is broken down into these compounds by **citrate lyase**.

$$\text{Citrate} + \text{CoA} + \text{ATP} \rightleftharpoons \text{acetyl CoA} + \text{oxaloacetate} + \text{ADP} + P_i \qquad \text{(equation 11.20)}$$
$$\Delta G^{\circ\prime} = 2 \text{ kcal} \cdot \text{mol}^{-1}$$

Thus, acetyl CoA appears in the cytosol at the expense of ATP.

The linking together of acetyl groups deriving from acetyl CoA to form a fatty acid is not favored thermodynamically. Hence, an even more activated form of the acetyl group is required, and that is **malonyl CoA**. This compound is synthesized from acetyl CoA and bicarbonate by the catalytic action of **acetyl CoA carboxylase** (another biotin-containing enzyme) at the expense of yet another ATP.



Acetyl CoA **Malonyl CoA**

$\Delta G^{\circ\prime} = -1.3 \text{ kcal} \cdot \text{mol}^{-1}$ (equation 11.21)

Although the malonyl group has three carbons, only two (the ones in black) are used in fatty acid synthesis.

Fatty acid synthesis from malonyl CoA is catalyzed by **fatty acid synthase (FAS)**. This remarkable **multifunctional enzyme** catalyzes all the reactions required for synthesis, as substrates pass from one active site to another, with all active sites located in separate domains of a single polypeptide chain. In fact, a fatty acid does not leave the enzyme until synthesis is completed. Thus, FAS is even more impressive than the bifunctional enzyme we encountered in section 10.26.

In short, fatty acid synthesis begins with the covalent linking of an acetyl group from acetyl CoA and a malonyl group from malonyl CoA to two distinct sulfhydryl groups in FAS (figure 11.16). Then the acetyl group is transferred to the middle carbon of the malonyl group, and the carboxyl group of the latter is detached as CO_2. This action results in the formation of a four-carbon group, which is subsequently hydrogenated (NADPH, introduced in section 2.5 and figure 2.8, serves as the hydrogen donor), dehydrated, and hydrogenated a second time (again with NADPH as the hydrogen donor). The end of this first round of fatty acid synthesis produces an acyl group of four C, called the **butyryl group**.

For the second round to begin, the butyryl group shifts to where the acetyl group had been on FAS. This shift permits a second malonyl group to be linked to the enzyme and two of its carbons to be added to the butyryl group. Thus, every round results in elongation of the aliphatic chain by two C (figure 11.17) according to the reaction

$$\text{C}_n\text{-acyl FAS} + \text{malonyl CoA} + 2\ \text{NADPH} + 3\ \text{H}^+ \rightarrow \text{C}_{n+2}\text{-acyl FAS} + \text{CoA} + \text{CO}_2 + 2\ \text{NADP}^+ + \text{H}_2\text{O} \qquad \text{(equation 11.22)}$$

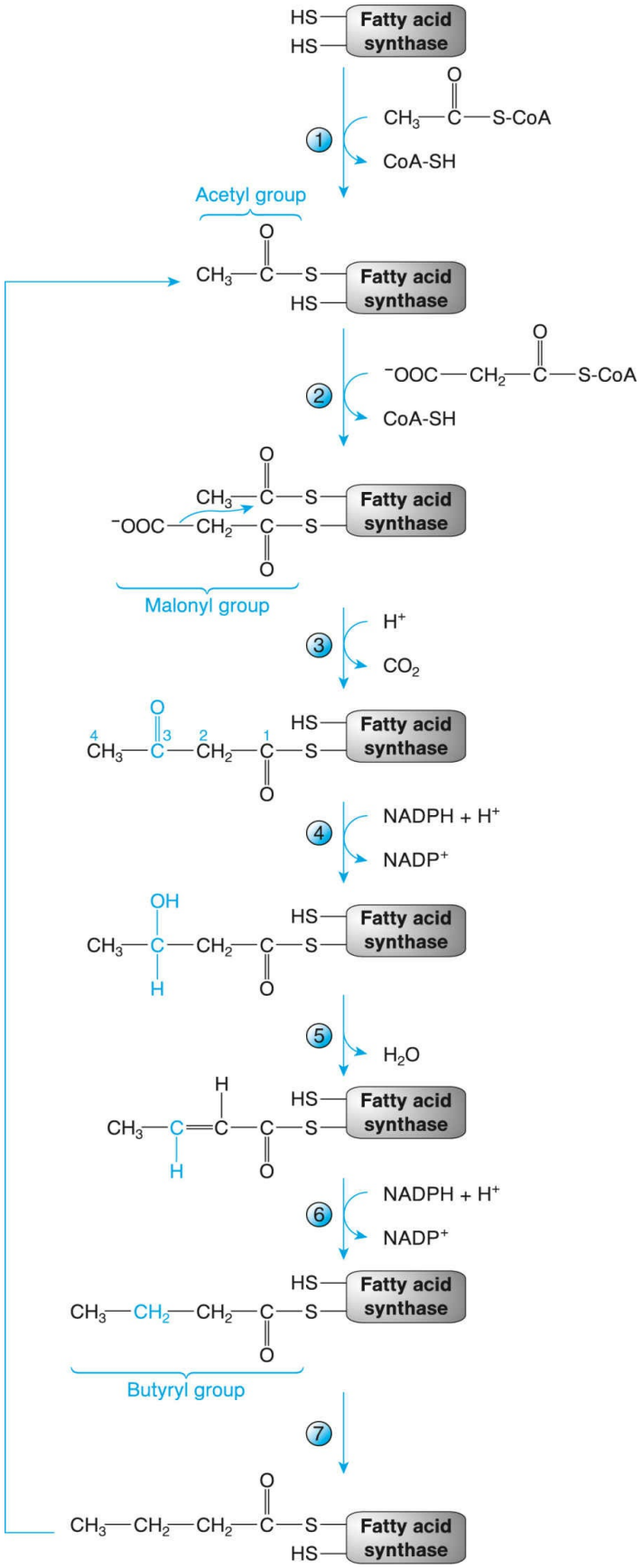

HS
HS
Fatty acid synthase
CH3—C—S-CoA
O
1
CoA-SH
Acetyl group
CH3—C—S
HS
Fatty acid synthase
2
⁻OOC—CH2—C—S-CoA
CoA-SH
CH3—C—S
⁻OOC—CH2—C—S
Fatty acid synthase
Malonyl group
3
H+
CO2
4 3 2 1
CH3—C—CH2—C—S
HS
Fatty acid synthase
4
NADPH + H+
NADP+
OH
CH3—C—CH2—C—S
H
HS
Fatty acid synthase
5
H2O
H
CH3—C=C—C—S
HS
Fatty acid synthase
6
NADPH + H+
NADP+
CH3—CH2—CH2—C—S
HS
Fatty acid synthase
Butyryl group
7
CH3—CH2—CH2—C—S
HS
Fatty acid synthase

Figure 11.16 **The reactions of fatty acid synthesis.** The first round of fatty acid synthesis starts with the attachment of an acetyl group *(1)* and a malonyl group *(2)* to two discrete sites on fatty acid synthase. After two acetyl groups are linked *(3)*, hydrogenated *(4)*, dehydrated *(5)*, and hydrogenated again *(6)*, a butyryl group emerges. This group is transferred to the other site of the synthase *(7)*, so that the second round can begin (upward arrow at left) with the addition of a second malonyl group. One O is replaced by two H on C3 (in color)—the reverse of what happens in β oxidation (figure 11.13).

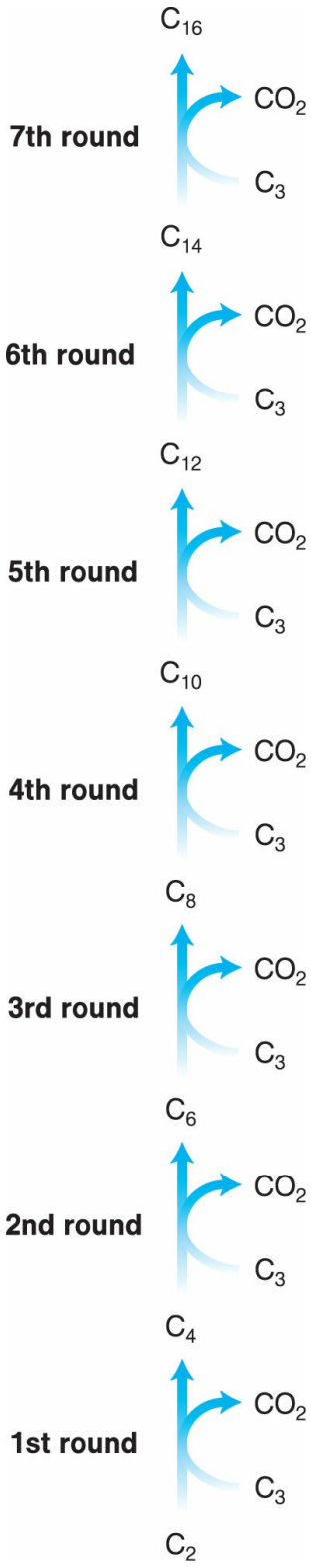


Figure 11.17 **Palmitate synthesis.** Starting with an acetyl group (C_2 at the bottom), seven rounds of fatty acid synthesis result in the formation of palmitate (C_{16}) through the sequential addition of acetyl groups deriving from malonyl groups (C_3) after removal of CO_2. Synthesis is

presented from bottom to top to facilitate comparison with degradation (figure 11.14).

Elongation usually stops at the end of the seventh round with the formation of a palmitoyl group. This group is subsequently detached from FAS by hydrolysis (also catalyzed by a domain of the multifunctional enzyme), resulting in the release of palmitate (figure 11.18). The enzyme, free of acyl groups at both of its sites, is ready to catalyze the synthesis of another palmitate molecule. Thus, the overall reaction of palmitate synthesis is

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ H^+ \rightarrow C_{16}H_{31}O_2^- + 7\ CO_2 + 8\ \text{CoA} + 14\ NADP^+ + 6\ H_2O \qquad \Delta G^{\circ\prime} = -130.4\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 11.23)}$$

The reaction is highly exergonic, but this comes at a price, since palmitate synthesis requires the reducing power—and consumption—of 14 NADPH. Also, remember that the 7 malonyl CoA needed for palmitate synthesis are made from 7 acetyl CoA at the expense of 7 ATP (equation 11.19).

It is instructive to compare palmitate synthesis from 8 acetyl CoA to palmitate degradation into 8 acetyl CoA (figure 11.19). To do so, we must first consider that, apart from the 7 ATP already mentioned, 8 more are needed for palmitate synthesis to get 8 acetyl CoA out of the mitochondria and into the cytosol (reaction 11.19). This addition makes a total of 15 ATP. By contrast, 2 ATP are required to activate palmitate and get β oxidation started, but β oxidation produces 7 NADH and 7 $FADH_2$, yielding a total of about 28 ATP (2.5 per NADH and 1.5 per $FADH_2$) through the electron-transport chain and oxidative phosphorylation. Once more, we see that a biosynthetic process is more expensive than the reverse degradation process is profitable. (Remember the comparison of glycolysis and gluconeogenesis in section 10.24.)

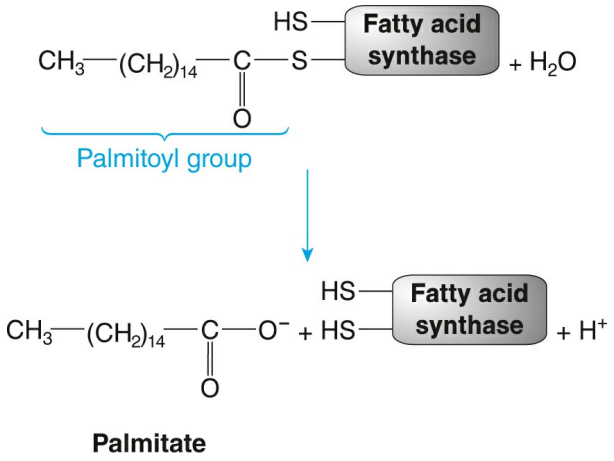


Figure 11.18 **Termination of fatty acid synthesis.** When a palmitoyl group has been synthesized on fatty acid synthase, hydrolysis produces palmitate and an enzyme free of acyl groups, like the one at the top of figure 10.16.

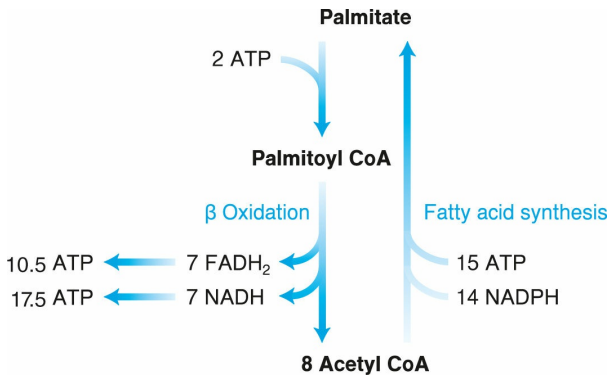


Figure 11.19 **Comparing β oxidation to fatty acid synthesis.** The conversion of palmitate into acetyl CoA in the mitochondria requires 2 ATP and produces 7 NADH (yielding 17.5 ATP) and 7 $FADH_2$ (yielding 10.5 ATP), whereas the conversion of mitochondrial acetyl CoA into palmitate in the cytosol requires 15 ATP and 14 NADPH.

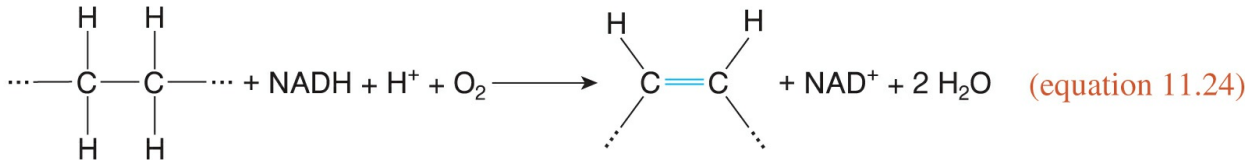
Don't be misled into comparing palmitate synthesis with its complete oxidation (which yields about 106 ATP). The end product of the oxidation process is CO_2, not acetyl CoA (the starting material of fatty acid synthesis).

11.14 Synthesis of Fatty Acids Other Than Palmitate

As we saw in the previous section, fatty acid synthase composes primarily palmitate. How then do our cells synthesize fatty acids other than palmitate?

Shorter fatty acids (comprising a small percentage of the total fatty acid pool of cells) can be formed when an acyl group leaves fatty acid synthase at the end of fewer than seven rounds. Longer fatty acids are synthesized by **elongases** located primarily in the endoplasmic reticulum membrane. These enzymes extend palmitate and other fatty acids by catalyzing the same reactions that fatty acid synthase does.

The endoplasmic reticulum membrane is the site of another modification of fatty acids, that is, the introduction of double bonds. This introduction is performed by protein complexes that include **desaturases**, so named because they convert saturated fatty acids into unsaturated ones. These complexes, acting before or after elongases, remove two hydrogens from adjacent carbons in an acyl CoA. The hydrogen acceptor in this case is one oxygen atom from O_2; the other O reacts with NADH or NADPH to yield NAD^+ or $NADP^+$. Thus two H_2O are formed.



(equation 11.24)

Humans and other mammals lack genes encoding desaturases that can introduce double bonds beyond C10 of a fatty acid. Thus, although we can synthesize any saturated fatty acid, as well as the two monounsaturated ones of table 5.2 (since their double bond is at C9), we are unable to synthesize the polyunsaturated ones, because they contain double bonds beyond C10. Nevertheless, polyunsaturated fatty acids are important components of membrane phospholipids and precursors to a variety of important biomolecules in the human body. The only way of ensuring their adequacy is by consuming organisms (such as plants and fish) that do synthesize them.

Two of the polyunsaturated fatty acids, linoleate and α-linolenate, are termed **essential fatty acids**, in the sense that it is necessary to obtain them from the diet. The term does not usually extend to the remaining polyunsaturated fatty acids, because it is thought that we can synthesize them from linoleate or α-linolenate through a combination of elongations and desaturations. In particular, we can synthesize ω6 fatty acids from linoleate (itself being an ω6 fatty acid), and we can synthesize ω3 fatty acids from α-

linolenate (itself being an ω3 fatty acid).

Now that I have described fatty acid synthesis as well as triacylglycerol synthesis, let me comment on a term that you might encounter in the scientific literature: **lipogenesis**. This rather vague term is used by some authors to describe fatty acid synthesis, by others to describe triacylglycerol synthesis from fatty acids and glycerol, and by yet others to describe both processes in sequence. Because of this ambiguity, I will not use the term.

11.15 Exercise Speeds Up Fatty Acid Oxidation in Muscle

Exercise speeds up the oxidation of fatty acids in muscle primarily by increasing their concentration. This increase is due to

- stimulation of lipolysis in adipose tissue,
- augmented blood flow to the active muscles (resulting in augmented delivery of fatty acids),
- (possibly) enhanced translocation of FABP-PM and FAT/CD36 from an intracellular reservoir to the plasma membrane (in a manner similar to the translocation of GLUT4 in response to exercise or insulin), and
- stimulation of lipolysis in the muscles themselves.

It is also possible that exercise enhances the translocation of FAT/CD36 from its intracellular reservoir to the outer mitochondrial membrane, where it facilitates the delivery of fatty acids to acyl CoA synthetase (also located at the outer mitochondrial membrane; section 11.1).

Thanks to these changes, more fatty acids are converted into acyl CoA and enter the mitochondria, where they are broken down into acetyl CoA through β oxidation. The latter process is accelerated as the concentrations of the substrates for its reactions are increased, one after another. In addition, β oxidation is accelerated during exercise as its products—acetyl CoA, $FADH_2$, and NADH—are avidly consumed in the accelerated citric acid cycle, electron-transport chain, and oxidative phosphorylation (see sections 10.13 and 10.18).

While speeding up fatty acid oxidation, exercise may slow down fatty acid synthesis in muscle by affecting acetyl CoA carboxylase. The enzyme is inhibited by phosphorylation, which is catalyzed by an **AMP-activated protein kinase**. The kinase, which is activated by the increase in [AMP] during exercise, phosphorylates and deactivates acetyl CoA carboxylase. Thus, less malonyl CoA may be synthesized, and less substrate may be available for fatty acid synthesis. A decrease in malonyl CoA might also speed up fatty acid oxidation, since malonyl CoA inhibits carnitine acyltransferase I, the enzyme that lets fatty acids enter the mitochondria. Although attractive, the hypothesis that exercise affects fatty acid metabolism through a decrease in malonyl CoA has not been verified experimentally.

The AMP-activated protein kinase is different from the cAMP-dependent protein kinase A that participates in the cAMP cascade.

The maximal rate of ATP resynthesis through the oxidation of fatty acids coming from the hydrolysis of myocellular triacylglycerols is only 0.3 mmol per kilogram muscle per second. The corresponding rate for the fatty acids deriving from adipose tissue is even lower, just 0.2 mmol · kg^{-1} · s^{-1}, because these fatty acids enter the muscle fibers rather slowly. Both values are lower than the maximal rate of ATP resynthesis through carbohydrate oxidation (0.5 mmol · kg^{-1} · s^{-1}; section 10.22). As a result, lipids cannot support hard exercise. In contrast, they are the major energy source at rest and during light exercise. During moderate-intensity exercise, lipids compete with carbohydrates for first place in ATP resynthesis and gradually move to second place as exercise intensity rises. We will return to the issue of how exercise intensity, along with other factors, determines the choice of energy sources during exercise in chapter 14.

Carnitine Supplementation

Because fatty acid oxidation makes an important contribution to energy supply during endurance exercise, several efforts to increase aerobic endurance performance have been directed at increasing the availability

of fatty acids in the mitochondria. One of the means employed is carnitine supplementation based on the rationale that boosting the carnitine concentration in the active muscles will increase the rate of fatty acid transport into the mitochondria and, hence, their utilization for energy supply. Although many athletes involved in endurance activities apparently take carnitine supplements as **ergogenic aids** (that is, substances or methods used to increase sport performance), most well-designed scientific studies on the matter have shown no effect on performance.

A major reason for the inefficiency of carnitine supplements appears to be that taking carnitine alone fails to raise the muscle carnitine content. However, Benjamin Wall and associates have shown that taking carnitine with carbohydrates (specifically, 2.7 g of L-carnitine and 160 g of a mixture of mono-, oligo-, and polysaccharides daily) for 24 weeks raised the muscle carnitine content and resulted in increased aerobic endurance performance. This helping hand of carbohydrates is due to their stimulatory effect on insulin secretion (section 10.29 and figure 10.45). Insulin, in turn, appears to facilitate the entry of carnitine from the bloodstream into muscle fibers in a way that has not been fully characterized.

Another hypothesis, based on the role of carnitine in facilitating fatty acid transport into the mitochondria, is that carnitine supplements cause loss of body fat and body weight. In fact, L-carnitine is one of the many advertised **fat burners**. However, most studies on the topic have not shown any fat-lowering effect of carnitine supplementation.

11.16 Changes in the Plasma Fatty Acid Concentration and Profile During Exercise

The plasma concentration of fatty acids depends on their rate of appearance, primarily from adipose tissue, and their rate of disappearance, notably toward muscle and the liver (figure 11.11). In contrast to the glucose concentration, the fatty acid concentration in plasma is not subject to strict control. Resting

values range from 0.3 to 0.9 mmol · L^{-1}.

Exercise increases the demand for fatty acids in muscle fibers. If the increased demand is not met by an increased hydrolysis of myocellular triacylglycerols, then the fatty acid concentration in the cytosol decreases. This decrease makes the concentration gradient across the sarcolemma (a gradient that is always inward) steeper and tends to draw in more fatty acids from plasma, thus decreasing their concentration there. On the other hand, the acceleration of lipolysis in adipose tissue tends to increase the fatty acid concentration in plasma.

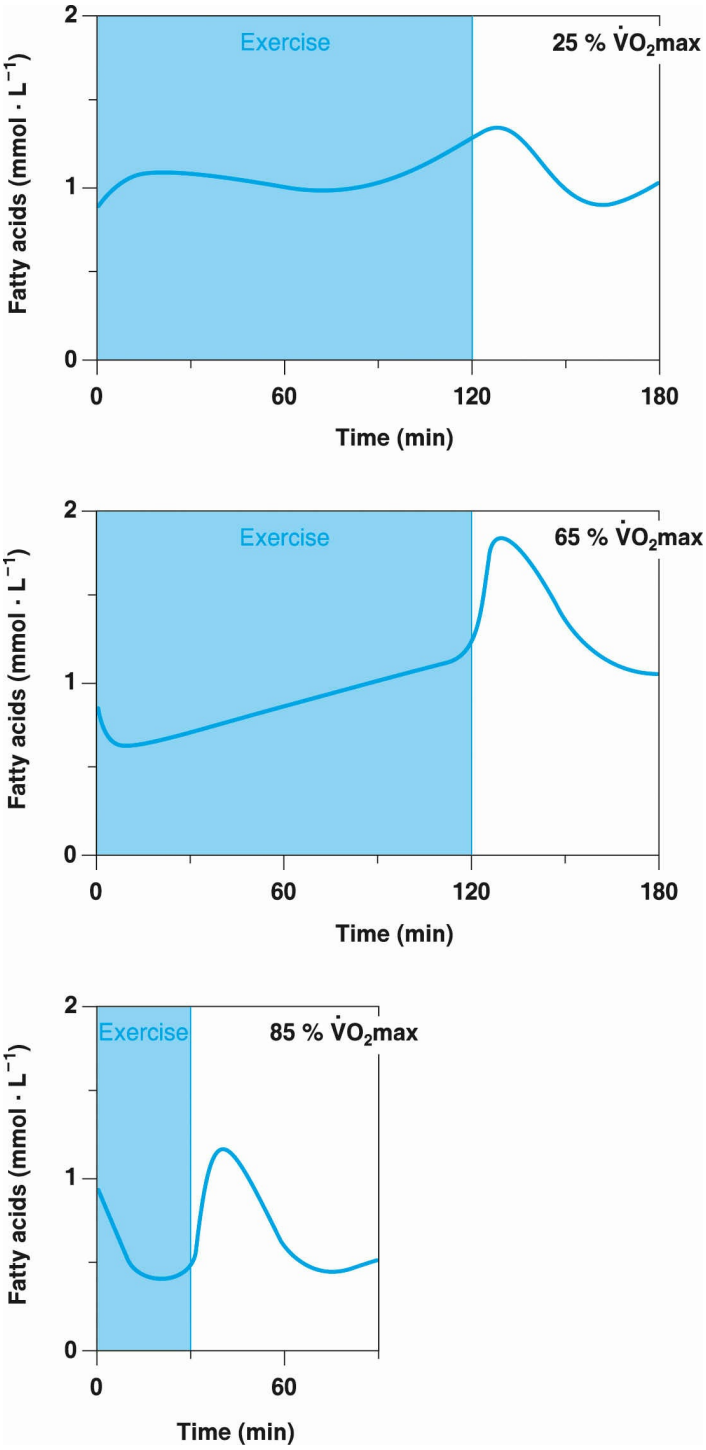


Figure 11.20 **Plasma fatty acid concentration during exercise.** Exercise intensity affects how the plasma fatty acid concentration changes. The graphs show average values for endurance cyclists who exercised at 25%, 65%, and 85% of $\dot{V}O_2$max. Exercise lasted 120 min at the first two intensities but only 30 min at the third intensity because of exhaustion on the part of the participants.

Adapted by permission from J.A. Romijn et al., “Regulation of Endogenous Fat And

Carbohydrate Metabolism in Relation to Exercise Intensity And Duration," *American Journal of Physiology* 265 (1993): E380-E391.

Thus, which way the plasma concentration of fatty acids goes during exercise depends on which of the factors just listed prevail. This question, in turn, depends on exercise intensity. During light exercise, the concentration rises (figure 11.20) because the demand for fatty acids in muscle is low and the lipolytic rate in adipose tissue is high. Moderate-intensity exercise is sometimes characterized by an initial drop, because the increase in fatty acid degradation is faster than the hormonal stimulation of lipolysis. However, as lipolysis catches up with fatty acid degradation, the concentration rises, and it can exceed 2 mmol $\cdot$ L^{-1} in very prolonged exercise tasks.

Finally, hard exercise drives the fatty acid concentration in plasma below baseline and keeps it there. The main reason for this drop is a decrease in the blood flow to adipose tissue due to vasoconstriction, which, together with vasoconstriction in the viscera, counterbalances the increased blood flow to the active muscles (mentioned in section 10.9). Because fatty acids need to bind to plasma albumin in order to circulate (section 11.8), vasoconstriction traps them in the interstitial space and prevents them from appearing in the circulation.

Soon after the end of exercise, the plasma concentration of fatty acids rises because the decrease in their use by the muscles is almost instantaneous, whereas the cessation of the hormonal stimulation of lipolysis takes some minutes. (In section 14.26, I describe how the cAMP cascade is silenced after exercise.) Thus, adipose tissue continues to supply increased amounts of fatty acids to blood even as the demand has returned to the resting level. The jump in the plasma fatty acid concentration is higher after hard exercise because, in addition, the blood flow to adipose tissue returns to normal through vasodilation, which permits the fatty acids previously trapped in the interstitial fluid to appear in the bloodstream.

How the plasma fatty acid concentration responds to exercise may depend on the training state as well. Trained individuals experience a slightly lower increase as compared with untrained ones during moderate-intensity exercise.

Researchers usually measure plasma fatty acids as a whole; that is, they do not separately measure palmitate, oleate, linoleate, and so on. This approach

is partly justified, since almost all individual fatty acids increase in plasma during moderate-intensity exercise. However, they do not increase proportionately. Instead, unsaturated fatty acids seem to increase more than saturated ones, resulting in an elevation of the proportion of the former to the latter. This shift in the profile, or distribution, of plasma fatty acids goes in the direction of their major source, the triacylglycerols of adipose tissue, which usually have a higher proportion of unsaturated to saturated acyl groups. We do not know why such a gap exists between the profiles of adipose tissue acyl groups and plasma fatty acids, but exercise serves to bridge it partially and transiently.

11.17 Interconversion of Lipids and Carbohydrates

From our discussion of carbohydrate and lipid metabolism in exercise so far, it must have become obvious that carbohydrates possess the advantage of faster ATP resynthesis (sections 10.22 and 11.15), whereas lipids possess the advantage of larger energy depots (sections 10.2 and 11.3). Thus, an athlete would love to have carbohydrate stores the size of body fat, which, unfortunately, is impossible. The question then arises: Can fat be converted into carbohydrate?

Let’s explore this question based on the biochemical knowledge you have acquired in chapter 10 and the present chapter. As we have seen, the most abundant of fats, triacylglycerols, are hydrolyzed to glycerol and fatty acids through the pathway of lipolysis (section 11.5). Glycerol can be converted into glucose in the liver and kidneys (section 11.8), but this does not exert much effect on the provision of carbohydrates because glycerol is a minor part of a triacylglycerol. In fact, ten grams of triacylglycerols give rise to just about one gram of glucose.

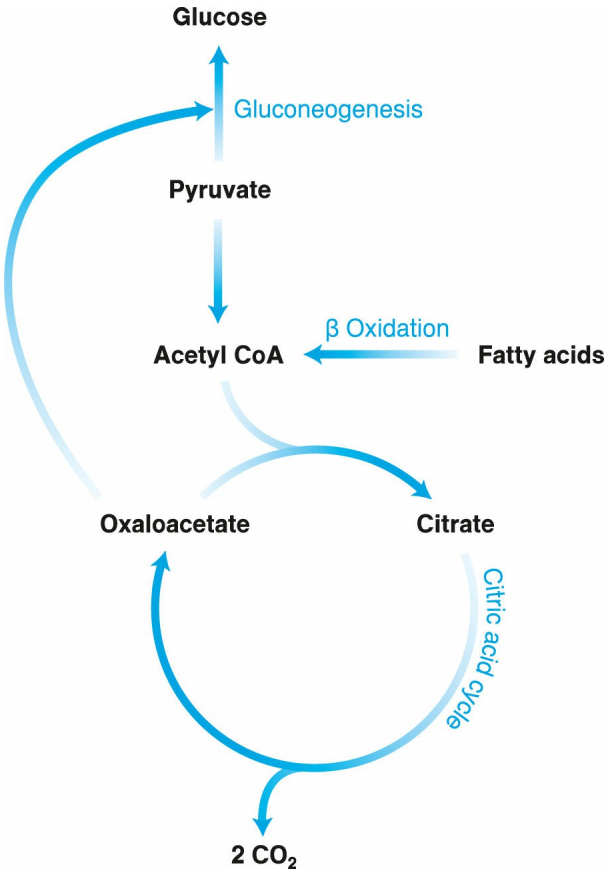


Figure 11.21 **Fatty acids do not form glucose.** Fatty acids are broken down into acetyl CoA, which cannot be converted into pyruvate and, hence, glucose. Acetyl CoA can reach oxaloacetate, a precursor of glucose, through the citric acid cycle, but this does not represent a net conversion, since the carbons entering the cycle are lost as carbon dioxide.

Fatty acids, on the other hand, which comprise the bulk of a triacylglycerol, are unable to produce glucose. Why? Although fatty acid metabolism meets carbohydrate metabolism at acetyl CoA (figure 11.21), acetyl CoA cannot be converted into pyruvate (a starting point of gluconeogenesis). The reason is that the reaction catalyzed by the pyruvate dehydrogenase complex (reaction 10.9) is irreversible, as I have pointed out in section 10.10.

What if acetyl CoA went all the way around the citric acid cycle up to oxaloacetate, which can give rise to glucose through gluconeogenesis (figures 10.36 and 10.38)? This looks like a reasonable course; however, there is no net conversion of acetyl CoA into oxaloacetate, since two carbons enter the cycle by way of the acetyl group and two exit as CO_2.

Thus, we are unable to convert fatty acids into glucose. In contrast, we can convert glucose into fatty acids through acetyl CoA. Moreover, glucose yields L-glycerol 3-phosphate for triacylglycerol synthesis, as described in section 11.4. Hence, glucose *can* form triacylglycerols (figure 11.22), though only a minimal amount of it is converted into fatty acids under physiological conditions.

It follows from this discussion that lipids cannot adequately replenish carbohydrates. Therefore, one must ensure carbohydrate sufficiency in the body through proper nutrition.

11.18 Brown Adipose Tissue

As mentioned in section 11.3, our bodies contain a small amount of brown adipose tissue in addition to white adipose tissue. The two tissues differ in several aspects. First, brown fat is much less prevalent in the human body than white fat: It ranges from about 150 g in newborn infants to just about 50 g in adults. Second, brown adipocytes contain more mitochondria than do white ones. This and the fact that brown fat contains more capillaries account for the tissue's dark appearance and name, since mitochondria are rich in heme-containing proteins of the electron-transport chain (section 10.14) and heme absorbs visible light in the mitochondria just as it does in the erythrocytes.

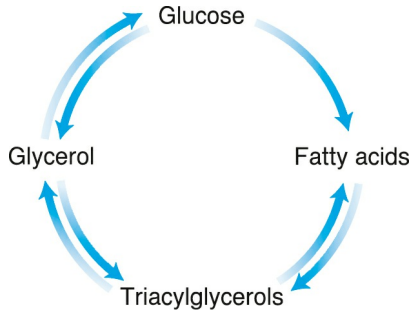


Figure 11.22 Interconversion of lipids and carbohydrates. Animals, including humans, can convert glucose into triacylglycerols, but they cannot convert the largest part of triacylglycerols (the fatty acids) into glucose.

An additional difference is that brown adipocytes contain many small lipid

droplets rather than one large droplet, as white adipocytes do. Finally, brown adipocytes express the gene of **uncoupling protein 1 (UCP1)**, or **thermogenin**, an integral protein of the inner mitochondrial membrane. UCP1 acts as a proton channel, letting protons flow down their electrochemical gradient, that is, from the intermembrane space to the mitochondrial matrix (figure 11.23). Remember that this gradient is due to the flow of electrons along the electron-transport chain and that the return of protons to the mitochondrial matrix through ATP synthase is what powers ATP resynthesis (section 10.16).

By providing an alternative route for protons, *UCP1 uncouples oxidative phosphorylation from the electron-transport chain* (hence the name *uncoupling protein*), thus depriving the mitochondria of much of the energy they need to form ATP. In effect, rather than being stored in ATP, this energy dissipates as heat. Heat production is brown fat's primary function and is termed **nonshivering thermogenesis** for distinction from shivering thermogenesis, introduced in section 8.11. This process gives UCP1 its alternative name, that is, *thermogenin*.

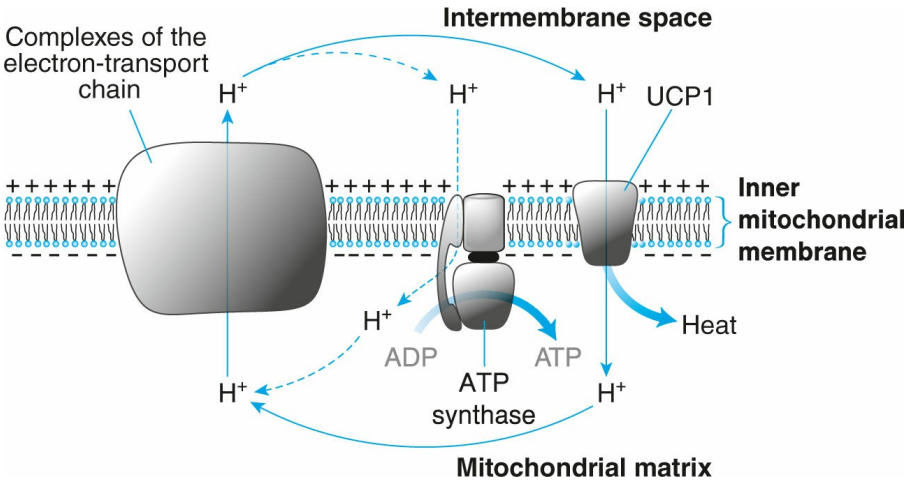


Figure 11.23 **Trading ATP for heat.** Mitochondria in brown adipocytes generate heat rather than ATP. UCP1, an integral protein of their inner membrane, provides an alternative route for the entry of protons into the mitochondrial matrix (dashed lines), just like short-circuiting a battery (the mitochondrion). This entry diminishes ATP synthesis and results in heat production.

Nonshivering thermogenesis is particularly important for newborn infants,

who are more vulnerable to heat loss and hypothermia (low body temperature) than adults are for a variety of reasons, including larger body surface area (a determinant of heat loss) relative to body volume (a determinant of heat production). Nonshivering thermogenesis is activated by cold exposure. The energy for heat production derives not only from the triacylglycerols in brown adipocytes (which are hydrolyzed to glycerol and fatty acids as they are in white adipocytes) but also from blood-borne fatty acids and glucose that enter the cells. These energy sources are oxidized to CO_2, producing NADH and $FADH_2$ on the way as usual. NADH and $FADH_2$ then deliver their electrons to O_2 through the electron-transport chain, providing the energy to pump protons out of the mitochondria. However, rather than fueling ATP synthesis, most of this energy is converted into heat, as just explained.

Because brown fat increases energy expenditure through heat production, it has become an attractive target for body-weight loss and, possibly, other health-related effects. Exercise is one of the means that have been tested to increase brown fat mass, thermogenic activity, or both. Although, in theory, a variety of exercise-related factors, including β-adrenergic stimulation, can speed up catabolism and energy expenditure in brown fat, there is little evidence that this happens in humans. In addition, evidence that training elicits a conversion of white into brown adipocytes in humans is lacking. In fact, contrary to expectations, reports with lead authors Vibha Singhal and Maarten Vosselman concluded that—as with total body fat—brown fat mass, activity, or both were lower in endurance athletes of both sexes as compared with nonathletes of similar age and BMI.

11.19 Plasma Lipoproteins

In section 11.1, I introduced lipoproteins as globular aggregates of lipids and proteins that circulate in the lymphatic and blood vessels. I also mentioned that, in addition to the chylomicrons discussed there, other classes of lipoproteins also exist. Lipoproteins allow the transport of lipids other than fatty acids (which employ albumin, as we saw in section 11.8) in plasma for delivery to tissues. It is now time to meet these classes and learn how

exercise affects their metabolism.

Lipoproteins of all classes are qualitatively similar; hence, their structure can be described as that of a chylomicron (figure 11.3). That is, they all contain thousands of lipid molecules and a few apoprotein molecules. Triacylglycerols and cholesterol esters, being hydrophobic, are hidden inside a lipoprotein particle. Phospholipids and cholesterol, being amphipathic, stay at the surface and form a monolayer with their polar head groups facing outward and their hydrophobic tails pointing to the center. The apoproteins hold the lipids in place and serve as recognition sites for enzymes and receptors.

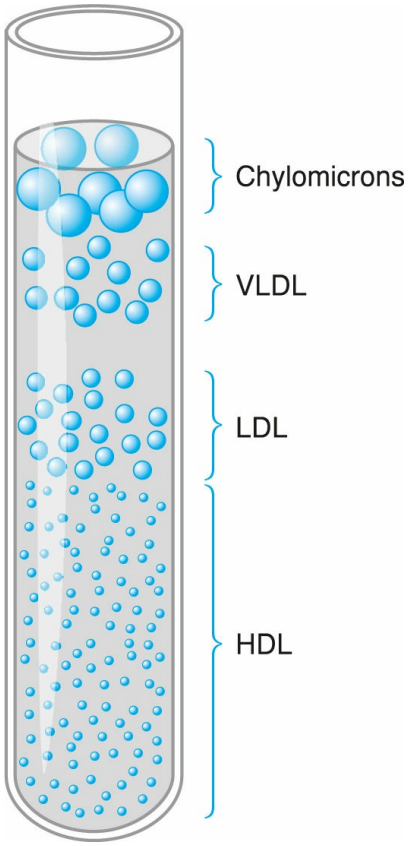


Figure 11.24 **Lipoprotein separation.** By centrifuging a small amount of plasma in a test tube at high speed for many hours, plasma lipoproteins can be separated. Lipoproteins of high density move to the bottom, and lipoproteins of low density stay at the top. HDL particles happen to be the smallest (on average, 10 nm in diameter), followed by LDL (25 nm), VLDL (50 nm), and chylomicrons (200 nm).

How, then, do lipoproteins differ from each other? Their basic

distinguishing feature is density—that is, the ratio of mass to volume—and, as a result, they can be separated by centrifugation, a laboratory technique introduced in section III.7. If we centrifuge a sample of plasma for sufficient time and at sufficient centrifugal force, the lipoproteins in it will arrange themselves in zones of different densities (figure 11.24).

Differences in lipoprotein density result from differences in the proportion of lipid to apoprotein mass, because the density of lipids is about 0.9 g · mL^{-1}, whereas that of proteins exceeds 1.2 g · mL^{-1}. (Just think how oil floats on water, which has a density of 1 g · mL^{-1}, and how a protein powder supplement sinks to the bottom of a glass of water.) Thus, the higher the proportion of apoproteins to lipids in a lipoprotein particle, the higher its density. The major lipoprotein classes, in order of increasing density, are chylomicrons, **very low-density lipoproteins (VLDL)**, **low-density lipoproteins (LDL)**, and **high-density lipoproteins (HDL)**. Table 11.1 summarizes their features.

Table 11.1 The Main Plasma Lipoproteins

Class	Density (g · mL^{-1})	Percentage composition by mass				Major apoproteins
		Triacylglycerols	Cholesterol and cholesterol esters	Phospholipids	Proteins	
Chylomicrons	0.92-0.94	88	4	6	2	B-48, C-II, E
VLDL	0.96-1.00	60	15	15	10	B-100, C-II, E
LDL	1.02-1.06	6	48	23	23	B-100
HDL	1.06-1.21	5	20	25	50	A

Each lipoprotein particle is a mixture of biomolecules, not a specific biomolecule. As a result, a lipoprotein does not have an exact composition. It just happens that each of the four lipoprotein classes encompasses particles of a rather uniform lipidcomposition and of characteristic apoproteins. Thus, the proportions of lipoprotein components in table 11.1 are indicative and may differ slightly among bibliographic sources. To distinguish the apoproteins, we use the letters A, B, C, and E, followed, in some cases, by an Arabic or Latin numeral. For example, we have apoprotein B-100.

11.20 A Lipoprotein Odyssey

Each lipoprotein class plays a distinct biological role in the complex process of transport and distribution of lipids throughout the body. As discussed in section 11.1, chylomicrons carry dietary fat, which consists primarily of triacylglycerols, to extrahepatic tissues, where triacylglycerols are broken down and absorbed. This action results in the shrinking and conversion of chylomicrons into what are known as **chylomicron remnants**, which contain cholesterol, cholesterol esters, phospholipids, and apoproteins.

The liver takes up chylomicron remnants by **endocytosis**—that is, formation of intracellular vesicles by invagination of the plasma membrane—and dismantles them. The liver has a profound biosynthetic and secretory activity, which extends to lipids. It is the main organ of fatty acid synthesis (described in sections 11.13 and 11.14) and cholesterol synthesis (through a pathway that we will not consider), as well as an important site of triacylglycerol synthesis. Thus, fatty acids deriving from food (*exogenous*, as we call them) or synthesized in the liver (*endogenous*) are incorporated in triacylglycerols, packaged in VLDL particles along with cholesterol and cholesterol esters, and released to the circulation.

VLDL deliver hepatic triacylglycerols to extrahepatic tissues. They do so through the catalytic action of lipoprotein lipase (section 11.1 and figure 11.4), which acts on VLDL just as it does on chylomicrons. By gradually losing their triacylglycerols, the VLDL particles shrink to become **intermediate-density lipoproteins (IDL)**. About half of them are taken up by the liver (again through endocytosis), whereas the other half lose almost all of their triacylglycerols by the further action of lipoprotein lipase to become LDL.

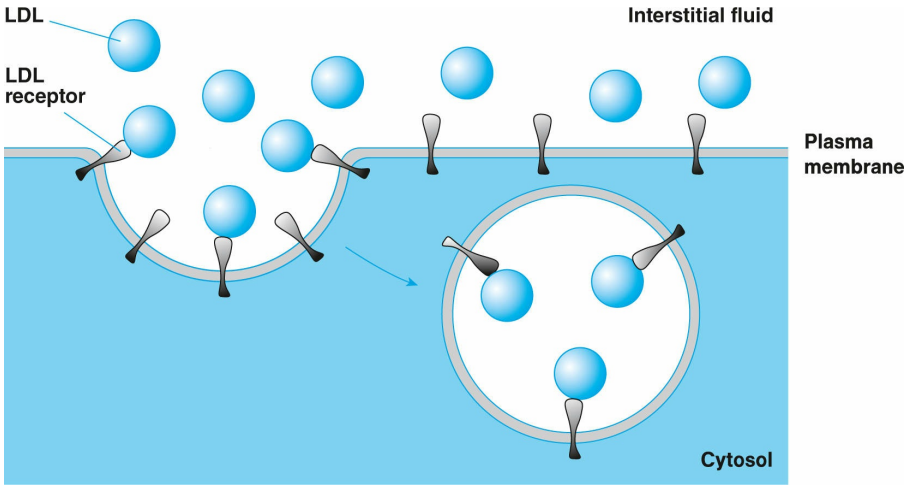
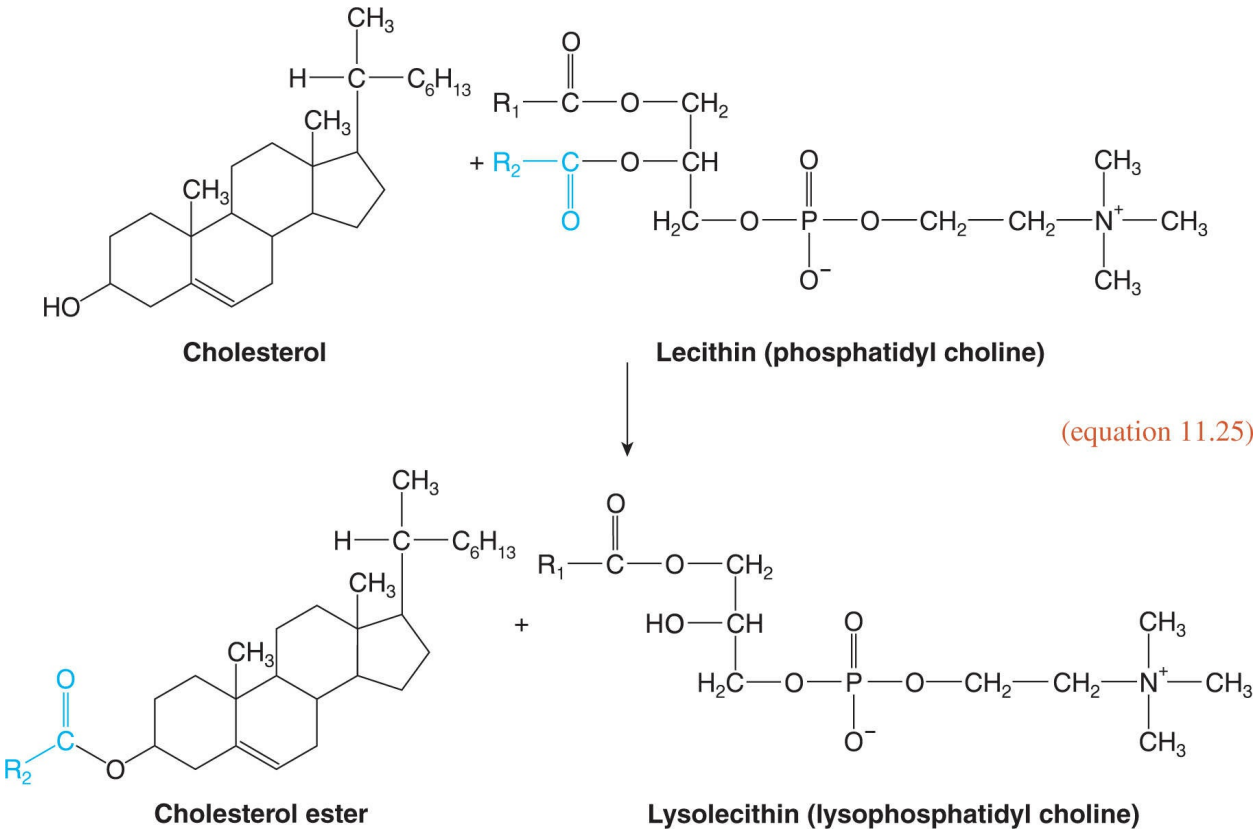


Figure 11.25 **LDL endocytosis.** LDL particles in the interstitial fluid surrounding a cell bind to molecules of the LDL receptor in the plasma membrane. This binding causes the membrane to invaginate and swallow the LDL particles inside a vesicle.

LDL are the main carriers of cholesterol (both free and esterified) in plasma. Cholesterol is delivered to cells through endocytosis of the entire LDL particle (figure 11.25), a process that has been characterized more adequately than the endocytosis of other lipoproteins. Apoprotein B-100, lying at the surface of LDL, binds to a receptor in the plasma membrane, causing the membrane to cave in and enclose the complex of LDL with the receptor. Once in the cytoplasm, the LDL particles are attacked by degradative enzymes, which release cholesterol and the other components of LDL for use by the cell.

HDL carry cholesterol in blood too, but their mission differs from that of LDL. HDL set out as **nascent HDL** from the liver and small intestine to collect cholesterol that is released to the bloodstream from dead cells and restructured membranes. Cholesterol is first incorporated in the shell of nascent HDL; it is then esterified through the attachment of an acyl group deriving from **phosphatidyl choline**, a phospholipid we encountered in section 5.9 and figure 5.15. Phosphatidyl choline is commonly known as **lecithin**, and that name is used when describing this transfer.



By losing an acyl group from position 2 of glycerol, lecithin is converted into **2-lysolecithin**, or **2-lysophosphatidyl choline** (a 2-lysophospholipid, as introduced in section 11.2). The enzyme catalyzing the reaction is **lecithin-cholesterol acyl transferase (LCAT)**. The cholesterol ester formed, being hydrophobic, is sequestered to the core of the lipoprotein particle. In this manner, HDL become engorged with cholesterol esters, which they then recycle in two ways.

- They deliver cholesterol esters to the liver by endocytosis.
- They pass cholesterol esters on to chylomicrons and VLDL with the aid of **cholesterol ester transfer protein** in exchange for triacylglycerols.

Thus, HDL accomplish what is known as **reverse cholesterol transport**.

Figure 11.26 summarizes the life cycle of the lipoprotein classes, and table 11.2 summarizes their biological roles.

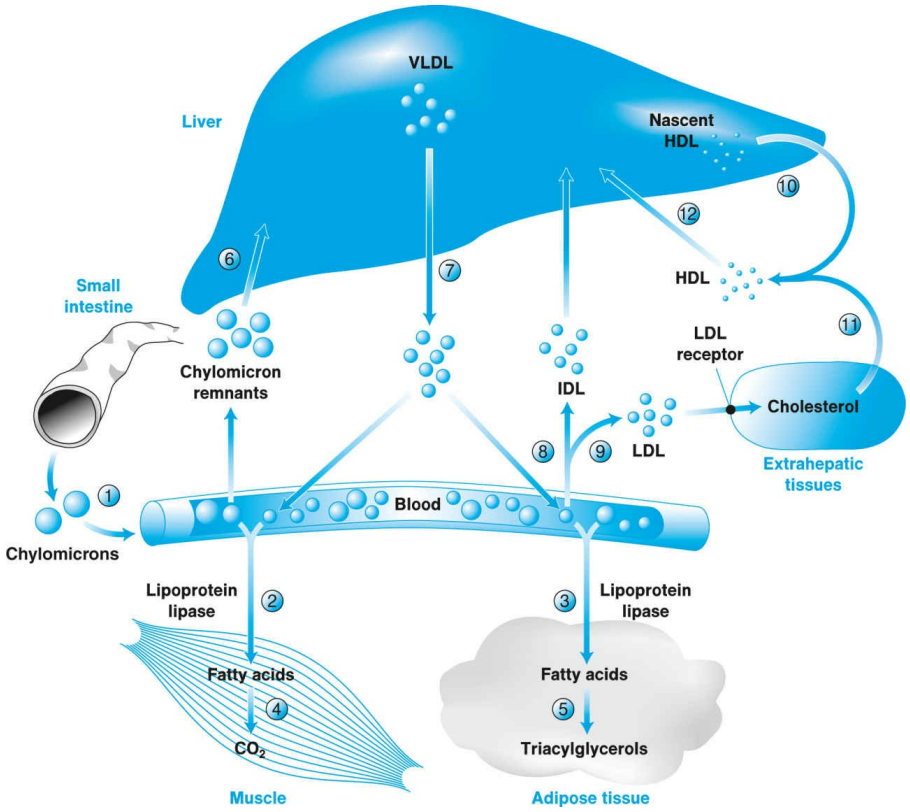


Figure 11.26 **Lipoprotein circulation.** Dietary lipids are packaged in chylomicrons inside the intestinal lining and are sent to the circulation *(1)*. Lipoprotein lipase breaks down chylomicron triacylglycerols in the capillaries of muscles *(2)* and adipose tissue *(3)*, letting the products in. Usually, fatty acids are burned in muscle *(4)* and form triacylglycerols in adipose tissue *(5)*. Chylomicron remnants are taken up by the liver *(6)*, which synthesizes and secretes VLDL *(7)*. VLDL follow the fate of chylomicrons, offering the components of their triacylglycerols to muscle and adipose tissue. Whatever escapes the action of lipoprotein lipase is converted into IDL *(8)*, which return to the liver, or LDL *(9)*, which bind to the LDL receptor and deliver their free and esterified cholesterol to extrahepatic tissues. Finally, nascent HDL from the liver *(10)* and the small intestine (not shown, for clarity) are fed with surplus cholesterol from extrahepatic tissues *(11)* to become mature HDL, which deliver their cholesterol esters to the liver *(12)* or trade them for triacylglycerols with chylomicrons or VLDL (also not shown, for clarity).

Table 11.2 Main Biological Roles of Lipoproteins

Chylomicrons	Transport of dietary triacylglycerols to extrahepatic tissues
VLDL	Transport of hepatic triacylglycerols to extrahepatic tissues
LDL	Transport of cholesterol and cholesterol esters to extrahepatic tissues
HDL	Transport of cholesterol and cholesterol esters from extrahepatic tissues to the liver, chylomicrons, and VLDL

11.21 Effects of Exercise on the Plasma Triacylglycerol Concentration

The plasma triacylglycerol concentration is of interest regarding health. Specifically, it relates to a pathological condition termed **atherosclerosis**, which is a leading cause of **cardiovascular disease (CVD)** and which we will discuss in section 15.1. In the present and next sections, we will explore how exercise affects two of the factors that promote atherosclerosis, that is, high plasma triacylglycerol and cholesterol concentrations. Then, in section 15.2, we will expand this knowledge to other ways in which exercise fights CVD.

The plasma triacylglycerol concentration is affected markedly by heredity and nutrition—factors that lie outside the scope of this book. What about exercise? Examination of its effects on plasma triacylglycerols offers the opportunity to verify the last three principles of exercise metabolism outlined in section III.2. It also affords an opportunity to apply the distinction between the acute and chronic effects of exercise.

Acute Effects

Let's begin with the effects of a single exercise session on plasma triacylglycerols. A review of the literature reveals that most of the studies on the topic have not found a significant change. However, some studies showed a reduction. As Faidon Magkos concludes, it seems that for plasma triacylglycerols to decrease, exercise must involve a rather high energy expenditure, that is, at least 500 kcal (in one long or multiple short bouts performed within a day). In such cases, the reduction ranges from 15% to 30% but is not detectable immediately or shortly after exercise. Instead, it requires 12 to 18 hours to become significant, and it persists for two to three days (figure 11.27). Thus, plasma triacylglycerols do not appear to serve as any considerable energy source during exercise.

If we exercise again before the plasma triacylglycerol concentration returns to baseline, the concentration remains low. It is understood that all measurements are performed in the postabsorptive state so that dietary

triacylglycerols do not interfere. Researchers fulfill this parameter by asking study participants to fast for about 12 hours before providing a blood sample.

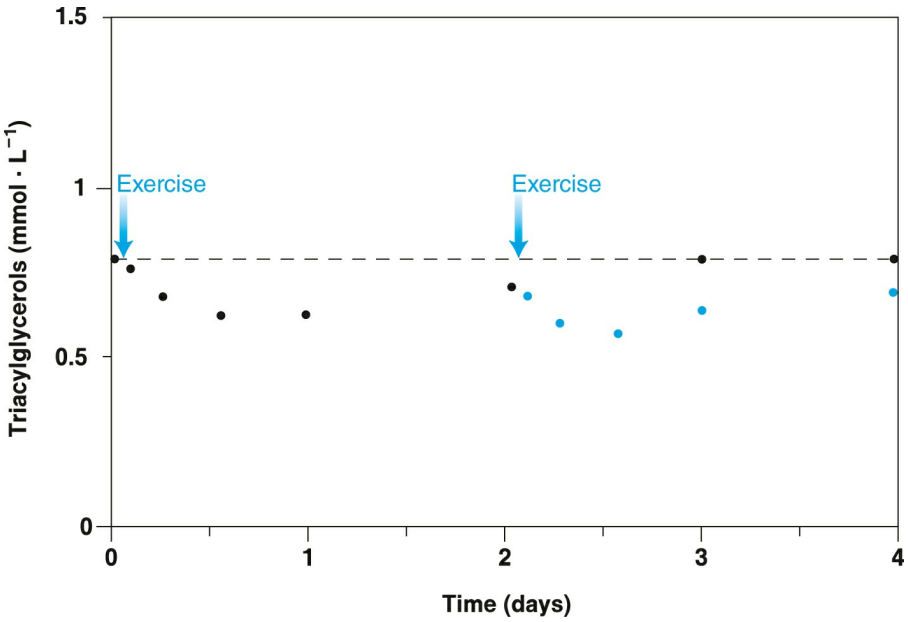


Figure 11.27 **Effect of exercise on plasma triacylglycerols.** An exercise bout of large energy expenditure can decrease the fasting plasma triacylglycerol concentration after a lag of about 15 hours. The concentration returns to baseline (broken line) over a period of two to three days. If we exercise again in the meantime, the concentration is kept low (colored dots). The dots (both black and colored) are not connected with lines because the concentration rises transiently after meals.

The reduction in plasma triacylglycerols after exercise results from their increased (by about 40%) removal from the bloodstream. This removal, in turn, may be due to a change in the composition and morphology of the VLDL particles synthesized by the liver: They are fewer, but each contains more triacylglycerols. This composition makes them more susceptible to the action of lipoprotein lipase, resulting in faster clearance of their triacylglycerols from the circulation. The increase of lipoprotein lipase in the muscle capillaries, which is frequently found after exercise, may contribute to the triacylglycerol-lowering effect of exercise but does not appear to be the primary reason for it.

Most studies of how acute exercise influences the plasma triacylglycerol concentration have employed endurance exercise. Nevertheless, resistance exercise and interval exercise (to be described in detail in section 14.17) may

be as effective in lowering plasma triacylglycerols—or even more effective, in the sense that they produce a similar effect with lower energy expenditure.

Exercise can lower not only the fasting but also the postprandial triacylglycerol concentration in plasma. Numerous studies, reviewed by Eric Freese and associates in a quantitative manner, have shown that **postprandial lipemia**—that is, the rise in the plasma triacylglycerol concentration after a meal of moderate or high fat content—is diminished when one has exercised before the meal, whether on the same day or on the preceding day (figure 11.28). This effect may be due to decreased VLDL secretion from the liver, prolonged activation of lipoprotein lipase in the muscles, or both. Of the different exercise types, interval exercise seems to be the most effective in reducing postprandial lipemia, followed by endurance exercise, followed by resistance exercise. Women exhibit a larger reduction in postprandial lipemia than do men.

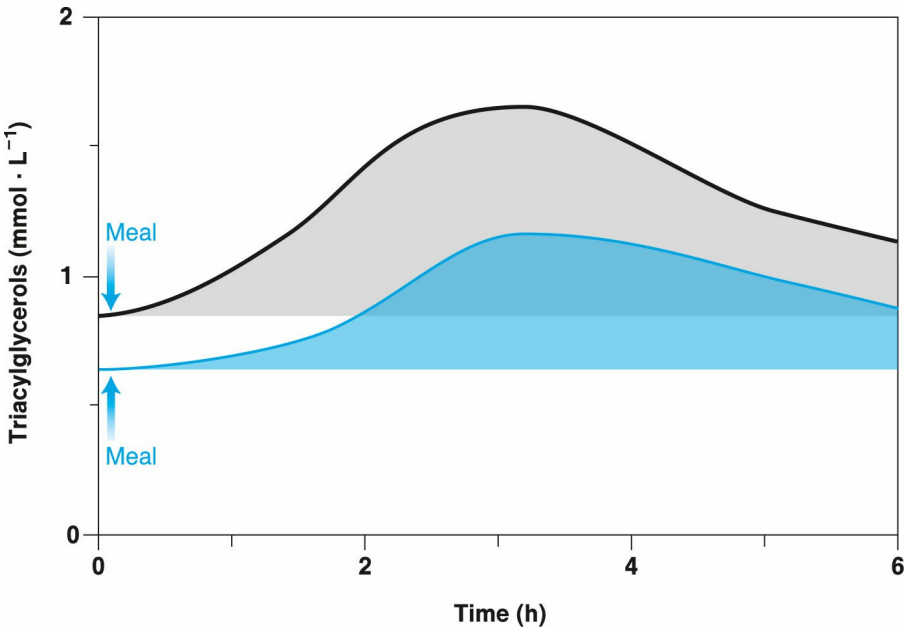


Figure 11.28 Exercise lowers postprandial lipemia. The plasma triacylglycerol concentration rises after we consume a meal and stays elevated for several hours, until the dietary lipids are delivered to the tissues. If we have exercised before the meal, both the fasting concentration (at 0 h) and the postprandial concentration are lower (colored line) as compared with the values in the absence of previous exercise (black line). In addition, the so-called incremental area under the curve (shaded) is less after exercise.

The importance of lowering postprandial lipemia lies in the reduction of

the triacylglycerol load in blood after a meal, which is another risk factor (independent of the fasting triacylglycerol concentration) for CVD. Unfortunately, and similarly to the lowering effect on the fasting concentration, the beneficial effect of exercise on postprandial lipemia is lost two days after exercise.

Chronic Effects

What are the effects of regular exercise on the plasma triacylglycerol concentration? The answer comes from both observational and interventional studies, as delimited in section III.6. Observational studies have shown that endurance athletes (such as long-distance runners), soccer players, and, generally, individuals engaging in regular endurance exercise have lower plasma triacylglycerol concentrations than untrained individuals. In contrast, the values of strength athletes (such as weightlifters) do not differ from the values of untrained individuals.

In addition, many interventional studies have demonstrated the ability of regular exercise to lower the plasma triacylglycerol concentration in both men and women. George and Kristi Kelley have summarized these effects in a quantitative manner through a series of meta-analyses. Specifically, they have found average reductions ranging from 5% to 9% after **endurance training** lasting at least eight weeks or **resistance training** lasting at least four weeks.

These effects may not seem large, but they are clinically significant in reducing the risk of CVD. Additional reductions may be achieved by limiting food consumption: Combining endurance training and dieting for at least four weeks reduced plasma triacylglycerols by 13% according to the Kelleys.

Synopsis

Acute exercise of large energy expenditure and of various types (endurance, resistance, interval) reduces the plasma triacylglycerol concentration for about two days after a lag of about 15 hours. Endurance or resistance training for several weeks produces a small, though clinically relevant, reduction in plasma triacylglycerols.

11.22 Effects of Exercise on the Plasma Cholesterol Concentration

Most studies examining the plasma cholesterol concentration make no distinction between the free and esterified forms, as what seems to matter is the sum of the two. Thus, for the sake of brevity, from here on I will include cholesterol esters in the term *cholesterol* unless I state otherwise. The plasma cholesterol concentration, then, constitutes a risk factor for CVD because it promotes atherosclerosis, just as the plasma triacylglycerol concentration does.

Another important factor in regard to atherosclerosis is the distribution of cholesterol between the two lipoprotein classes that are its main carriers, that is, LDL and HDL. LDL are the main **atherogenic** lipoproteins, whereas HDL hinder the development of atherosclerosis, thus protecting the blood vessels. It appears that HDL play their protective role by removing cholesterol from extrahepatic tissues, including the vessel walls.

Because of their opposite effects on health, LDL and HDL are known as **bad** and **good cholesterol**, respectively.

Exercise can change the concentration of plasma cholesterol in total and in each of its lipoprotein carriers. As in the case of triacylglycerols, the changes are neither spectacular nor unequivocal; nevertheless, they are worth exploring.

Acute Effects

Let’s start again with the acute effects of exercise. Studies have reported a variety of effects, the most frequent one being an increase in the HDL cholesterol concentration after an endurance exercise session of large energy expenditure (in accordance with the lowering of the triacylglycerol concentration). The rise in HDL cholesterol usually mirrors the drop in triacylglycerols: It culminates about one day after exercise and disappears about two days later (figure 11.29). The link between these opposite changes

probably lies in the increased clearance of triacylglycerols from the circulation (described in the preceding section), which causes the VLDL particles to shrink. This shrinkage, in turn, creates a surplus of shell lipids (free cholesterol and phospholipids), which are transferred to HDL. In addition, exercise activates LCAT, which, as we have seen (section 11.20), feeds the HDL particles.

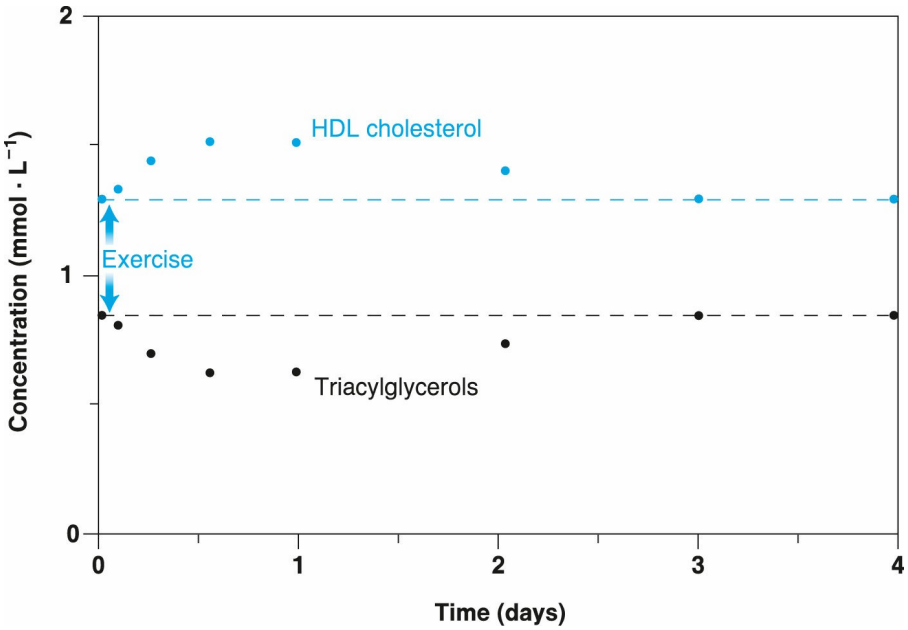


Figure 11.29 **Opposite changes in blood lipids with exercise.** Postexercise plasma concentrations of HDL cholesterol and triacylglycerols usually move in opposite directions. HDL cholesterol increases, whereas triacylglycerols decrease, and both return to their baselines (broken lines) within a few days.

Chronic Effects

Chronic exercise can influence both the concentration of total cholesterol in plasma and its distribution between LDL and HDL. Endurance athletes and other persons who engage in endurance training have lower total and LDL cholesterol concentrations than untrained persons. HDL cholesterol, on the other hand, is high in endurance trained individuals and in individuals who perform physical work regularly as part of their occupation. By contrast, the total, HDL, and LDL cholesterol concentrations in strength athletes do not differ from those of untrained persons.

Interventional studies have confirmed the beneficial effects of endurance training on plasma cholesterol. According to meta-analyses performed by George and Kristi Kelley, programs lasting at least eight weeks lower total cholesterol by 2% and LDL cholesterol by 3% while raising HDL cholesterol by 2% to 3%. Resistance training programs lasting at least four weeks lower total cholesterol by 3% and LDL cholesterol by 5%, but they have no effect on HDL cholesterol. Steven Mann and coworkers recommend at least 30 minutes of moderate-intensity exercise daily, five times per week, progressing to hard exercise and combined with moderate-intensity or hard resistance training three times per week.

As in the case of triacylglycerols, the effects of training on plasma cholesterol are small. Nevertheless, they contribute to reducing the risk of CVD. Larger reductions in total and LDL cholesterol (by 6%) can be achieved by combining endurance training and restriction of food consumption for at least four weeks. However, this combination negates the positive effect of endurance training on HDL cholesterol.

Synopsis

An endurance exercise session of high energy expenditure can increase HDL cholesterol. Endurance or resistance training for several weeks produces small, though clinically relevant, reductions in total and LDL cholesterol. In addition, endurance training raises HDL cholesterol.

All told, *the plasma lipid concentrations of trained individuals (particularly, endurance-trained individuals) lower their risk of atherosclerosis.* For the sake of brevity, we say that trained individuals have a healthy **lipidemic profile**, including low triacylglycerols, total cholesterol, and LDL cholesterol, as well as high HDL cholesterol. (For specific values of a healthy lipidemic profile, see sections 17.6 through 17.8.)

Some authors misname the lipidemic profile as *lipid profile*. However, the latter can refer to any lipids in any tissue. One should not forget the ending *-emic* to specify a reference to blood.

11.23 Exercise Speeds Up Ketone Body Metabolism

As we saw in section 11.9, the acetyl CoA produced from β oxidation enters the citric acid cycle, where the acetyl group is finally oxidized to CO_2. It is obvious that sufficient oxaloacetate must be present for the acetyl group to enter the cycle (refer to figure 10.27). Now remember that in the liver (as discussed in section 10.24), oxaloacetate also participates in gluconeogenesis (figure 11.30*a*), which is accelerated during exercise (section 10.26). This acceleration can cause a shortage of oxaloacetate—a shortage that can be accentuated by inadequate dietary intake of carbohydrates, which lowers hepatic glycogen and blood glucose (figure 11.30*b*). The latter results in increased secretion of glucagon, the very hormone that stimulates gluconeogenesis.

The lack of oxaloacetate in the liver diverts acetyl CoA to **ketogenesis**, a metabolic pathway that ends in the production of **ketone bodies** in the mitochondria (figure 11.31). In the first reaction of the pathway, two acetyl CoA are joined to form **acetoacetyl CoA**. (This reaction is the reversal of the fourth reaction in the final round of β oxidation of an even-number fatty acid, in which acetoacetyl CoA is split into two acetyl CoA; figure 11.12.) In the second reaction of ketogenesis, acetoacetyl CoA receives another acetyl CoA and water to produce **3-hydroxy-3-methylglutaryl CoA**, which, in the third reaction, loses acetyl CoA to form **acetoacetate**, the first ketone body. This four-carbon compound can be viewed as the product of the net linking of two acetyl groups. In the fourth reaction, part of acetoacetate is reduced to **D-3-hydroxybutyrate**, the second ketone body, with NADH serving as the reducing agent. Finally, a minute portion of acetoacetate is decarboxylated (usually without any enzyme involved) to yield **acetone**, the third ketone body.

In ketogenesis, ketone bodies are formed from acetyl CoA.

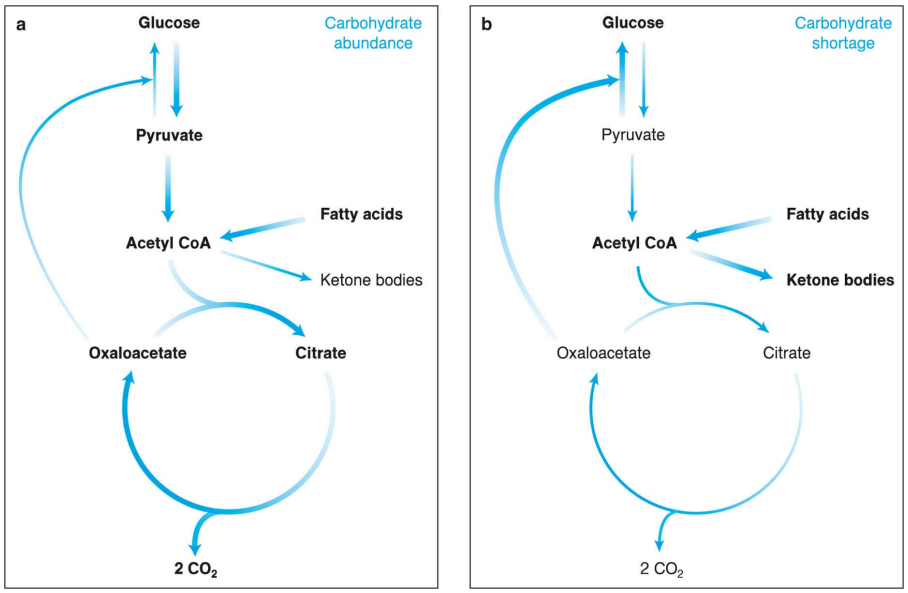


Figure 11.30 **Fats burn in the flame of carbohydrates.** High and low carbohydrate states in the body promote different metabolic pathways in the liver. In the two panels presented here, boldface signifies an abundant metabolite, whereas regular print signifies a scarce metabolite. Thick arrows denote a high rate and thin arrows a low rate. *(a)* When carbohydrates are adequate, there is sufficient oxaloacetate to support the entrance of the acetyl CoA stemming from the breakdown of fatty acids into the citric acid cycle. Thus, fatty acids are fully burned to CO_2. Ketone body formation is minimal, and the rate of gluconeogenesis from pyruvate or oxaloacetate is low. *(b)* Exercise or a shortage of carbohydrates stimulates gluconeogenesis, diverting oxaloacetate to glucose synthesis. Then there is not enough oxaloacetate to join acetyl CoA in the formation of citrate, and the accumulation of acetyl CoA leads to increased ketone body production.

Ketone bodies exit the liver mitochondria and diffuse from the hepatocytes to blood. Acetone is usually exhaled from the lungs, whereas acetoacetate and 3-hydroxybutyrate are taken up by extrahepatic tissues, including muscle. There they can be used as fuel after entering the mitochondria and following the pathway of **ketolysis**. In the first reaction of the pathway (figure 11.32), D-3-hydroxybutyrate is oxidized to acetoacetate by reversal of the fourth reaction of ketogenesis. In the second reaction, acetoacetate is converted into acetoacetyl CoA by receiving coenzyme A from succinyl CoA. Finally, acetoacetyl CoA is split into two acetyl CoA by reversal of the first reaction of ketogenesis.

In ketolysis, ketone bodies are broken down into acetyl CoA.

Acetyl CoA can then be oxidized in the citric acid cycle and yield ATP through the electron-transport chain and oxidative phosphorylation. This action is feasible in most extrahepatic tissues because, in contrast to the liver, they do not synthesize glucose; hence, they have adequate oxaloacetate. Thus the liver can provide an energy source to the active muscles through lipid metabolism, as it does through carbohydrate metabolism (sections 10.27 and 10.28).

The concentration of ketone bodies in blood and the ketolytic rate in muscle increase during prolonged exercise, although neither becomes high enough to render acetoacetate and 3-hydroxybutyrate major fuels for the active muscles. As summarized by Mark Evans and collaborators, the contribution of ketone bodies to energy expenditure during exercise ranges from 2% to 10%.

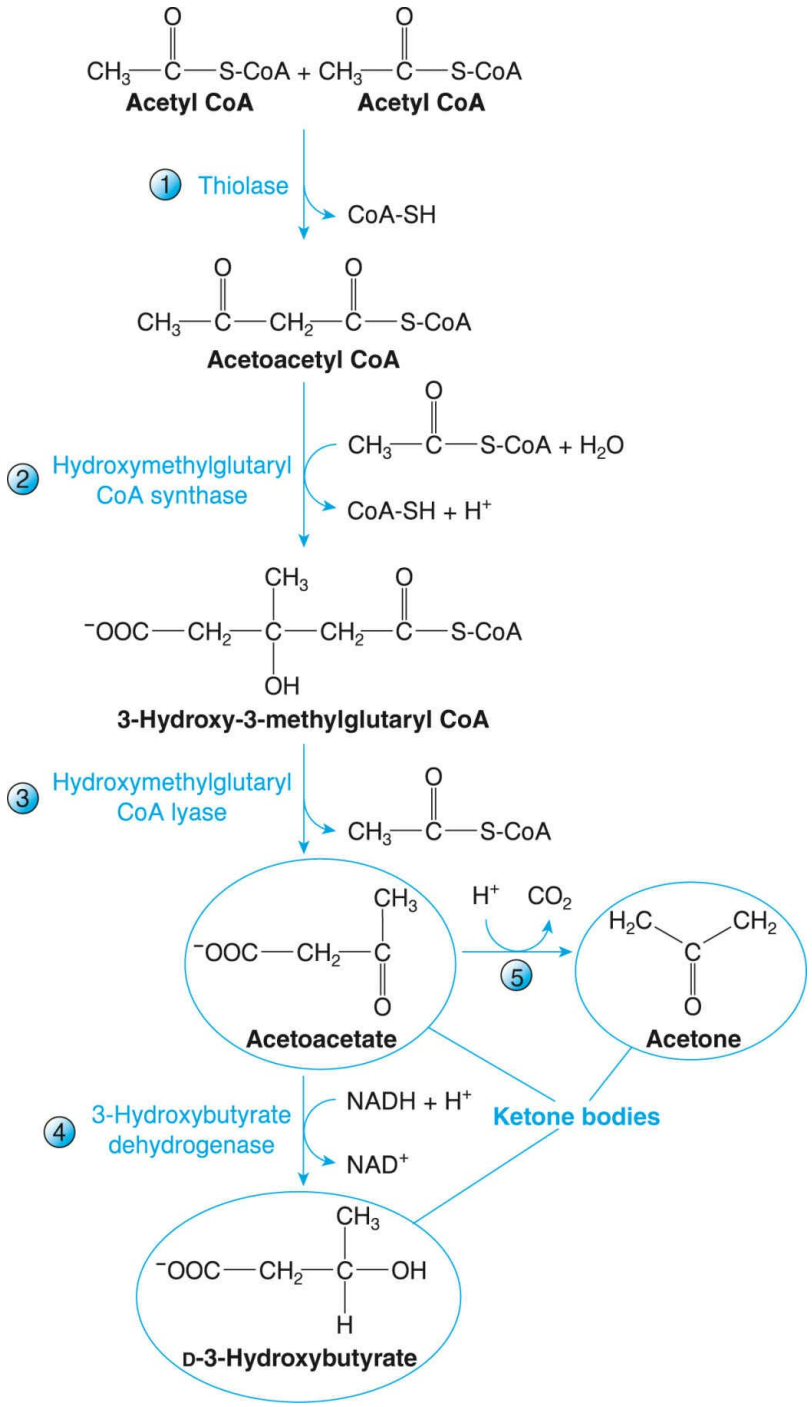


Figure 11.31 **Ketogenesis.** Ketone bodies are synthesized from two acetyl CoA in the liver.

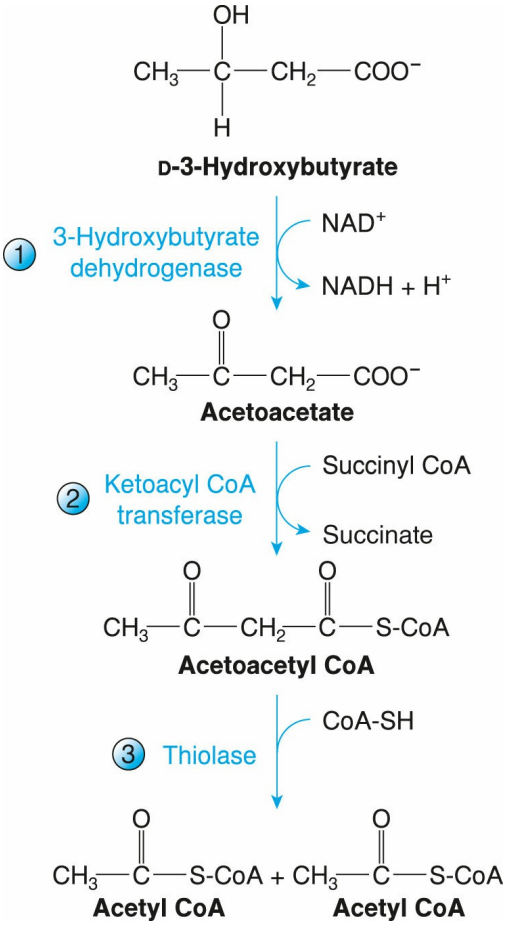


Figure 11.32 **Ketolysis.** Ketone bodies are broken down into two acetyl CoA in extrahepatic tissues.

Summary

The lipids that serve as energy sources during exercise include the triacylglycerols of adipose tissue and muscle, as well as the fatty acids of plasma and muscle. Adipose-tissue triacylglycerols are the largest energy depots in the body. They are synthesized from dietary fatty acids and glucose entering the adipocytes after a meal, and they are hydrolyzed to fatty acids and glycerol through the pathway of lipolysis in the postabsorptive state and during exercise. Exercise speeds up lipolysis thanks primarily to the increase in epinephrine and (when it happens) the decrease in insulin secretion. Fatty acids produced from lipolysis in

adipocytes exit to the bloodstream and are taken up by the active muscles, whereas those produced from the breakdown of myocellular triacylglycerols are used on the spot. Fatty acids are degraded only aerobically in the mitochondria, where the long-chain ones enter with the help of carnitine. Fatty acids are first oxidized to acetyl CoA through the pathway of β oxidation and then to CO_2 through the citric acid cycle. $FADH_2$ and NADH produced in the two pathways cash in their reducing power in the electron-transport chain and oxidative phosphorylation. Fatty acid oxidation increases during exercise and yields more energy than does carbohydrate oxidation (for example, palmitate yields about 106 ATP, against 30 ATP from glucose), albeit at a lower rate. As a result, fatty acids serve as a major energy source in light and moderate-intensity efforts but not during hard exercise.

Triacylglycerols, phospholipids, cholesterol, and cholesterol esters are carried in plasma by globular aggregates called *lipoproteins* and divided into chylomicrons, VLDL, LDL, and HDL. Chylomicrons transport primarily triacylglycerols synthesized in the small intestine from dietary fat to extrahepatic tissues; VLDL transport triacylglycerols synthesized in the liver to extrahepatic tissues; LDL transport cholesterol to extrahepatic tissues; and HDL transport cholesterol from extrahepatic tissues to the liver, chylomicrons, and VLDL. The concentration of plasma lipids does not change much during or after exercise. However, regular endurance exercise can decrease plasma triacylglycerols, total cholesterol, and LDL cholesterol, while increasing HDL cholesterol. Resistance training elicits similar changes except for the latter. These changes lower the risk of atherosclerosis.

Ketone bodies (primarily acetoacetate and 3-hydroxybutyrate) form in the liver from fatty acids when there is a dearth of carbohydrates. Ketone bodies are transported to extrahepatic tissues, where they are burned for energy production. Ketogenesis increases during exercise, but the contribution of ketone bodies to the energy requirements of exercise is small.

Problems and Critical Thinking Questions

1. What do the digestion, absorption, and distribution of dietary triacylglycerols, phospholipids, and cholesterol esters have in common?
2. (Integrative problem) Order the following energy sources of an average man and an average woman from the largest to the smallest.
    a. Man's glycogen
    b. Woman's glycogen
    c. Man's triacylglycerols
    d. Woman's triacylglycerols
3. Which enzymes catalyzing triacylglycerol hydrolysis have we met? How do they differ?
4. What are the protagonists of lipolysis in adipose tissue and muscle? What is the role of each one?
5. What changes in concentration speed up lipolysis in adipose tissue during exercise?
6. Calculate the ATP yield of the oxidation of fatty acids with 12, 14, and 18 carbons.
7. (Integrative problem) Although arachidonate (table 5.2) has double bonds beyond C10, it is not classified as an essential fatty acid, because our cells can synthesize it from linoleate. Suggest how.
8. The concentrations of which plasma lipids change with endurance training, and in which direction?
9. (Integrative problem) Suppose that the model man described in sections 10.2 and 11.3 performed moderate-intensity exercise for one hour, spending 400 kcal. Also suppose that this energy derived equally from the aerobic breakdown of carbohydrates and lipids. (For the sake of simplicity, assume that other sources made negligible contributions.)

a. How many grams of carbohydrates and of lipids did the man burn?

b. What percentage of his body glycogen and of his lipid stores did he consume?

10. (Integrative problem) Following up on the preceding problem, calculate the ATP yield (in mmol) from each of the two sources. For the sake of simplicity, assume that carbohydrates are in the form of glycogen, with a molecular mass of 162 Da for the glucosyl unit, and that lipids are in the form of palmitic acid, with a molecular mass of 256 Da. How do the two ATP yields compare?
11. Answer problem 9 for the model woman described in sections 10.2 and 11.3, who spent 300 kcal by performing moderate-intensity exercise for one hour.
12. Answer problem 10 for the model woman.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

lipid
triacylglycerol
phospholipid
cholesterol
fatty acid
triacylglycerol digestion
bile acid
emulsion
cholecystokinin
pancreatic lipase
2-monoacylglycerol
triacylglycerol absorption

acyl coenzyme A, acyl CoA
palmitoyl group
palmitoyl CoA
acyl CoA synthetase
monoacylglycerol acyltransferase
diacylglycerol acyltransferase
triacylglycerol synthetase complex
triacylglycerol distribution
lipoprotein
chylomicron
apolipoprotein, apoprotein
lipoprotein lipase
glycerol
monoacylglycerol lipase, MGL
glycerophospholipid
pancreatic phospholipase A_2
2-lysophospholipid
pancreatic cholesterol esterase
acyl CoA:cholesterol acyltransferase
white adipose tissue, white fat
brown adipose tissue, brown fat
adipocyte
perilipin
perilipin 1
L-glycerol 3-phosphate
glycerol phosphate dehydrogenase
glycerol phosphate acyltransferase
phosphatidate
lipolysis
adipose triacylglycerol lipase, ATGL

hormone-sensitive lipase, HSL
1,3-diacylglycerol
2,3-diacylglycerol
1-monoacylglycerol
epinephrine
adrenal medulla
β-adrenergic receptor
cAMP cascade
protein kinase A, PKA
comparative gene identification 58, CGI–58
α_2-adrenergic receptor
phosphodiesterase 3B
myocellular triacylglycerol
reesterification
albumin
glycerol kinase
fatty acid-binding protein at the plasma membrane, FABP-PM
fatty acid translocase, FAT
cluster of differentiation 36, CD36
fatty acid transport protein, FATP
cytosolic fatty acid-binding protein, FABP-C
β oxidation
L-carnitine
acylcarnitine
carnitine acyltransferase I
carnitine–acylcarnitine translocase
carnitine acyltransferase II
carnitine palmitoyltransferase I
carnitine palmitoyltransferase II
enoyl CoA

L-3-hydroxyacyl CoA
3-ketoacyl CoA
propionyl CoA
citrate lyase
malonyl CoA
acetyl CoA carboxylase
fatty acid synthase, FAS
multifunctional enzyme
butyryl group
elongase
desaturase
essential fatty acid
lipogenesis
AMP-activated protein kinase
ergogenic aid
fat burner
uncoupling protein 1, UCP1, thermogenin
nonshivering thermogenesis
very low-density lipoprotein, VLDL
low-density lipoprotein, LDL
high-density lipoprotein, HDL
chylomicron remnant
endocytosis
intermediate-density lipoprotein, IDL
nascent HDL
phosphatidyl choline, lecithin
2-lysolecithin, 2-lysophosphatidyl choline
lecithin-cholesterol acyl transferase, LCAT
cholesterol ester transfer protein
reverse cholesterol transport

atherosclerosis
cardiovascular disease, CVD
postprandial lipemia
endurance training
resistance training
atherogenic
bad cholesterol
good cholesterol
lipidemic profile
ketogenesis
ketone body
acetoacetyl CoA
3-hydroxy-3-methylglutaryl CoA
acetoacetate
D-3-hydroxybutyrate
acetone
ketolysis

References and Suggested Readings

Eichmann TO, Kumari M, Haas JT, Farese RV Jr, Zimmermann R, Lass A, Zechner R (2012). Studies on the substrate and stereo/regioselectivity of adipose triglyceride lipase, hormone-sensitive lipase, and diacylglycerol-*O*-acyltransferases. *Journal of Biological Chemistry* 287: 41446-41457.

Evans M, Cogan KE, Egan B (2017). Metabolism of ketone bodies during exercise and training: Physiological basis for exogenous supplementation. *Journal of Physiology* 595: 2857-2871.

Frayn KN (2009). Fat as fuel: Emerging understanding of the adipose tissue–skeletal muscle axis. *Acta Physiologica* 199: 509-518.

Freese EC, Gist NH, Cureton KJ (2014). Effect of prior exercise on postprandial lipemia: An updated quantitative review. *Journal of Applied Physiology* 116: 67-75.

Iqbal J, Hussain MM (2009). Intestinal lipid absorption. *American Journal of Physiology, Endocrinology, and Metabolism* 296: E1183–E1194.

Kelley GA, Kelley KS (2006). Aerobic exercise and lipids and lipoproteins in men: A meta-analysis of randomized controlled trials. *Journal of Men's Health and Gender* 3: 61-70.

Kelley GA, Kelley KS (2009). Impact of progressive resistance training on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. *Preventive Medicine* 48: 9-19.

Kelley GA, Kelley KS (2012). Comparison of aerobic exercise, diet or both on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. *Clinical Nutrition* 31: 156-167.

Kelley GA, Kelley KS, Tran ZV (2004). Aerobic exercise and lipids and lipoproteins in women: A meta-analysis of randomized controlled trials. *Journal of Women's Health* 13: 1148-1164.

Kimmel AR, Sztalryd C (2016). The perilipins: Major cytosolic lipid droplet-associated proteins and their roles in cellular lipid storage, mobilization, and systemic homeostasis. *Annual Reviews of Nutrition* 36: 471-509.

Knapp M, Górski J (2017). The skeletal and heart muscle triacylglycerol lipolysis revisited. *Journal of Physiology and Pharmacology* 68: 3-11.

Magkos F (2009). Basal very low-density lipoprotein metabolism in response to exercise: Mechanisms of hypotriacylglycerolemia. *Progress in Lipid Research* 48: 171-190.

Mann S, Beedie C, Jimenez A (2014). Differential effects of aerobic exercise, resistance training and combined exercise

modalities on cholesterol and the lipid profile: Review, synthesis, and recommendations. *Sports Medicine* 44: 211-221.

Mittendorfer B, Horowitz JF, Klein S (2002). Effect of gender on lipid kinetics during endurance exercise of moderate intensity in untrained subjects. *American Journal of Physiology Endocrinology and Metabolism* 283: E58-E65.

Noland RC (2015). Exercise and regulation of lipid metabolism. *Progress in Molecular Biology and Translational Science* 135: 39-74.

Petridou A, Chatzinikolaou A, Avloniti A, Jamurtas A, Loules G, Papassotiriou I, Fatouros I, Mougios V (2017). Increased triacylglycerol lipase activity in adipose tissue of lean and obese men during endurance exercise. *Journal of Clinical Endocrinology and Metabolism* 102: 3945-3952.

Singhal V, Maffazioli GD, Ackerman KE, Lee H, Elia EF, Woolley R, Kolodny G, Cypess AM, Misra M (2016). Effect of chronic athletic activity on brown fat in young women. *PLoS ONE* 11: e0156353.

van Hall G (2015). The physiological regulation of skeletal muscle fatty acid supply and oxidation during moderate-intensity exercise. *Sports Medicine* 45: S23-S32.

Vosselman MJ, Hoeks J, Brans B, Pallubinsky H, Nascimento EB, van der Lans AA, Broeders EP, Mottaghy FM, Schrauwen P, van Marken Lichtenbelt WD (2015). Low brown adipose tissue activity in endurance-trained compared with lean sedentary men. *International Journal of Obesity* 39: 1696-1702.

Wall BT, Stephens FB, Constantin-Teodosiu D, Marimuthu K, Macdonald IA, Greenhaff PL (2011). Chronic oral ingestion of L-carnitine and carbohydrate increases muscle carnitine content and alters muscle fuel metabolism during exercise in humans. *Journal of Physiology* 589: 963-973.

CHAPTER 12

Protein Metabolism in Exercise

Learning Objectives

After reading this chapter, you should be able to do the following:

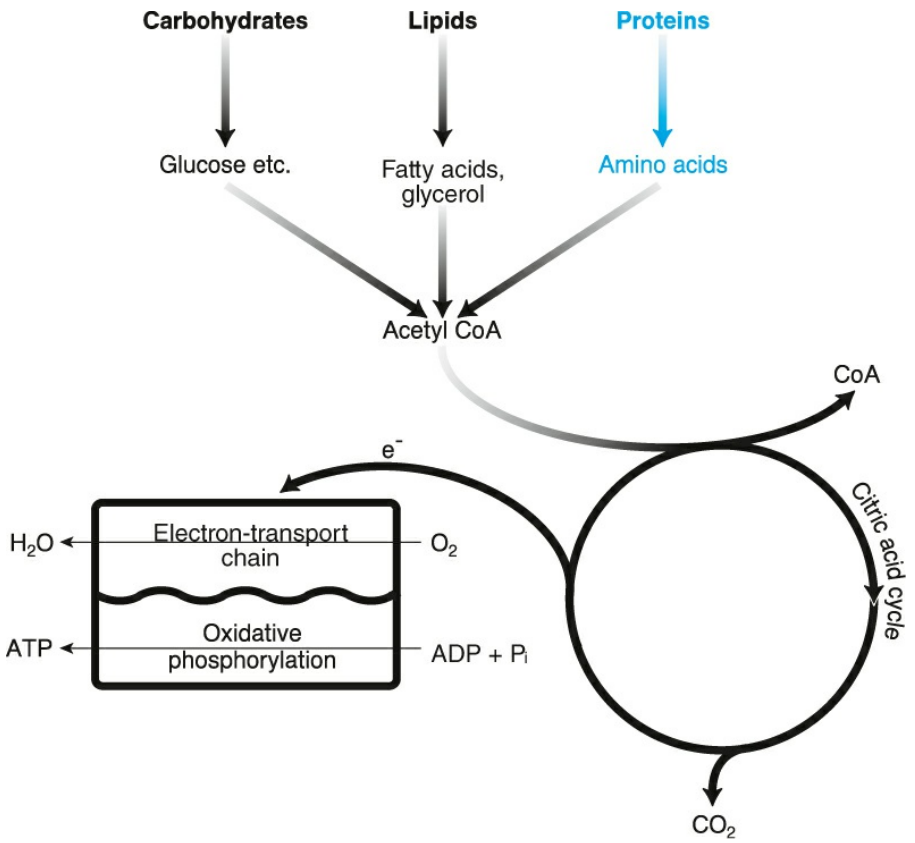
- Discuss how we process dietary proteins in the body.
- Describe the protein content of the human body.
- Compare how different exercise types affect protein turnover in muscle.
- Describe amino acid degradation and synthesis.
- Explain amino acid metabolism in muscle and liver during exercise.
- Discuss the glucose-alanine cycle.
- Describe the features of the urea cycle, the fate of urea, and how the urea cycle is affected by exercise.
- State the contribution of proteins to the energy expenditure of exercise and calculate protein use in exercise.
- Contrast the chronic effects of different types of training on muscle protein metabolism.
- Discuss the various aspects of protein and amino acid supplementation in exercise and training.

In chapter 3, we explored proteins in terms of both structure and function. The chapters that followed were replete with them: We met a plethora of enzymes, contractile proteins, transporters, receptors, and peptide hormones. Their participation in numerous biochemical processes confirms the pivotal role of proteins in the functions of life. Add to this the fact that, in contrast to carbohydrates and lipids, we do not have protein depots in the body to compensate for a possible shortage, and you will understand why the survival, health, and performance of an organism depend heavily on the integrity of its proteins.

The present chapter deals with how exercise affects protein metabolism by examining the acute and chronic changes in the rates of protein and amino acid synthesis and breakdown.

12.1 Processing of Dietary Proteins

Proteins are present in most foods of the human diet. Good sources include meat of all kinds, dairy products, eggs, legumes, nuts, and cereals. The amino acid sequence of dietary proteins differs from that of our own proteins, even if they bear the same name. For example, human myosin heavy and light chains differ in primary structure from the corresponding chains in beef and pork. In addition, an intact protein in our food cannot cross the walls of the gastrointestinal tract and become part of the proteome in a tissue. Hence, dietary proteins need to be digested before they can nourish us.



You are here: proteolysis.

Protein digestion begins in the stomach, where food is mixed with the gastric juice thanks to the activity of the smooth muscle forming the stomach wall. Food causes the stomach to secrete **gastrin**, a hormone that stimulates the secretion of hydrochloric acid (HCl) and **pepsinogen**, two components of the gastric juice that are essential for **proteolysis**, that is, protein breakdown. Let's see what they do.

Hydrochloric acid is secreted by gastric glands and accumulates in the gastric juice. Being a strong acid, it dissociates fully into H^+ and Cl^-, producing a pH of 1.5 to 3.5. Such a low pH denatures proteins (section 3. 6) and unfolds them, making their peptide bonds more accessible to digestive enzymes (figure 12.1).

If a low pH promotes the denaturation and hydrolysis of dietary proteins, why does it not also destroy the proteins in the cells lining the stomach? These cells are protected by a layer of mucus, secreted by the stomach wall, which keeps HCl at bay. You can understand how harmful the acidity of the stomach contents is to the rest of the body by

recalling what an unpleasant and prolonged sensation vomit elicits in the oral mucosa (which is not made to handle highly acidic fluids).

Pepsinogen, on the other hand, is a protein that generates **pepsin**. This **protease** (the term is introduced in section 8.4) catalyzes the hydrolysis of peptide bonds lying preferably at the amino or carboxyl side of phenylalanine, tryptophan, or tyrosine. Unsurprisingly, pepsin is maximally active at pH 1 to 2. (Remember the dependence of enzyme activity on pH; section 3.16.)

How does pepsinogen generate pepsin? Like HCl, pepsinogen is secreted by gastric glands. Upon encountering the acidic environment of the gastric juice, pepsinogen unfolds and catalyzes the cleavage of part of its own polypeptide chain. What remains is pepsin, which then hydrolyzes other pepsinogen molecules to accelerate its own production.

Why do gastric glands get into the trouble of synthesizing a precursor of pepsin rather than pepsin directly? If pepsin was made in the glands, it would hydrolyze cellular proteins encountered on its way to secretion, thus destroying its creator. However, by synthesizing an inactive precursor, the stomach safeguards itself and lets the active enzyme emerge in the gastric juice, on the other side of the protective mucus (much like producing a bomb in one place and detonating it in another).

When the stomach contents pass into the duodenum, their low pH causes it to secrete bicarbonate (HCO_3^-) to the intestinal lumen and the hormone **secretin** to the circulation. Secretin, in turn, causes the pancreas to release more bicarbonate into the intestinal lumen through the pancreatic duct. Bicarbonate from both sources serves to neutralize the stomach contents through a reversal of reactions 3.3. This neutralization is necessary for protection of the intestinal wall against acidity and for proper functioning of the digestive enzymes secreted by the pancreas, which are maximally active at pH values ranging from 6 to 9.

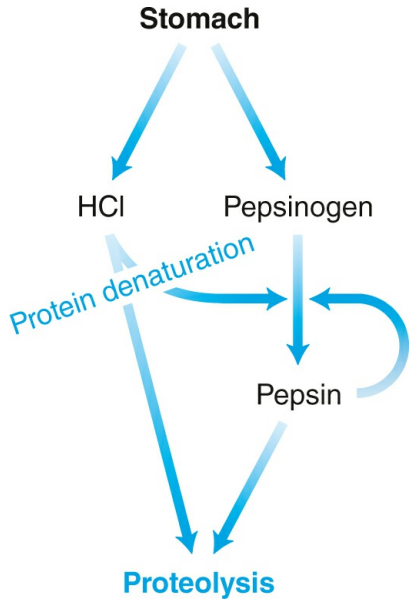


Figure 12.1 **Protein digestion in the stomach.** The breakdown of dietary proteins begins in the stomach with the concerted action of hydrochloric acid and pepsin. HCl creates a highly acidic environment that denatures proteins and facilitates their hydrolysis while fostering the conversion of pepsinogen into pepsin, the active protease. Pepsin facilitates its own production from pepsinogen and breaks down dietary proteins into shorter peptides.

In previous chapters, we have considered the pancreatic enzymes involved in the digestion of carbohydrates and lipids: α-amylase (section 10.1), pancreatic lipase (section 11.1), and pancreatic phospholipase A_2, and pancreatic cholesterol esterase (both in section 11.2). Now it is time to meet the pancreatic enzymes that complement the stomach's pepsin in protein digestion: **trypsin**, **chymotrypsin**, **elastase**, and **carboxypeptidase**. These proteases are synthesized in pancreatic cells as inactive precursors known collectively as **zymogens** (meaning "generators of enzymes"); they are presented in table 12.1. The synthesis of zymogens, rather than the active enzymes, protects the pancreas against degradation of its own proteins.

Table 12.1 Zymogens and Proteases of the Digestive Tract

Synthesizing organ	Zymogen	Protease
Stomach	Pepsinogen	Pepsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Proelastase	Elastase
Pancreas	Procarboxypeptidase	Carboxypeptidase

The trigger for the secretion of pancreatic zymogens to the duodenum is **cholecystokinin**, the very hormone that causes bile secretion in response to a fatty meal (section 11.1). The reason is that cholecystokinin-secreting cells in the duodenum are sensitive to peptides as well as fat. Cholecystokinin then causes the pancreas to secrete the zymogens.

Key to the activation of pancreatic zymogens is **enteropeptidase**, an enzyme synthesized by epithelial cells of the duodenum and present in their plasma membrane as an integral protein, with its active site facing the intestinal lumen. Enteropeptidase hydrolyzes a specific peptide bond in trypsinogen as the latter enters the duodenum from the pancreas, thus producing trypsin (figure 12.2). Trypsin (introduced in section 8.4) then acts on other trypsinogen molecules (just as pepsin does on pepsinogen) to accelerate its own production. In addition to acting on trypsinogen, trypsin takes over the task of activating the remaining zymogens, thus producing chymotrypsin, elastase, and carboxypeptidase. Once produced in the duodenum, the four enzymes accompany the dietary proteins along the small intestine, hydrolyzing them on the way.

The four pancreatic proteases have different specificities: Trypsin specializes in peptide bonds lying at the carboxyl side of arginine or lysine. Chymotrypsin breaks peptide bonds located preferably at the carboxyl side of phenylalanine, tryptophan, or tyrosine. Elastase hydrolyzes peptide bonds lying at the carboxyl side of alanine, glycine, or valine. These residues abound in elastin, the structural protein of our connective tissue (section 3.8).

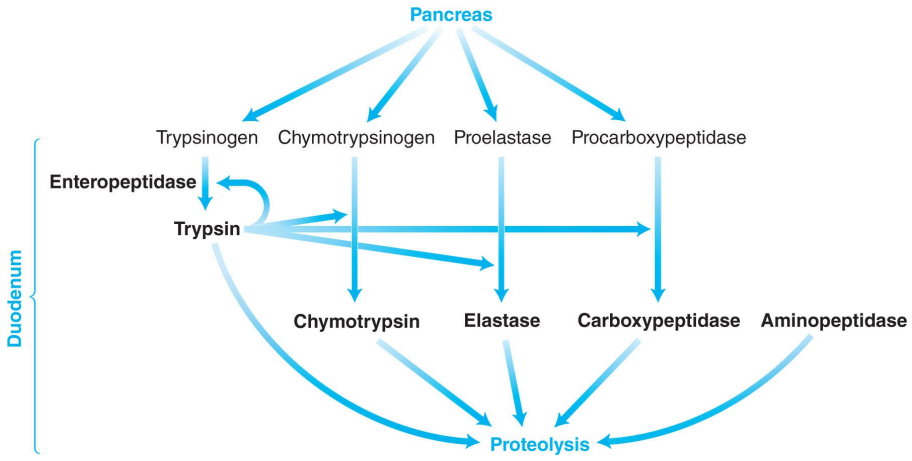


Figure 12.2 **Protein digestion in the small intestine.** The breakdown of dietary proteins is

completed in the small intestine with the concerted action of six enzymes (in black boldface), four of which (trypsin, chymotrypsin, elastase, and carboxypeptidase) are produced in the duodenum from pancreatic zymogens.

Carboxypeptidase catalyzes the removal of the *C*-terminal residue in a peptide by hydrolysis of the last peptide bond. Thus, it differs from the other proteases in table 12.1, which can hydrolyze peptide bonds anywhere in a peptide (as long as the peptide bonds meet the indicated specificity criteria). Another enzyme, **aminopeptidase**, catalyzes the removal of the *N*-terminal residue in a peptide by hydrolysis of the first peptide bond. Like enteropeptidase, aminopeptidase is synthesized by epithelial cells of the small intestine and anchored to their plasma membrane.

Aminopeptidase and carboxypeptidase are termed **exopeptidases**, because they catalyze the removal of terminal residues from a polypeptide chain (figure 12.3). By contrast, pepsin, enteropeptidase, trypsin, chymotrypsin, and elastase are **endopeptidases**. If endopeptidases seem superfluous when exopeptidases are available, consider that endopeptidases speed up protein digestion by breaking up long polypeptide chains into shorter ones, on which multiple exopeptidase molecules can act at the same time. Thus, both endopeptidases and exopeptidases are necessary for efficient protein digestion.

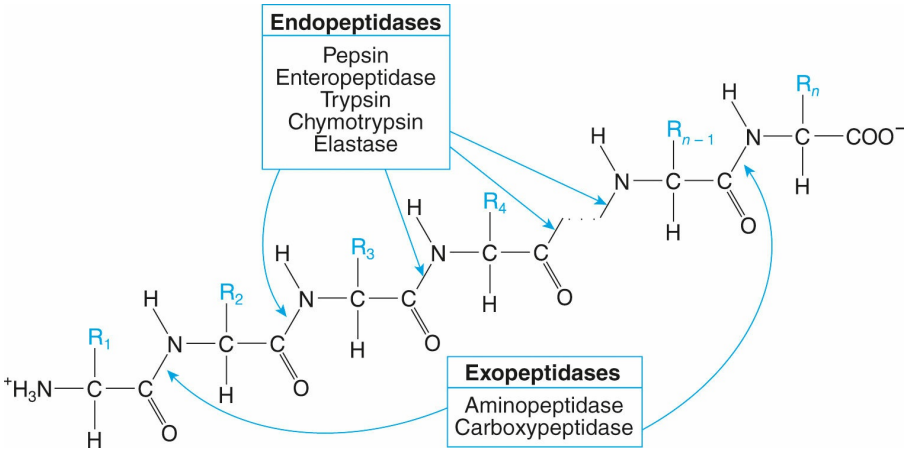


Figure 12.3 Endopeptidases and exopeptidases in concert. Five endopeptidases collaborate with two exopeptidases along the digestive tract to dismantle dietary proteins into their constituent amino acids.

As a result of the concerted action of proteases in the stomach and small intestine, dietary proteins are hydrolyzed to amino acids, though some dipeptides and tripeptides remain due to incomplete digestion. The products of protein digestion enter the enterocytes through transport proteins in the plasma membrane just as monosaccharides do (section 10.1). Then they exit the enterocytes through the side facing the capillaries of the villi (figure 10.3) and enter the circulation. They are first taken up by the liver, which keeps some for its own needs and releases the rest to blood, to be taken up by extrahepatic tissues. Dietary dipeptides and tripeptides may be hydrolyzed to their constituent amino acids by peptidases in any of the body's tissues. Thus, finally, all tissues are nourished with dietary amino acids.

12.2 Protein Content of the Human Body

Being essential for nearly all biological processes, proteins are present in every cell in the body. They are also present outside of the cells, in extracellular fluids and solids. ZiMian Wang and coworkers have estimated the total body protein to be 16% of body mass in lean men and 14% in lean women. The difference results from the fact that adipose tissue, which is proportionally more prevalent in females than in males (section 11.3), is poor in proteins, since most of its mass is occupied by triacylglycerols. It follows that overweight and obese individuals have lower percentages of total body protein than do lean individuals, since larger proportions of their body mass belongs to fat.

Table 12.2 Percentage Distribution of Total Proteins Among the Compartments of the Human Body

Compartment	Men	Women
Cellular	79	75
Extracellular solids	19	23
Extracellular fluids	2	2

Data based on Wang et al. (2003).

Let's convert the percentages of body protein into protein mass. The

model man weighing 75 kg (sections 10.2 and 11.3) has 16% of his body mass as protein, or 12 kg. The model woman weighing 62 kg has 14% of her body mass as protein, or 8.7 kg. Most of the body proteins belong to cells (table 12.2). Another source is extracellular solids, of which collagen and elastin are the major protein constituents. Finally, extracellular fluids, such as the interstitial fluid and plasma, contribute minimally.

12.3 Protein Turnover

Proteins in a living organism take part in an ongoing cycle termed **protein turnover** (figure 12.4). This cycle includes protein synthesis, or translation (an anabolic process), and protein degradation, or proteolysis (a catabolic process). The balance of the two processes can be positive, negative, or zero, depending on whether the rate of protein synthesis is higher than, lower than, or equal to the rate of proteolysis, respectively. As is the case with carbohydrates and lipids, protein balance depends heavily on the dietary state, being positive in the postprandial (fed) state and negative in the postabsorptive (fasted) state.

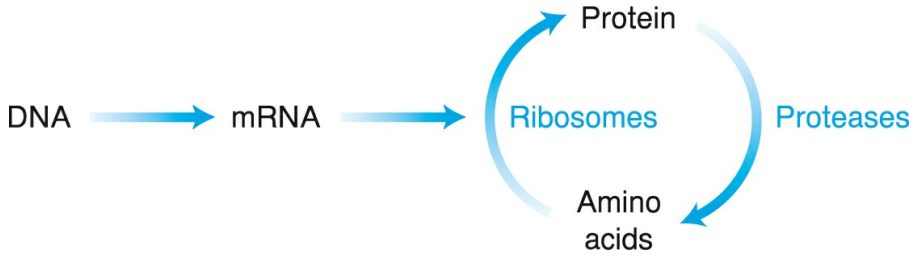


Figure 12.4 Life cycle of a protein. The synthesis and breakdown of a protein follow different metabolic pathways. Synthesis is more complex, because it requires the linking of amino acids in a precise sequence defined by DNA and transmitted to the ribosomes by mRNA. Breakdown is catalyzed by proteases.

Protein synthesis takes place in the ribosomes of cells, which translate the mRNA resulting from the transcription of a gene, as discussed in chapter 4. Proteolysis, on the other hand, is catalyzed by proteases, which, like the digestive ones that we considered in section 12.1, hydrolyze cellular proteins to peptides or amino acids. Many proteases are soluble cytosolic proteins. Another group is gathered in **lysosomes**, the cellular organelles introduced in

section 1.15 and figure 1.9 as sites of decomposition of cellular components (and not just proteins). Cellular components are targeted to lysosomes through a process termed **autophagy** (meaning "self-devouring" in Greek). Autophagy consists in the formation of a membranous vesicle around cytoplasmic components that may include entire organelles such as mitochondria. The vesicle then fuses with a lysosome, to which it delivers its contents for degradation.

A different major proteolytic process begins in the cytosol with the earmarking of a protein through the covalent attachment of ubiquitin chains to its molecule. **Ubiquitin** is a polypeptide (76 amino acid residues, 8.5 kDa) that, when attached to proteins, renders them recognizable by the **proteasome**, a bulky complex of proteins (more than 60 subunits, more than 2,000 kDa) hydrolyzing other proteins to small peptides. Both the attachment of ubiquitin to a protein and the operation of the proteasome are powered by ATP hydrolysis. Other proteases then hydrolyze the peptides released from the proteasome to amino acids.

On average, a healthy and adequately nourished adult has (in the whole body and over the day) about equal rates of protein synthesis and proteolysis. As a result, the individual has zero protein balance, and his or her protein mass remains stable.

12.4 Effects of Exercise on Protein Turnover

Exercise changes the rates of protein synthesis and proteolysis in muscle, and the changes depend on exercise type. Most research has been conducted with individuals in the postabsorptive state (typically, after an overnight fast), in which, according to Philip Atherton and collaborators, the rate of muscle protein synthesis is, on average, 0.05% per hour, while the rate of muscle protein breakdown is 0.09% per hour. This combination results in a negative protein balance of 0.04% $\cdot$ h^{-1}. Let's examine, then, how exercise changes these baseline values, starting with resistance exercise.

If we perform a resistance exercise session, the rate of muscle protein synthesis increases gradually, with a lag of one or more hours after the end of the exercise, and peaks two to six hours postexercise at a value two to four

times the baseline. Because of technical difficulties, it is unclear whether or how protein metabolism changes *during* resistance exercise.

The rate of muscle protein synthesis remains above baseline for up to two days after resistance exercise, as long as we ingest adequate amounts of proteins to provide substrates for protein synthesis. The anabolic stimulus seems to be stronger on the myofibrillar proteins (such as myosin and actin) than on other muscle proteins.

When different intensities of resistance exercise are compared, while keeping the total work the same, the anabolic effect increases with intensity until it plateaus between 60% and 90% of 1RM. Nevertheless, light exercise (30% of 1RM) can be as effective as hard exercise if both are performed to what is termed *momentary muscular failure*, that is, inability to complete another repetition in a set while maintaining proper form. In this case, however, the total work that one produces at low intensity is more than the total work one produces at high intensity.

Let’s turn now to the effect of resistance exercise on the rate of muscle proteolysis. It follows a similar temporal pattern to protein synthesis, that is, it rises gradually after exercise and peaks within a few hours, but the increase is only 30% to 50%. As a result, the postabsorptive gap between protein degradation and synthesis narrows (figure 12.5).

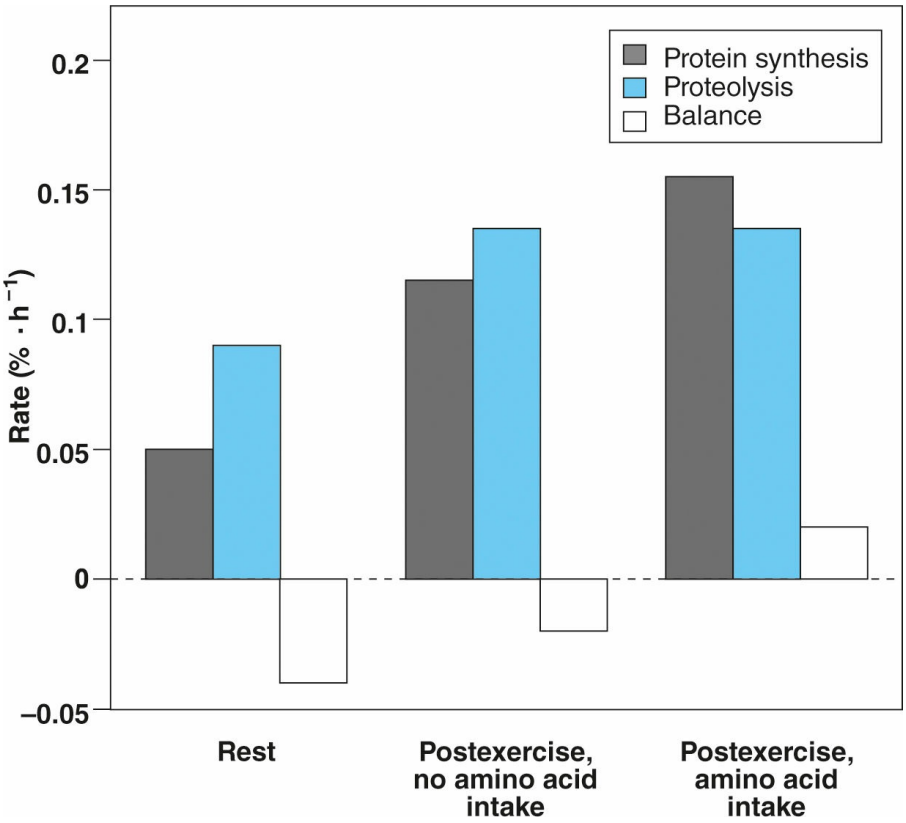


Figure 12.5 How resistance exercise affects protein turnover. The rate of protein synthesis is lower than that of proteolysis at rest in the fasted state (in which most relevant research has been done), resulting in negative protein balance. A few hours after resistance exercise, the rate of protein synthesis rises spectacularly, whereas the rate of proteolysis rises less. Even so, protein balance remains negative if one remains in the fasted state. However, amino acid intake raises the rate of protein synthesis even further (while not affecting the rate of proteolysis), resulting in positive protein balance. The graph reflects a consensus of findings from studies reviewed by Atherton and collaborators, as well as Vinod Kumar and collaborators.

What is more, if we consume proteins or an amino acid supplement soon after resistance exercise, the anabolic response is even stronger and the rate of protein synthesis exceeds that of proteolysis, since the latter is not affected considerably by dietary intake. This response results in a shift of protein balance from negative to positive.

Endurance exercise has received less attention than resistance exercise regarding its effect on muscle protein turnover. It seems that, shortly after or within a few hours of an endurance exercise session, the rate of muscle protein synthesis rises to usually no more than double the baseline. It is unclear whether or how the rate changes *during* endurance exercise.

After endurance exercise, the rate of muscle protein synthesis remains above baseline for about one day, again provided we ingest adequate amounts of proteins. The anabolic stimulus seems to be stronger on mitochondrial than on myofibrillar proteins.

The rate of muscle proteolysis increases *during* endurance exercise to such a degree that the negative baseline protein balance becomes more negative. This condition favors the increased availability of amino acids for use in the active muscles. The negative protein balance remains after exercise (figure 12.6) unless we consume proteins or an amino acid supplement. In that case, the rate of protein synthesis exceeds that of proteolysis, resulting in a shift of protein balance from negative to positive.

Although proteins comprise a large part of muscle mass (about 20%, or 200 g · kg^{-1}), the 20 amino acids that make up proteins are present in free form (not incorporated in proteins) in minute amounts—approximately 3 g · kg^{-1}, or 20 mmol · kg^{-1}. Of these, glutamine has the lion's share (about 60%), followed by glutamate (14%) and alanine (4%). The total amino acid concentration in the cytosol of muscle fibers increases during endurance exercise thanks to the enhancement of proteolysis mentioned earlier.

The exercise stimulus can also be sensed by connective tissue proteins. As reviewed by Michael Kjaer and associates, some, but not all, studies have shown that resistance exercise increases not only the rate of protein synthesis inside the fibers of an active muscle but also the rate of collagen synthesis in the tendon, in the muscle's connective tissue, or in both.

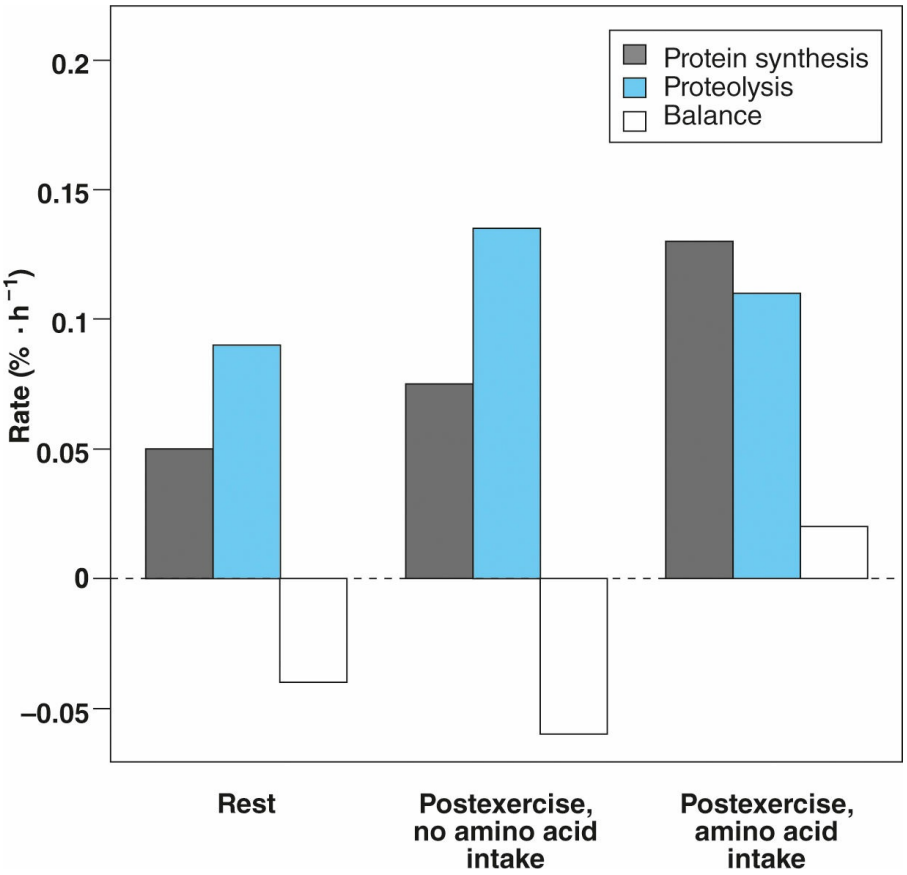
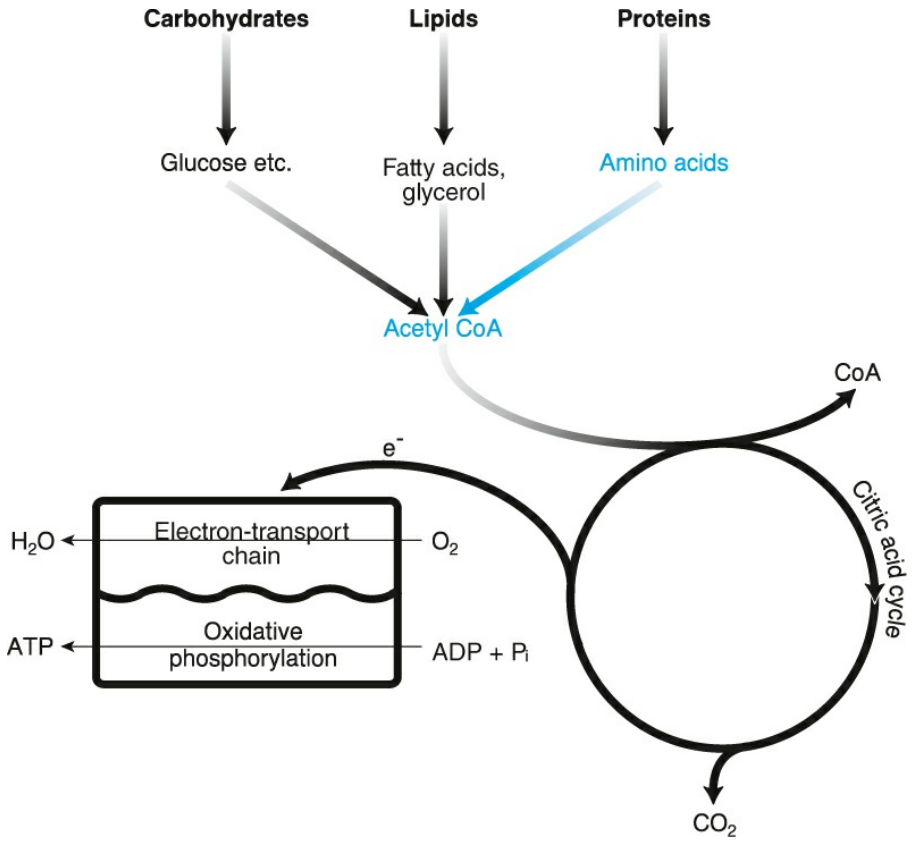


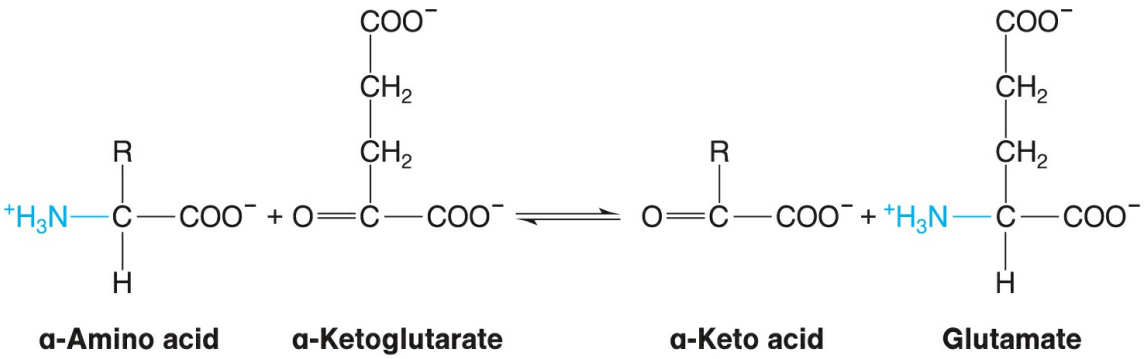
Figure 12.6 **How endurance exercise affects protein turnover.** After endurance exercise, the rate of protein synthesis rises, but the rate of proteolysis rises more. As a result, the negative baseline protein balance becomes more negative if one remains in the fasted state. However, amino acid intake raises the rate of protein synthesis above the rate of proteolysis, resulting in positive protein balance. The graph was constructed similarly to that of figure 12.5.



You are here: amino acid degradation.

12.5 Amino Acid Degradation

Amino acids can be broken down to produce energy. Although each one follows its own catabolic route, all routes start with the removal of the α-amino group. Two amino acids (serine and threonine) dispose of it as ammonium (figure 12.7) through deamination. So does glutamine in the liver (see section 12.8), kidneys, intestine, and central nervous system. The remaining amino acids, except glutamate, transfer their α-amino groups to α-ketoglutarate, a compound of the citric acid cycle (section 10.12 and figure 10.27), which is thus converted into glutamate. The general equation of the transfer reaction is



(equation 12.1)

The reaction is a **transamination** catalyzed by an **aminotransferase**, or **transaminase**. There are several aminotransferases, each of which specializes in one or a few amino acids. All contain pyridoxal phosphate, a derivative of vitamin B_6 (section 6.1) in their active sites.

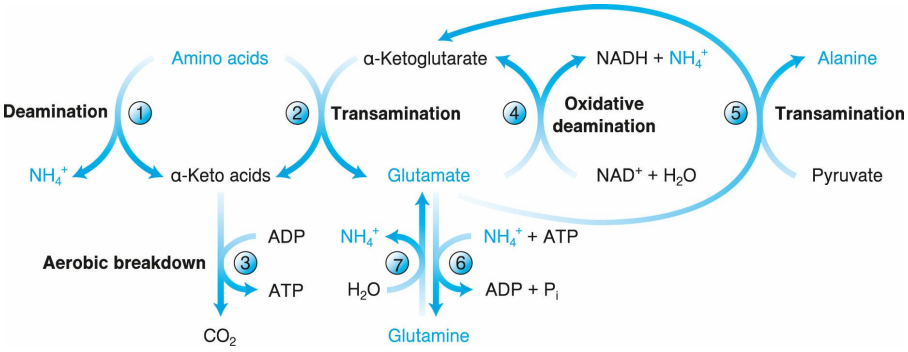


Figure 12.7 **Overview of amino acid catabolism.** Two or three amino acids discard their amino groups by deamination *(1)*, producing α-keto acids and ammonium. The remaining amino acids, except glutamate, transfer their amino groups to α-ketoglutarate by transamination *(2)*, producing α-keto acids and glutamate. The α-keto acids can be degraded aerobically through the citric acid cycle to yield ATP *(3)*. Glutamate can dispose of its amino group by oxidative deamination *(4)* or transfer the amino group to pyruvate by transamination *(5)*. Both processes regenerate α-ketoglutarate. Alternatively, glutamate can be converted into glutamine through acquisition of another amino group *(6)*. Glutamine then disposes of this amino group as ammonium in certain organs and tissues *(7)*. Compounds in color carry amino groups.

The **α-keto acids** produced in reaction 12.1 are (or can be) converted into intermediate compounds of carbohydrate or lipid metabolism that we have considered in chapters 10 and 11. These compounds are as follows:

- Pyruvate

- Acetyl CoA
- Acetoacetyl CoA, which is involved in fatty acid metabolism (figures 11.31 and 11.32)
- Succinyl CoA, fumarate, and oxaloacetate, all of which are compounds of the citric acid cycle (figure 10.27)

What is the fate of the amino group that is loaded to glutamate in reaction 12.1? Glutamate is one of the few amino acids that can discard the amino group in the form of ammonium. This discard happens according to the following reactions:

$$\text{Glutamate} + NAD^+ + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + NADH + H^+ \qquad \Delta G^{\circ\prime} = 9.1 \text{ kcal} \cdot \text{mol}^{-1}$$

(equation 12.2)

or

$$\text{Glutamate} + NADP^+ + H_2O \rightleftharpoons \alpha\text{-ketoglutarate} + NH_4^+ + NADPH + H^+ \qquad \Delta G^{\circ\prime} = 9.3 \text{ kcal} \cdot \text{mol}^{-1}$$

(equation 12.3)

These reactions are catalyzed by **glutamate dehydrogenase**, an enzyme located in the mitochondrial matrix. The reactions constitute **oxidative deamination**, as glutamate both loses the amino group and is oxidized by NAD^+ or $NADP^+$. The product, α-ketoglutarate, can serve again as amino group acceptor in transamination reactions or can enter the citric acid cycle.

What remains of an amino acid after it has lost its amino group is often referred to as the *carbon skeleton*. Figure 12.8 summarizes the fates of the carbon skeletons of the 20 amino acids. By entering pathways of carbohydrate and lipid metabolism, amino acids can be catabolized aerobically to CO_2, yielding ATP. The yield varies from one amino acid to another (table 12.3) and averages about 22 ATP.

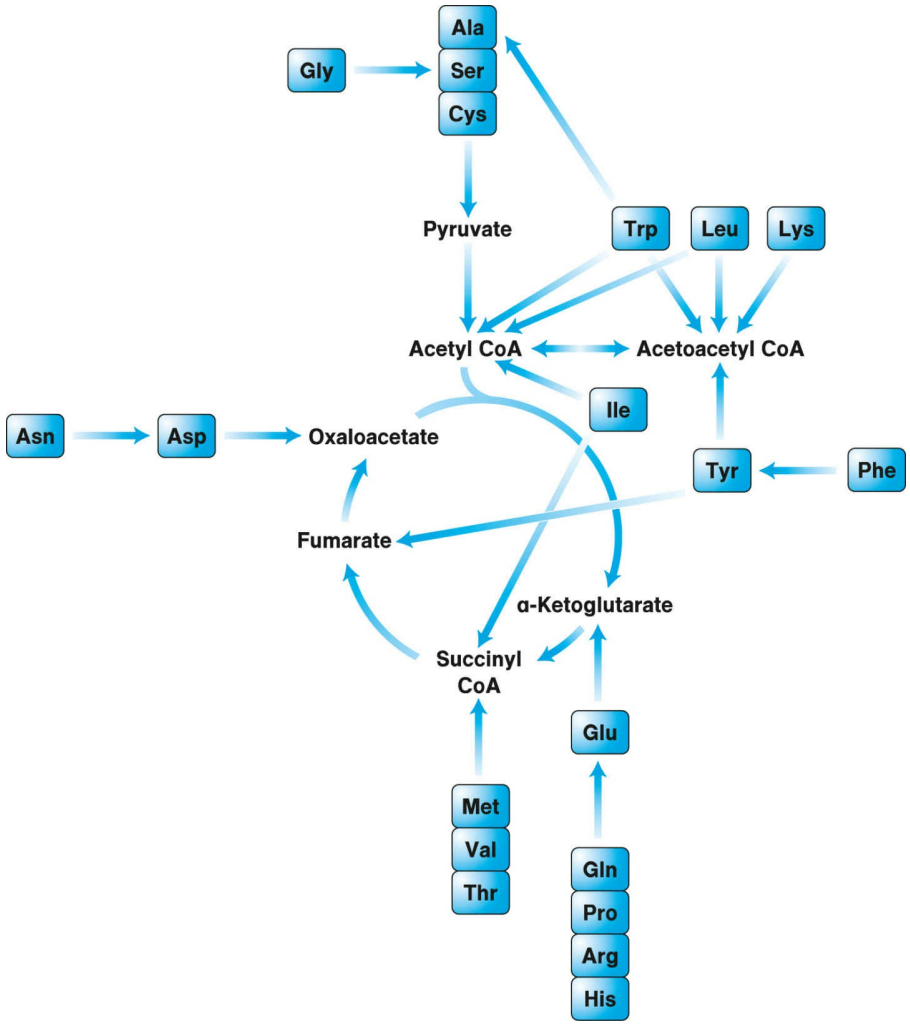


Figure 12.8 **Fates of the carbon skeletons of amino acids in humans.** After ridding themselves of their α-amino groups, the 20 amino acids of proteins (enclosed in boxes) follow catabolic routes that lead to intermediate compounds of carbohydrate and lipid metabolism.

Table 12.3 Energy Yield of the Complete Oxidation of Amino Acids

Amino acid	ATP	Amino acid	ATP	Amino acid	ATP
Ala	12.5	Gly	12.5	Pro	25
Arg	25	His	22.5	Ser	12.5
Asn	12.5	Ile	29	Thr	19
Asp	12.5	Leu	31	Trp	45
Cys	12.5	Lys	22.5	Tyr	33
Gln	22.5	Met	19	Val	19
Glu	22.5	Phe	30.5	Average	22

Values have been calculated by the author on the basis of figure 12.8 and of details of the metabolic pathways for each amino acid that are not presented.

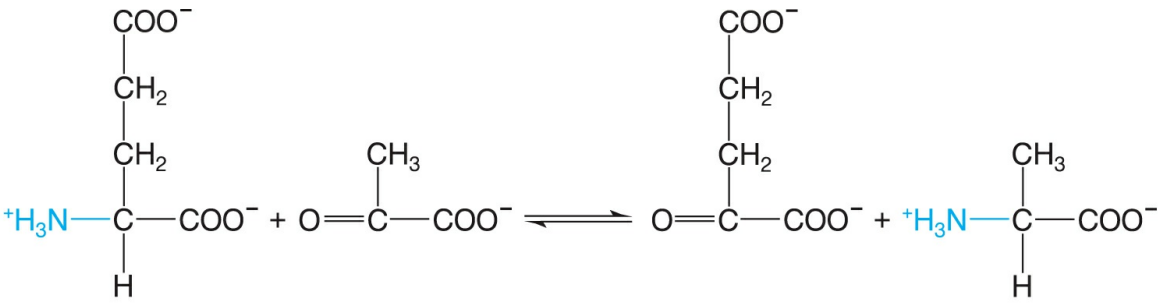
Reactions 12.2 and 12.3 relieve glutamate and, by extension, most amino acids of their amino groups permanently but also create a new problem: ammonium accumulation. Indeed, if too much ammonium is released into the bloodstream, it builds up in the brain, where high concentrations of it are toxic. To prevent this problem, extrahepatic tissues (including muscle) divert part of glutamate away from oxidative deamination and ammonium production and toward receipt of ammonium and production of another amino acid, glutamine, at the expense of ATP.

$$\text{Glutamate} + NH_4^+ + \text{ATP} \rightleftharpoons \text{glutamine} + \text{ADP} + P_i + H^+ \qquad \Delta G^{\circ\prime} = -3.6 \text{ kcal} \cdot \text{mol}^{-1}$$

(equation 12.4)

The reaction is catalyzed by **glutamine synthetase**. Glutamine is harmless, exits to the bloodstream, and travels to the liver for further processing, as we will see in sections 12.6 and 12.7.

Another amino acid that, like glutamine, assumes much of the burden of transferring amino groups from extrahepatic tissues to the liver is alanine. It does so through the following reaction:



$\Delta G^{\circ\prime} = 0 \text{ kcal} \cdot \text{mol}^{-1}$ (equation 12.5)

This is a transamination reaction but in reverse direction relative to general reaction 12.1, in which you would have to replace α-amino acid with alanine and α-keto acid with pyruvate. (Notice the structural similarity of pyruvate and alanine.) In other words, instead of transferring its amino group to α-ketoglutarate and producing glutamate, alanine is produced when pyruvate receives the amino group of glutamate. The reaction is catalyzed by **alanine aminotransferase**, one of the most important aminotransferases.

You realize from all this that the predominance (mentioned in the previous section) of glutamate, glutamine, and alanine among muscle amino acids is not accidental, since these three are the major recipients of amino groups from the remaining seventeen. Glutamate holds the central position in the intracellular network of amino group disposal, as it appears in all five reactions presented in this section. Specifically, it collects amino groups through reaction 12.1 (in which it is a product) and dispatches them through the remaining ones (in which it is a substrate).

Table 12.4 Essential and Nonessential Amino Acids for Adult Humans

Essential (9)	Nonessential (11)
Histidine	Alanine
Isoleucine	Arginine
Leucine	Asparagine
Lysine	Aspartate
Methionine	Cysteine
Phenylalanine	Glutamate
Threonine	Glutamine
Tryptophan	Glycine

Valine	Proline
	Serine
	Tyrosine

12.6 Amino Acid Synthesis

Besides obtaining amino acids from the hydrolysis of dietary proteins, our bodies also synthesize them. As is the case with degradation, the synthesis of each amino acid follows a separate route. Glutamate plays a central role here too by serving as donor of amino groups to α-keto acids in transamination reactions that are the reverse of reaction 12.1. These reactions can thus be viewed as part of both catabolic and anabolic pathways.

Animals cannot synthesize all 20 protein amino acids. Humans, in particular, lack the genes that encode enzymes needed to synthesize 9 of them (table 12.4), which are thus termed **essential amino acids** (by analogy to essential fatty acids; section 11.14). The remaining 11 amino acids are termed **nonessential**. Unless we obtain all of the essential amino acids in adequate amounts through the diet, protein synthesis suffers, because almost every protein contains all 20 amino acids.

Unlike animals, plants and many microorganisms can synthesize all amino acids. Herbivores obtain their essential amino acids by eating plants, carnivores do so by eating herbivores, and humans do so by eating foods of both plant and animal origin.

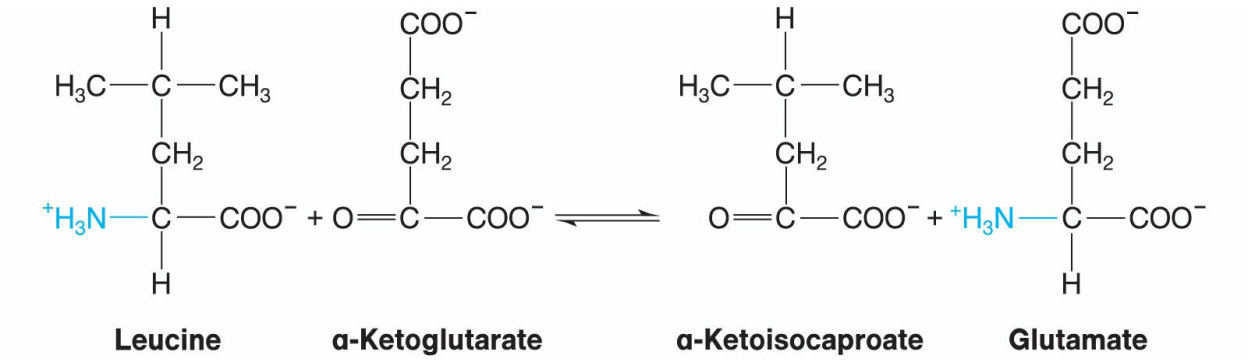
12.7 Effects of Exercise on Amino Acid Metabolism in Muscle

Amino acid metabolism in muscle has been studied more during endurance than during resistance exercise. The rise in the amino acid concentration in the cytosol of active fibers (section 12.4) favors amino acid breakdown, which is also favored by the activation of glutamate dehydrogenase, the enzyme catalyzing reactions 12.2 and 12.3. This enzyme is inhibited by ATP and GTP but activated by ADP and GDP. Therefore, the decreases in ATP and GTP and the increases in ADP and GDP during exercise activate glutamate dehydrogenase and speed up glutamate deamination. Because

glutamate deamination regenerates α-ketoglutarate, it promotes transamination and the removal of amino groups from amino acids (see reaction 12.1). This action, in turn, opens the door to amino acid oxidation.

Despite these changes, oxidation of most muscle amino acids does not seem to increase during exercise. However, oxidation of three of them does increase—namely, leucine, isoleucine, and valine. All three have branched aliphatic side chains (figure 3.2), and they are known collectively as **branched-chain amino acids (BCAA)**. BCAA are unique in that they bypass degradation by the liver after a meal, because the liver lacks the specific aminotransferase needed to convert them into α-keto acids, as reported by Andrew Sweatt and colleagues. Thus, a higher proportion of dietary BCAA, as compared with other amino acids, becomes available to extrahepatic tissues, including muscle.

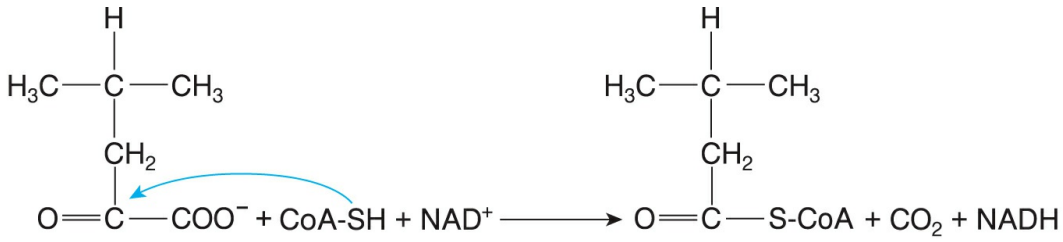
Muscle BCAA are first transaminated to **branched-chain α-keto acids** according to the general reaction 12.1. As an example, leucine produces **α-ketoisocaproate**:



$\Delta G^{\circ\prime} = -0.1$ kcal · mol^{-1} (equation 12.6)

Investigators usually measure α-ketoisocaproate as an index of BCAA catabolism. The muscle concentration of branched-chain α-keto acids increases during moderate-intensity exercise.

Next, branched-chain α-keto acids are decarboxylated by the **branched-chain α-keto acid dehydrogenase complex**. The decarboxylation reaction for α-ketoisocaproate is as follows:



α-Ketoisocaproate **Isovaleryl CoA**

$\Delta G^{\circ\prime} = -7.4$ kcal · mol^{-1} (equation 12.7)

In humans, the branched-chain α-keto acid dehydrogenase complex is found mainly in the skeletal muscles (which contain 60% of its total amount in the body) and is activated during moderate-intensity exercise. This enzyme complex resembles the pyruvate dehydrogenase complex that we considered in sections 10.10 and 10.11 in terms of the reaction catalyzed (compare reactions 10.9 and 12.7), location (the mitochondrial matrix), and control by reversible phosphorylation. Thus, an increase in its substrates—the branched-chain α-keto acids, analogous to pyruvate in the pyruvate dehydrogenase complex—and a decrease in ATP during exercise favor the active *a* form of branched-chain α-keto acid dehydrogenase (cf. figure 10.26).

The products of branched-chain α-keto acid decarboxylation participate in reactions leading to their complete oxidation to CO_2. In this way, they offer energy for exercise.

Although the total concentration of muscle amino acids increases during endurance exercise, the muscle glutamate concentration decreases. This decrease probably results from the central role played by glutamate in amino acid metabolism, as presented in section 12.5, and, in particular, its consumption in reactions that dispose of amino groups.

Beyond energy provision, researchers have proposed that the breakdown of muscle amino acids during exercise might raise the concentration of compounds of the citric acid cycle, which might in turn promote the aerobic catabolism of carbohydrates and lipids by offering more substrates for the processing of the acetyl groups entering the cycle. However, findings show this not to be the case. Thus, amino acid catabolism in muscle during exercise does not appear to facilitate ATP resynthesis through carbohydrate or lipid catabolism.

In addition to amino acids, ammonium (produced from amino acid

deamination) increases in muscle during exercise. We need to consider, however, that ammonium is also produced from AMP deamination, which is accelerated during hard exercise (section 9.5). It is believed that *AMP is the main source of ammonium during hard exercise, whereas amino acids are the main source of ammonium during moderate-intensity exercise.*

12.8 Effects of Exercise on Amino Acid Metabolism in the Liver

The liver has a free amino acid content similar to that of muscle, that is, about 3 g · kg^{-1} (section 12.4). Part of this pool is used for glucose synthesis. How? As you can see in figure 12.8, the carbon skeletons of five amino acids end up in pyruvate, either in whole (Ala, Cys, Gly, and Ser) or in part (Trp). The carbon skeletons of two more amino acids end up in oxaloacetate (Asn and Asp). Both pyruvate and oxaloacetate are substrates for gluconeogenesis (figure 10.36). Thus, if the carbon skeletons of these seven amino acids are not degraded, they can form glucose.

Table 12.5 Classification of Amino Acids on the Basis of Their Metabolic Fate

Glucogenic (14)	Ketogenic (2)	Glucogenic and ketogenic (4)
Alanine	Leucine	Isoleucine
Arginine	Lysine	Phenylalanine
Asparagine		Tryptophan
Aspartate		Tyrosine
Cysteine		
Glutamate		
Glutamine		
Glycine		
Histidine		
Methionine		
Proline		
Serine		
Threonine		
Valine		

Note: Several amino acids are known to follow more than one metabolic route, and this

diversity may change their character. As a result, there is no full agreement in the literature as to which amino acids are glucogenic and which are ketogenic. The routes that prevail in humans serve as the basis for this table (and figure 12.8, for that matter).

In addition, the carbon skeletons of 11 amino acids are converted into α-ketoglutarate (Arg, Gln, Glu, His, and Pro), succinyl CoA (Met, Thr, Val, and, partly, Ile), or fumarate (partly Phe and Tyr). Because α-ketoglutarate, succinyl CoA, and fumarate yield oxaloacetate through the citric acid cycle, these 11 amino acids are added to the 7 listed in the previous paragraph as sources of glucose. This leaves just 2 amino acids (Leu and Lys) that are incapable of producing glucose, because their carbon skeletons are converted into acetoacetyl CoA. The latter can be broken down into acetyl CoA (section 11.23 and figure 11.32), which cannot produce glucose (section 11.17 and figure 11.21).

Thus, the carbons of most amino acids (18) find their way to glucose. These are the fully (14) or partly (4) **glucogenic amino acids** (table 12.5). During exercise, the increased secretion of glucagon and the decreased secretion of insulin (when it happens) speed up gluconeogenesis (sections 10.26 and 10.29), with the result that more hepatic amino acids are used for glucose synthesis.

The two amino acids that are degraded into acetoacetyl CoA are termed (fully) **ketogenic**, since acetoacetyl CoA forms ketone bodies (section 11.23 and figure 11.31). The four partly glucogenic amino acids are also partly ketogenic (for the parts of their carbon skeletons that end up in acetyl CoA or acetoacetyl CoA).

Amino acids from muscle can also serve as precursors for glucose synthesis in the liver. As mentioned in section 12.5, glutamine and alanine transport amino groups safely from the muscles to the liver. There, glutamine is converted back to glutamate (a glucogenic amino acid) through the catalytic action of **glutaminase**.

$$\text{Glutamine} + H_2O \rightarrow \text{glutamate} + NH_4^+ \qquad \Delta G^{\circ\prime} = -2.7 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 12.8)}$$

The ammonium produced poses no threat, since it can be efficiently eliminated through the urea cycle, which operates solely in the liver and which we will explore in the next section.

The fate of alanine deserves a more extended discussion. This amino acid is converted into pyruvate by a reversal of reaction 12.5 and through the catalytic action of (naturally) the same enzyme, alanine aminotransferase.

$$\text{Alanine} + \alpha\text{-ketoglutarate} \rightleftharpoons \text{pyruvate} + \text{glutamate} \qquad \Delta G^{\circ\prime} = 0 \text{ kcal} \cdot \text{mol}^{-1} \quad \text{(equation 12.9)}$$

Hepatocytes then use the pyruvate produced to synthesize glucose, which they release to the circulation. This flow of carbon atoms in the form of alanine from muscle to the liver contributes to euglycemia in the postabsorptive state, during which we experience a negative protein balance (section 12.3). The degradation of hepatic and muscle proteins in the postabsorptive state underlines the importance of euglycemia: Although proteins are valuable for survival, the body sacrifices a portion of them, because it cannot synthesize substantial amounts of glucose from the abundant triacylglycerols (section 11.17).

A common misconception is that the body begins to break down protein when it runs out of carbohydrate and fat. However, the adequacy of fat does not affect the protein status—let alone the fact that it is impossible to run out of fat. Protein status is affected, however, by carbohydrate adequacy. In particular, if liver glycogen is depleted because of inadequate carbohydrate intake, then the body is deprived of its major source of blood glucose and thus resorts to proteins.

The increase in muscle alanine production during exercise and the resulting increased liberation of alanine in the bloodstream result in its higher contribution to glucose synthesis in the liver. Part of the glucose produced is taken up by the exercising muscles, thus completing the **glucose-alanine cycle** (figure 12.9). The glucose-alanine cycle resembles the Cori cycle (figure 10.41), differing only in that it involves alanine in place of lactate.

The importance of the glucose-alanine cycle for muscle metabolism is dual. First, it relieves muscle of amino groups. Second, every turn of the cycle yields two ATP for muscle (the yield of glycolysis) and charges six ATP to the liver (the cost of gluconeogenesis). Thus, as with the Cori cycle, muscle shifts part of its metabolic burden to the liver. In fact, the glucose-alanine cycle benefits muscle and burdens the liver more than the Cori cycle does: The two NADH produced in the muscle cytosol during glycolysis can yield three ATP through the electron-transport chain and oxidative

phosphorylation (section 10.17), whereas gluconeogenesis in the liver *demands* two NADH, thus depriving the hepatocytes of five potential ATP. In contrast, there is no net production or consumption of NADH in the interconversion of glucose and lactate (the compounds participating in the Cori cycle).

The difference in ATP yield per cytosolic NADH between muscle and liver results from the prevalence of different shuttles in the two organs (section 10.17).

On the other hand, by being converted into alanine, pyruvate misses the opportunity to be converted into lactate and quickly regenerate the NAD^+ needed for fast anaerobic production of ATP in glycolysis. At any rate, however, the glucose-alanine cycle requires several minutes to operate, which makes it useless in (necessarily brief) anaerobic efforts. Even in extended efforts, the importance of the cycle is limited. The amount of alanine entering the liver from muscle accounts for 4%, at most, of the glucose produced by the liver during prolonged moderate-intensity exercise. An equally low amount of glucose is contributed by the sum of the other glucogenic amino acids taken up by the liver.

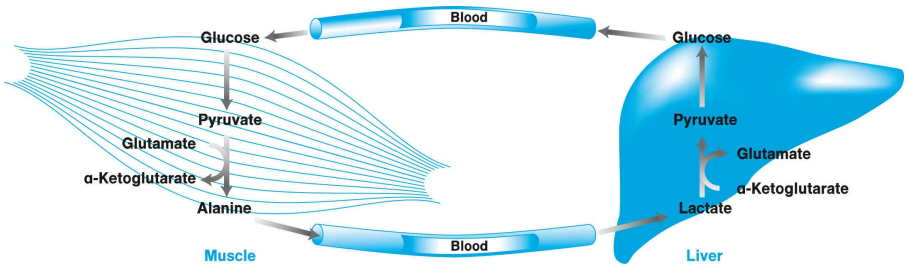


Figure 12.9 **The glucose-alanine cycle.** This cycle supplies an active muscle with glucose synthesized in the liver from alanine of muscle origin.

12.9 The Urea Cycle

Most (approximately 90%) of the nitrogen excreted from the human body is incorporated in **urea**, a simple nontoxic compound bearing two amino groups —a compound that we met in section 1.8. Urea is synthesized in the liver through a series of four reactions called the **urea cycle**. The cycle was

discovered in 1932 by the German-born British physician and biochemist Hans Krebs (the same researcher who later discovered the citric acid cycle) and the German biochemist Kurt Henseleit.

The urea cycle starts with **carbamoyl phosphate**, a compound of high phosphoryl-transfer potential, deriving from the linking of ammonium, bicarbonate, and a phosphoryl group at the expense of two ATP (figure 12.10). Urea is produced after four additional reactions involving two protein amino acids (aspartate and arginine) and three nonprotein ones (ornithine, citrulline, and argininosuccinate).

In the first reaction of the cycle, the carbamoyl group is linked with ornithine to form citrulline. In the second reaction, citrulline is linked to aspartate at the expense of another ATP, which is hydrolyzed to AMP and PP_i. The product of the second reaction, argininosuccinate, then splits into arginine and fumarate. Finally, arginine is hydrolyzed to urea and ornithine, thus completing the cycle. The synthesis of carbamoyl phosphate and the first reaction of the cycle take place in the mitochondrial matrix, whereas the remaining three reactions take place in the cytosol of the hepatocytes.

Notice a similarity of the first reaction of the urea cycle with that of the citric acid cycle (figure 10.26): The groups entering both cycles (carbamoyl and acetyl, respectively) are delivered by activated carriers (carbamoyl phosphate and acetyl CoA). It is like putting an artificial satellite into orbit around Earth with a carrier rocket.

The overall reaction of urea synthesis is

$$NH_4^+ + HCO_3^- + 3\ ATP + aspartate + H_2O \rightarrow urea + fumarate + 2\ ADP + AMP + 2\ P_i + PP_i + 4\ H^+ \qquad \Delta G^{\circ\prime} = -9.2\ kcal \cdot mol^{-1} \qquad \text{(equation 12.10)}$$

The reaction is exergonic thanks to the hydrolysis of three ATP (two to ADP and P_i and one to AMP and PP_i). This action contributes a total of –20.8 kcal · mol^{-1} to $\Delta G^{\circ\prime}$ (see reactions 2.5 and 2.6), not counting the extra energy that can be drawn from PP_i hydrolysis (see reaction 2.7). Because the hydrolysis of ATP to AMP and PP_i is considered as an expenditure of two ATP (section 11.10), the total cost of urea production is four ATP.

Without ATP hydrolysis, urea synthesis would be highly endergonic (with a $\Delta G^{\circ\prime}$ of –9.2 + 20.8 kcal · mol^{-1}, or 11.6 kcal · mol^{-1}) and, hence, not

favored. Thus, the body spends considerable energy to ensure the safe disposal of amino groups. Indeed, after urea is formed in the liver, it diffuses to blood. The kidneys then excrete it in urine, after which it was named.

The two amino groups of urea derive from ammonium and aspartate. Ammonium, in turn, derives from deamination reactions such as reactions 9.7, 12.2, 12.3, and 12.8. Aspartate can carry the amino groups of other amino acids through two consecutive

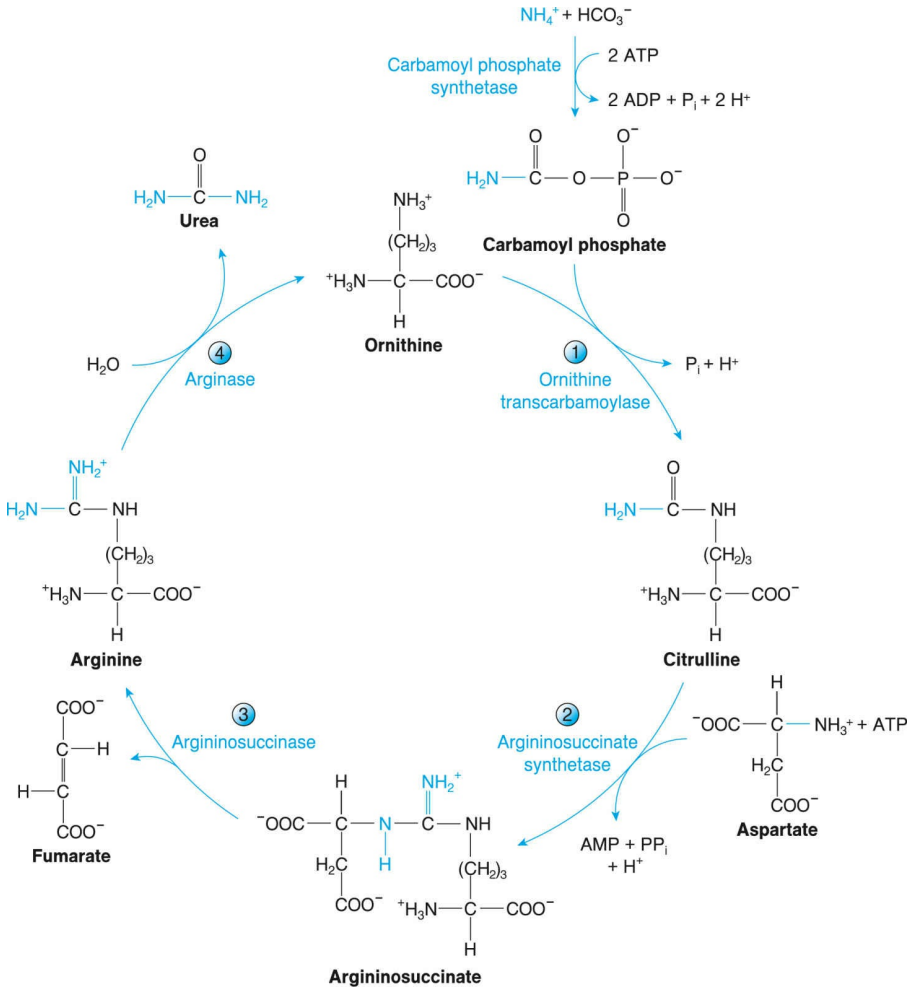


Figure 12.10 **The urea cycle.** Ammonium and the amino group of aspartate (both shown in color) are incorporated in urea in order to be safely excreted from the body.

transamination reactions (figure 12.11). The first is reaction 12.1, which transfers the amino group of an amino acid to glutamate. The second is

$$\text{Glutamate} + \text{oxaloacetate} \rightleftharpoons \alpha\text{-ketoglutarate} + \text{aspartate}$$
$$\Delta G^{\circ\prime} = -0.7 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 12.11)}$$

This reaction transfers the amino group to aspartate. The reaction is catalyzed by another important aminotransferase, namely **aspartate aminotransferase**.

The rate of urea production does not change during short hard exercise. A rise is measured, however, in prolonged efforts, and it appears to relate to exercise duration and intensity. Evidently, the increased urea production under such conditions is due to the accelerated removal of amino groups from alanine and other amino acids used in glucose synthesis in the liver. There are, however, studies that did not detect any significant rise in urea production during prolonged exercise. This lack of detection may be due to the small magnitude of the changes in amino acid metabolism.

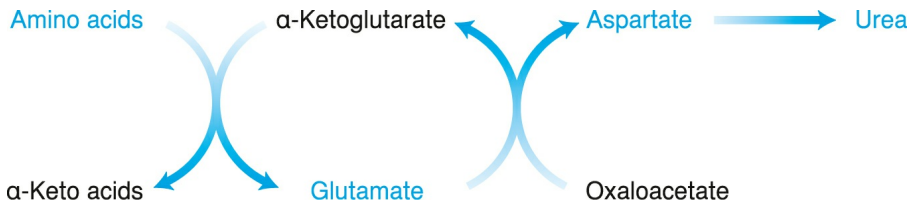


Figure 12.11 **Aspartate as an amino group acceptor.** Amino groups from various amino acids end up in aspartate through glutamate. Aspartate then disposes of its amino group in the urea cycle.

12.10 Plasma Amino Acid, Ammonia, and Urea Concentrations During Exercise

The total amino acid concentration in plasma is 3 to 4 mmol · L^{-1}. The most abundant plasma amino acids are glutamine and alanine, as one would expect from the discussion of their roles in section 12.5. Their concentrations are about 0.5 and 0.4 mmol · L^{-1}, respectively.

The total amino acid concentration in plasma is not affected considerably by exercise lasting less than one hour. At the level of individual amino acids, glutamate decreases, and alanine increases. These changes are like the ones measured in the exercising muscles (section 12.7). When exercise duration exceeds 2 hours, the plasma amino acid concentration drops by as much as 30%. This shows that, under such demanding conditions, there is an increase in their rate of disappearance toward the tissues relative to their rate of appearance in plasma.

Moderate-intensity or hard exercise is accompanied by a rise in the plasma ammonium concentration, which relates to the plasma lactate concentration. Finally, the plasma urea concentration also increases in exercises lasting at least half an hour and having an intensity of at least 60% of $\dot{V}O_2max$. However, the plasma urea concentration may not change during hard exercise despite an increased production, as the blood flow through the liver decreases; thus, there is not enough blood to carry urea out of the hepatocytes. For specific values of plasma urea and ammonia concentrations, see sections 17.10 and 17.11.

12.11 Contribution of Proteins to the Energy Expenditure of Exercise

As discussed in sections 12.4 and 12.7, the rates of proteolysis and BCAA degradation increase in the active muscles during endurance exercise. In addition, the rate of gluconeogenesis from amino acids rises in the liver (section 12.8). These changes point to elevated use of proteins for energy supply to the exercising muscles. However, as mentioned in several instances, the changes are small. It seems that the body spares proteins because they are of paramount importance for all biological functions and because the body lacks a protein reservoir that it could recruit in case of emergency.

All in all, the contribution made by proteins to the energy expenditure of prolonged (even extremely prolonged) exercise is small. Most studies estimate it at 3% to 6% of the total energy expenditure. The input of proteins in ATP resynthesis during short efforts of any intensity is negligible. The maximal rate of ATP resynthesis from amino acid oxidation is only 0.1 mmol per kilogram muscle per second, even lower than that from fatty acid oxidation (0.2 to 0.3 mmol $\cdot$ kg^{-1} $\cdot$ s^{-1}; section 11.15).

The fact that proteins contribute little to energy expenditure during exercise suggests that athletes do not need the excessive protein intakes that nonexperts often advocate. Indeed, most researchers agree that athletes are well advised to obtain about 10% to 15% of their energy from dietary proteins. (Carbohydrates, the main fuel for most sport activities, should

contribute more than 50% to energy intake, and lipids should be confined to about 30%.) More accurate recommendations for protein consumption take into account the weight of an individual. We will return to this issue in the highlight box at the end of the chapter, after we have explored how protein turnover is affected by training (in the next section).

12.12 Effects of Training on Protein Turnover

In section 12.4, we discussed the acute effects of exercise on protein turnover. What about the chronic effects? They are more spectacular than the acute ones, as you can judge from two extreme examples: muscle hypertrophy caused by **resistance training** (also known as strength training) and muscle atrophy caused by immobilization due to injury or surgery. It is no exaggeration to say that regular physical activity is the most powerful factor controlling muscle protein turnover. The effect is so striking that we speak of **muscle remodeling**, that is, reorganization of muscle structure and function, in response to training. Muscle remodeling plays an important role in maintaining both health and performance.

As you have realized from the discussion of how training influences the lipidemic profile (sections 11.21 and 11.22), and as we will see repeatedly in the following two chapters, *different training types elicit different adaptations*. Most research data on the adaptations of protein metabolism refer to resistance training, followed by endurance training. These adaptations are the subject of the present section. Then, in the next chapter, we will explore the biochemical mechanisms that mediate the adaptations of protein metabolism to training, which involve changes in gene expression in response to the mechanical, neural, hormonal, and metabolic stimuli generated by training.

Resistance Training

When accompanied by adequate protein intake, repeated sessions of resistance exercise result in an accumulation of muscle protein gains, primarily the myofibrillar proteins (figure 12.12). This accumulation causes a bulging of the myofibrils in the muscle fibers, followed by longitudinal

fission of the myofibrils and an increase in their number. Increases also occur in the volumes of the cytosol and sarcoplasmic reticulum.

These changes produce an increase in the cross-sectional area of muscle fibers as assessed by light microscopy. This increase is what we refer to as **muscle hypertrophy** (*hypertrophy* means "overfeeding" in Greek). Then increases occur in cross-sectional area and volume of the entire muscle (ideally, measured by scanning techniques such as magnetic resonance imaging, computed tomography, and ultrasound). These adaptations are measurable a few weeks after the beginning of training. Resistance training is also accompanied by an increase in maximal muscle strength, which traditionally has been attributed to muscle hypertrophy. However, this connection has been reasonably questioned by Scott Dankel and associates, who, instead, ascribe strength gains to the principle of specificity: Simply stated, performing a resistance exercise repeatedly makes you learn to perform it better.

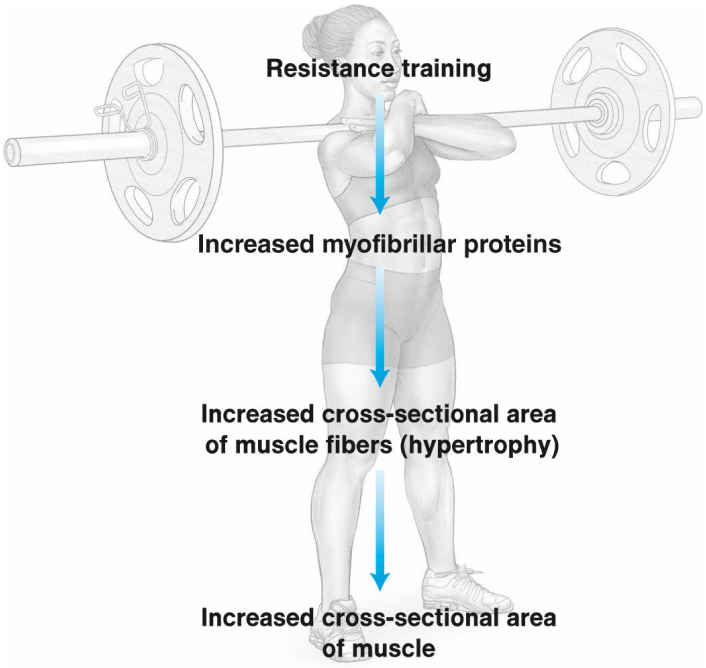


Figure 12.12 **Effect of resistance training on muscle proteins.**

Average rates of muscle hypertrophy range from about 0.1 to 0.2% per day, as reviewed by Mathias Wernbom and colleagues. The hypertrophic

stimulus weakens with time because of gradually smaller increases in muscle protein balance after each session of resistance exercise. After five to six months of resistance training, muscle cross-sectional area reaches a plateau that is 33% above baseline on average. Individual increases can exceed 50%. As for relative gains in maximal muscle strength, they can be similar to or larger or smaller than those in muscle cross-sectional area.

Of course, the cessation of muscle growth does not mean that it is pointless for a resistance-trained individual to carry on training. Indeed, discontinuing training, or *detraining*, leads to gradual loss of muscle mass gains, as discussed in section 14.27. The cessation of muscle growth simply means that there is a natural limit to an individual's muscle mass.

Adaptations to resistance training may differ between muscle fiber types. In response to hard training, type IIX fibers alter their gene expression pattern to become type IIA within a few weeks, resulting in their being practically undetectable after four months of training, as reviewed by Gregory Adams and Marcas Bamman. Whether the degree of hypertrophy in response to resistance training differs between fiber types remains controversial. In fact, studies reviewed by Brad Schoenfeld and colleagues in 2016, as well as by Adams and Bamman, have reported all possible results—that is, higher in type I, higher in type II, and no difference.

Considerable interest surrounds the question of which characteristics of a resistance training program ensure maximal gains in muscle mass and strength. Although a comprehensive answer lies beyond the scope of a book on exercise biochemistry, I should point out that consensus is currently lacking despite hundreds of reports on the matter in both the scientific and lay literature. Meta-analyses with various lead authors—including Tim Davies, Grant Ralston, and Schoenfeld—have proven helpful in discovering which factors are crucial and which are not, despite their limitations and the reservations that have been expressed (for example, by Paulo Gentil and coworkers). Here is a summary of findings.

- The number of weekly sets of a particular exercise relates to muscle hypertrophy in a dose–response manner; specifically, each additional set promotes muscle growth by 0.37% per week. Similarly, strength gains are higher with five or more sets than with fewer, although no

additional strength gains seem to exist above nine sets.

The dose–response relationship consists of the way a biological response (in this case, muscle hypertrophy) changes with changing levels, or doses, of a stimulus (in this case, weekly training sets).

- In order to maximize muscle hypertrophy, the major muscle groups should be trained on at least two days per week. Nevertheless, training a muscle group once per week still promotes robust muscle hypertrophy.
- Intensities above 60% of 1RM tend to produce greater muscle growth and strength gains than do intensities of 60% or below. Nevertheless, intensities between 20% and 60% of 1RM can produce substantial gains as long as sets are carried out to momentary muscular failure.
- The time taken to complete a repetition does not seem to influence the hypertrophic response over a wide range (0.5 to 8 s) if sets are carried out to failure. Thus, repetitions can be carried out at either a fast or a slow tempo.
- Although training to momentary muscular failure may favor a fair comparison of training programs with differing parameters, such as intensity or volume, it seems to cause slightly lower strength gains as compared with training that does not lead to failure. Thus the latter may be preferable, especially for novices, because it entails less discomfort and a lower risk of injury.

In resistance training, *volume* is another word for *work*. It is usually expressed as sets · repetitions · intensity.

- Eccentric training causes slightly higher hypertrophy than does concentric training. Thus, both eccentric and concentric muscle activity should be included in a resistance training program.

Sprint training also causes muscle hypertrophy, although the effect is smaller and requires about two months to appear. Regarding connective tissue (and similar to the controversial results regarding the effect of acute

exercise on collagen synthesis mentioned in section 12.4), it remains uncertain whether training causes tendon hypertrophy. As reviewed by Kjaer and associates, cross-sectional studies show hypertrophy of the Achilles tendon in habitual endurance runners, an effect that lowers the stress on the tendon and may reduce the risk of injury. However, a prolonged training intervention failed to elicit hypertrophy of the Achilles tendon. On the other hand, resistance training of the knee extensor muscles usually results in hypertrophy of the patellar tendon. Thus, it may be that different tendons respond differently to training.

Note that the number of muscle fibers does not change in hypertrophy. Such a rise in muscle fiber number, called **muscle hyperplasia** (meaning "excess formation"), has been found in animal models of resistance training. However, there is little evidence that it occurs in humans. In the case of atrophy, the cross-sectional area of human muscle fibers decreases, but, again, their number does not seem to change.

Muscle hypertrophy consists of an increase in muscle fiber size, whereas muscle hyperplasia consists of an increase in muscle fiber number.

Endurance Training

Endurance training is usually not accompanied by muscle hypertrophy unless it employs high intensities, in which case it may cause some increase in myofibrillar proteins and a small degree of hypertrophy. The characteristic adaptation of muscle protein metabolism to endurance training is different—namely, an increase in mitochondrial proteins (figure 12.13), as reviewed by Frank Booth and coworkers. These proteins include (in order of presentation in this book) enzymes of the citric acid cycle, proteins of the electron-transport chain, subunits of ATP synthase, carnitine acyltransferases I and II, enzymes of β oxidation, aminotransferases, and the branched-chain α-keto acid dehydrogenase complex.

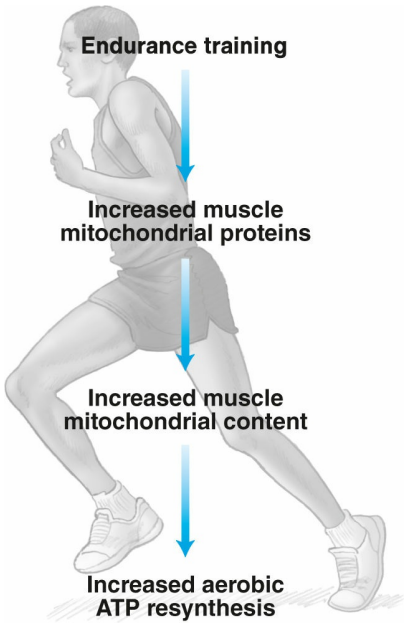


Figure 12.13 **Effect of endurance training on muscle proteins.**

The increase in muscle mitochondrial proteins with endurance training leads to an increase in the volume of the mitochondrial reticulum, the network of cellular mitochondria described in section 10.10. To express the volume of the mitochondria as a percentage of muscle fiber volume, researchers use the term **mitochondrial volume density**. However, the term *volume density* makes little physical sense, since volume does not have density (rather, volume is multiplied by density to produce mass). A more appropriate—and simpler—term would be *content*. **Mitochondrial content**, then, is about 4% (ranging from 2% to 6%) in untrained human skeletal muscle and can increase by about 50% (that is, to 6% on average, and ranging from 4% to 8%) after a few (for example, six) weeks of endurance training.

The overwhelming majority of the relevant scientific literature ascribes the exercise-induced increase in muscle mitochondrial content to generation of new mitochondria, a process termed **mitochondrial biogenesis**. However, Anne-Kristine Meinild Lundby and coworkers found that the increase in mitochondrial content after endurance training was due not to biogenesis but to mitochondrial enlargement, or **mitochondrial hypertrophy**. We can hope that future research resolves the question of whether endurance training

causes an increase in mitochondrial number, size, or both.

The exercise-induced increase in mitochondrial content allows the muscles to regenerate a larger portion of the ATP they need for exercise through the aerobic breakdown of carbohydrates, lipids, and proteins. This process, being more economical than the anaerobic breakdown of carbohydrates, increases resistance to fatigue. We will explore this important change, in sections 14.14 and 14.15, in the wider context of how endurance training affects the selection of energy sources during exercise.

Protein and Amino Acid Supplementation in Exercise and Training

At several points throughout this chapter, I have mentioned adequate intake of protein and amino acid as a prerequisite for positive changes in muscle protein balance with acute and chronic exercise. The question then arises: What is adequate intake of protein and amino acid for exercisers? Research has shown that athletes and, more generally, individuals who exercise regularly have higher protein requirements than individuals who do not. Thus, although the daily recommended dietary allowance for the general adult population is 0.8 g per kilogram body mass, multiple organizations—the American College of Sports Medicine, the former American Dietetic Association (now Academy of Nutrition and Dietetics), and Dietitians of Canada—issued a joint position statement, authored by Nancy Rodriguez and associates, recommending 1.2 to 1.4 $g \cdot kg^{-1}$ for persons who engage in endurance training and 1.2 to 1.7 $g \cdot kg^{-1}$ for persons who perform resistance training.

The higher protein requirements for exercisers can be explained by the need to counterbalance the increased protein breakdown after exercise (section 12.4 and figures 12.5 and 12.6) and, in the case of resistance training, to provide raw materials for muscle hypertrophy. The latter is the reason for the higher research interest in the protein requirements of resistance training than of endurance training.

Although considerable debate surrounds the question of whether protein supplementation is necessary to maximize the muscle growth and strength benefits of resistance training, most studies provide a positive answer.

As an example, a meta-analysis of 49 studies, with 1,863 participants, by Robert Morton and collaborators confirmed that protein supplementation during prolonged (at least six-week) resistance exercise improved muscle hypertrophy and maximal strength (1RM). Protein supplementation increased daily protein intake from 1.4 to 1.8 g · kg^{-1} on average, although the authors determined that, regarding hypertrophy, daily average protein intakes above 1.6 g · kg^{-1} resulted in no further gains. The contribution of protein supplementation to indices of hypertrophy ranged from 14% to 38%, and the contribution to strength gain was 10%. These results help to put protein supplementation in context, because they show that the lion's share of the adaptations belongs to training per se.

Protein supplementation can be achieved in a variety of ways, ranging from the increased consumption of foods rich in proteins, such as those listed in section 12.1, to commercial supplements for protein, amino acids, or both. Are some protein sources preferable? The key is to consume proteins that are highly digestible, deliver amino acids efficiently to muscle, and have a high proportion of essential to nonessential amino acids. (Because essential amino acids are not synthesized in the body, their adequacy may become a limiting factor of protein synthesis.) Among the essential amino acids, leucine possesses the highest anabolic potential. The highest known leucine content is found in whey protein (whey is the liquid that remains after milk has been curdled and strained to become cheese). Among plant foods, maize protein is believed to contain the highest proportion of leucine.

Are supplements of individual amino acids or groups of amino acids (such as the BCAA) superior to supplements of whole or hydrolyzed proteins? It appears not. Research has yet to produce sufficient evidence that any of the many marketed products of this kind possesses

higher anabolic potential. Thus, the solid advice for exercisers who wish to increase their protein intake is to select protein-rich foods or high-quality protein supplements.

Does it matter when protein is ingested relative to resistance exercise? For instance, it is a popular practice to take part of the daily protein allowance between one hour before the start and one hour after the end of an exercise session in order to optimize adaptations. However, a meta-analysis by Schoenfeld and colleagues in 2013 showed that such a practice conferred little benefit as compared with taking proteins earlier than two hours before, later than two hours after exercise, or both in training programs lasting at least six weeks. Thus, again, it seems that total daily protein intake is what matters the most in maximizing the adaptations to resistance training, although there is no scientifically justifiable reason to delay protein intake for many hours after exercise.

Summary

Proteins in the body undergo constant turnover: They are formed from and degraded into amino acids, which derive either from the digestion of dietary proteins in the stomach and small intestine or from biosynthesis in our cells. The balance of the two processes depends on the dietary state and exercise. Performing resistance exercise in the fasted state makes the negative baseline protein balance in the exercising muscles less negative, whereas consuming proteins or amino acids after exercise switches the protein balance to positive. Conversely, performing endurance exercise makes the muscle protein balance more negative; again, however, consuming proteins or amino acids after exercise switches the protein balance to positive.

Amino acids can contribute to the energy expenditure of exercise either by being oxidized in the exercising muscles or by being converted into glucose in the liver. Either way, they first

dispose of their α-amino groups, which end up in urea through the urea cycle, a pathway operating in the liver at the expense of four ATP. The carbon skeletons of the amino acids are then converted into pyruvate, acetyl CoA, acetoacetyl CoA, or compounds of the citric acid cycle. Amino acids that can yield glucose through gluconeogenesis are termed *glucogenic*, whereas those that cannot are termed *ketogenic*. The contribution of proteins to the energy expenditure of exercise is minor. Humans cannot synthesize 9 of the 20 amino acids of proteins; hence, these 9 are referred to as *essential*.

Chronic exercise produces more spectacular effects on protein turnover than does acute exercise. Adaptations depend on training type. Resistance training increases the amounts of muscle proteins and, in particular, the myofibrillar proteins. These increases are manifested as muscle hypertrophy. Daily protein intakes of about 1.6 g per kg body mass boost the hypertrophic and strength gains from resistance training. Endurance training, on the other hand, augments the mitochondrial proteins, resulting in increased mitochondrial content and increased capacity to resynthesize ATP from the aerobic breakdown of carbohydrates, lipids, and proteins.

Problems and Critical Thinking Questions

1. How do acute resistance and acute endurance exercise resemble each other regarding their effects on muscle protein turnover? (*Hint:* Compare figures 12.5 and 12.6.)
2. How do acute resistance and acute endurance exercise differ regarding their effects on muscle protein turnover?
3. Do the three BCAA belong to the same or different categories of amino acids regarding our ability to synthesize them?
4. Do the three BCAA belong to the same or different categories of amino acids regarding our ability to synthesize

glucose from them?

5. (Integrative problem) List the ways in which the liver provides energy sources for muscle during exercise.
6. (Integrative problem) As mentioned in section 12.9, carbamoyl phosphate (the starting compound of the urea cycle) is a compound of high phosphoryl-transfer potential. Indeed, the $\Delta G°'$ of its hydrolysis to carbamate and phosphate is –10.3 kcal · mol^{-1}. Where does this value place carbamoyl phosphate in the list of problem 10 in chapter 9, as updated after problem 3 in chapter 10?
7. Suppose that the model man I have used repeatedly in calculations of energy stores performed moderate-intensity exercise for one hour, spending 400 kcal (as in problem 9 in chapter 11). Now suppose that 6% of that energy derived from protein breakdown.
    a. How many grams of proteins did the man burn?
    b. What percentage of his body protein stores did he consume?
8. Answer the preceding problem for a model woman who spent 300 kcal on moderate-intensity exercise for one hour.
9. Muscle hypertrophy caused by resistance training probably results from accumulated positive muscle protein balance after each training session. But how does this balance compare with the rate of muscle hypertrophy? To answer this problem, measure the balance after exercise and protein intake in figure 12.5. Then compare it with the rates of muscle hypertrophy mentioned in section 12.12. If the values differ, suggest a possible explanation.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

protein digestion
gastrin
pepsinogen
proteolysis
pepsin
protease
secretin
trypsin
chymotrypsin
elastase
carboxypeptidase
zymogen
cholecystokinin
enteropeptidase
aminopeptidase
exopeptidase
endopeptidase
protein turnover
lysosome
autophagy
ubiquitin
proteasome
transamination
aminotransferase, transaminase
α-keto acid
glutamate dehydrogenase
oxidative deamination
glutamine synthetase
alanine aminotransferase
essential amino acid

nonessential amino acid
branched-chain amino acid, BCAA
branched-chain α-keto acid
α-ketoisocaproate
branched-chain α-keto acid dehydrogenase complex
glucogenic amino acid
ketogenic amino acid
glutaminase
glucose-alanine cycle
urea
urea cycle
carbamoyl phosphate
aspartate aminotransferase
resistance training
muscle remodeling
muscle hypertrophy
muscle hyperplasia
endurance training
mitochondrial volume density
mitochondrial content
mitochondrial biogenesis
mitochondrial hypertrophy

References and Suggested Readings

Adams GR, Bamman MM (2012). Characterization and regulation of mechanical loading-induced compensatory muscle hypertrophy. *Comprehensive Physiology* 2: 2829-2870.

Atherton PJ, Phillips BE, Wilkinson DJ (2015). Exercise and regulation of protein metabolism. *Progress in Molecular*

Biology and Translational Science 135: 75-98.

Booth FW, Ruegsegger GN, Toedebusch RG, Yan Z (2015). Endurance exercise and the regulation of skeletal muscle metabolism. *Progress in Molecular Biology and Translational Science* 135: 129-151.

Dankel SJ, Buckner SL, Jessee MB, Grant Mouser J, Mattocks KT, Abe T, Loenneke JP (2018). Correlations do not show cause and effect: Not even for changes in muscle size and strength. *Sports Medicine* 48: 1-6.

Davies T, Orr R, Halaki M, Hackett D (2016). Effect of training leading to repetition failure on muscular strength: A systematic review and meta-analysis. *Sports Medicine* 46: 487-502.

Gentil P, Arruda A, Souza D, Giessing J, Paoli A, Fisher J, Steele J (2017). Is there any practical application of meta-analytical results in strength training? *Frontiers in Physiology* 8: 1.

Kjaer M, Jørgensen NR, Heinemeier K, Magnusson SP (2015). Exercise and regulation of bone and collagen tissue biology. *Progress in Molecular Biology and Translational Science* 135: 259-291.

Kumar V, Atherton P, Smith K, Rennie MJ (2009). Human muscle protein synthesis and breakdown during and after exercise. *Journal of Applied Physiology* 106: 2026-2039.

Meinild Lundby AK, Jacobs RA, Gehrig S, de Leur J, Hauser M, Bonne TC, Flück D, Dandanell S, Kirk N, Kaech A, Ziegler U, Larsen S, Lundby C (2018). Exercise training increases skeletal muscle mitochondrial volume density by enlargement of existing mitochondria and not de novo biogenesis. *Acta Physiologica* 222: e12905.

Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, Aragon AA, Devries MC, Banfield L, Krieger JW, Phillips SM (2018). A systematic review, meta-analysis, and meta-regression of the effect of protein

supplementation on resistance training–induced gains in muscle mass and strength in healthy adults. *British Journal of Sports Medicine* 52: 376-384.

Ralston GW, Kilgore L, Wyatt FB, Baker JS (2017). The effect of weekly set volume on strength gain: A meta-analysis. *Sports Medicine* 47: 2585-2601.

Rodriguez NR, Di Marco NM, Langley S (2009). American College of Sports Medicine position stand: Nutrition and athletic performance. *Medicine and Science in Sports and Exercise* 41: 709-731.

Schoenfeld BJ, Aragon AA, Krieger JW (2013). The effect of protein timing on muscle strength and hyper trophy: A meta-analysis. *Journal of the International Society of Sports Nutrition* 10: 53.

Schoenfeld BJ, Grgic J, Ogborn D, Krieger JW (2017). Muscular adaptations in low- versus high-load resistance training: A meta-analysis. *European Journal of Sport Science* 16: 1-10.

Schoenfeld BJ, Ogborn D, Krieger JW (2015). Effect of repetition duration during resistance training on muscle hypertrophy: A systematic review and meta-analysis. *Sports Medicine* 45: 577-585.

Schoenfeld BJ, Ogborn D, Krieger JW (2016). Effects of resistance training frequency on measures of muscle hypertrophy: A systematic review and meta-analysis. *Sports Medicine* 46: 1689-1697.

Schoenfeld BJ, Ogborn D, Krieger JW (2017). Dose-response relationship between weekly resistance training volume and increases in muscle mass: A systematic review and meta-analysis. *Journal of Sports Sciences* 35: 1073-1082.

Schoenfeld BJ, Ogborn D, Vigotsky AD, Franchi M, Krieger JW (2017). Hypertrophic effects of concentric versus eccentric muscle actions: A systematic review and meta-analysis. *Journal of Strength and Conditioning Research* 31: 2599-

2608.

Sweatt AJ, Wood M, Suryawan A, Wallin R, Willingham MC, Hutson SM (2004). Branched-chain amino acid catabolism: Unique segregation of pathway enzymes in organ systems and peripheral nerves. *American Journal of Physiology Endocrinology and Metabolism* 286: E64-E76.

Wang Z, Shen W, Kotler DP, Heshka S, Wielopolski L, Aloia JF, Nelson ME, Pierson RN Jr, Heymsfield SB (2003). Total body protein: A new cellular level mass and distribution prediction model. *American Journal of Clinical Nutrition* 78: 979-984.

Wernbom M, Augustsson J, Thomeé R (2007). The influence of frequency, intensity, volume, and mode of strength training on whole muscle cross-sectional area in humans. *Sports Medicine* 37: 225-264.

CHAPTER 13

Effects of Exercise on Gene Expression

Learning Objectives

After reading this chapter, you should be able to do the following:

- Describe the stages in the control of gene expression.
- Discuss the evidence for the control of each stage of gene expression by exercise.
- Describe and explain the kinetics of a gene product after exercise.
- List experimental tools used by researchers in the study of gene expression.
- Describe which exercise-induced changes may modify gene expression.
- Discuss the possible mechanisms of exercise-induced muscle hypertrophy.
- Discuss the possible mechanisms of the exercise-induced increase in muscle mitochondrial content.
- Compare the different mechanisms of exercise-induced adaptations in terms of timing and stage in the control of gene expression that is primarily involved.

- Describe what epigenetics is and how exercise can influence epigenetic changes.

Skeletal muscles exhibit an astonishing ability to adapt, both structurally and functionally, to changing environment conditions, including exercise. This ability, often termed **muscle plasticity**, derives from changes in gene expression. Remember that we have defined gene expression as the presence of a gene product (that is, RNA or protein) in a cell, organ, or tissue (section 4.12). Changes in gene expression imposed by exercise serve as the focus of this chapter.

Our knowledge about how exercise affects gene expression lags our knowledge of how exercise affects energy metabolism. It is, one might say, like the knowledge of explorers charting a new territory (figure 13.1). As you read this chapter, you will notice more uncertainties than in other chapters and several question marks in the figures. But unknown territories are a great challenge for discoverers. Thus, gene expression in exercise is a scientific field attracting many investigators and one in which information is accumulating at a rapid rate.

In the sections that follow, I will present as much of this information as is pertinent to a textbook, placing emphasis on skeletal muscle, where most of the relevant research has been conducted. Note, however, that exercise affects gene expression in other tissues and organs as well.

Figure 13.1 **Terra incognita.** What we know about the effects of exercise on gene expression, and about the control of gene expression in general, resembles—in terms of both quantity and accuracy—what cartographers of past centuries knew about the shape of the continents. Shown here is part of a world map drawn by the Portuguese cartographer Diogo Ribeiro in 1529. Notice the absence of most of the western coastline of North and South America. Also notice, however, how much was known just 37 years after the discovery of the New World. The map is kept in the Vatican Library.

13.1 Stages in the Control of Gene Expression

When discussing the control of gene expression, we use the following terminology: We say that a gene is *induced,* or *upregulated*, if its product(s) increase(s); the corresponding process is **induction**, or **upregulation**. On the

other hand, we say that a gene is *repressed*, or *downregulated*, if its product(s) decrease(s); the corresponding process here is **repression**, or **downregulation**. Induction seems to be the predominant way of controlling gene expression in eukaryotes.

Cells control gene expression by regulating the rates of the processes included in the following list and depicted in figure 13.2. Most are familiar to you from chapter 4.

1. **Transcription.** As a first step in gene expression, nuclear DNA is transcribed into RNA (section 4.10).
2. **RNA processing.** After transcription, RNA molecules undergo extensive processing to become mRNA (section 4.13), rRNA (section 4.14), tRNA (section 4.16), and the novel kinds of RNA described in section 4.18.
3. **Transport from the nucleus to the cytosol.** Mature RNA molecules pass through the pores of the nuclear envelope to the surrounding cytosol (section 4.13).
4. **RNA degradation.** RNA molecules have rather high turnover rates: On average, within a few hours of formation, they are hydrolyzed by ribonucleases. It is believed that this action helps a cell to respond to rapidly changing needs.
5. **Translation.** Messenger RNA is translated into protein in the cytosol or in the endoplasmic reticulum (omitted in figure 13.2, for clarity) with the aid of rRNA and tRNA (sections 4.14 to 4.17), while miRNA control translation in a negative way (section 4.18).
6. **Proteolysis.** Although cellular proteins are more stable than mRNA, they too are degraded, as discussed in section 12.3.
7. **Post-translational modification and protein targeting.** Many proteins are not ready to play their biological roles right after they are synthesized. Instead, they require post-translational modification. Two examples are proline hydroxylation in collagen (section 6.1 and figure 6.4) and glutamate carboxylation in proteins that mediate blood clotting (section 6.2 and figure 6.7). Two other kinds of post-translational modification are the excision of one or more segments off the

polypeptide chain and **glycosylation**, that is, the covalent linking of one or more carbohydrate chains to the polypeptide chain, resulting in the formation of a glycoprotein. In addition, many proteins need to be transported to specific intracellular compartments (such as the mitochondria), which requires targeting. Often, a specific post-translational modification is a prerequisite for the correct targeting of a protein.

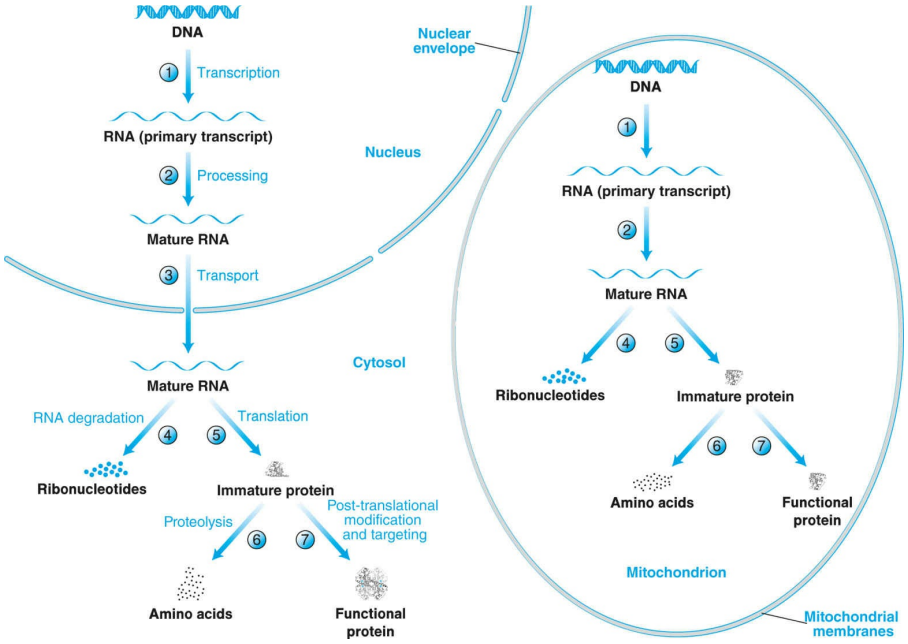


Figure 13.2 Control points in gene expression. The amounts of RNA and protein produced from the expression of nuclear genes are affected by the rates of transcription *(1)*, RNA processing *(2)*, exit of mature RNA to the cytosol through the pores of the nuclear envelope *(3)*, RNA degradation *(4)*, translation *(5)*, protein degradation *(6)*, and post-translational modification and targeting *(7)*. The same factors affect the amounts of RNA and protein produced from the expression of mitochondrial genes, except number 3, since the gene products do not leave the mitochondria.

Control of mitochondrial gene expression goes through the same stages except for transport, since the products of the genes hosted by the small mitochondrial DNA (section 4.6) stay in the mitochondria. Jyngyi Jessica Li and associates estimate that, of all the stages, the most important determinant of protein content in a cell is transcription, followed, in sequence, by translation, RNA degradation, and proteolysis.

Note that most of the processes discussed here consist of smaller steps,

which may be individually controlled. For example, the rate of translation depends on the rates of initiation, elongation, and termination (section 4.17). What is more, a change in one process may produce an effect that opposes the effect of the change in another process. For example, we have seen in section 12.3 that both translation and proteolysis (stages 5 and 6) are accelerated in muscle after exercise, which tends to increase and decrease protein mass, respectively. Such complexities explain part of the difficulty in studying gene expression.

Because of the involvement of so many stages and steps in gene expression, it is not justified to use this term to describe the plain measurement of RNA in a biological sample, as many authors do. Instead, one should speak of *RNA content*, or *RNA concentration*, or *RNA level*. Likewise, it is not justified to speak of *protein expression* in order to describe the mere measurement of a protein (let alone the fact that there is no such thing as protein expression; rather, there is gene expression at the protein level). Again, one should speak of *protein content,* or *protein concentration*, or *protein level*.

13.2 Stages in the Control of Gene Expression Affected by Exercise

Which of the stages itemized in the preceding section are affected by exercise, thus modifying gene expression? There is evidence that exercise is involved in all of the stages. Let's examine that evidence.

1. **Transcription.** As mentioned in the preceding section, transcription appears to be the most important control point of gene expression. However, because of technical difficulties, few studies have measured transcription rates in muscle after exercise. These few have revealed enhanced transcription rates of genes encoding structural proteins, transport proteins, and enzymes. This enhancement derives mainly from increased concentrations of transcription factors (section 4.11) in the nucleus or from structural alterations in transcription factors (for example, by phosphorylation) resulting in their increased binding to

DNA.

2. **RNA processing.** An impressive finding related to the effect of exercise on RNA processing is that hard resistance exercise, stretching, or electrical stimulation causes skeletal muscle to produce an unusual form of the peptide hormone **insulin-like growth factor 1 (IGF1)**, which is termed **mechano growth factor**, or **MGF**. MGF derives from the IGF1 gene by alternative splicing of the primary transcript (section 4.13), synthesis of a different mRNA, and, hence, synthesis of a different protein. The biological role of MGF is currently unclear.
3. **Transport from the nucleus to the cytosol.** The possibility that exercise may affect mRNA export from the nucleus is exemplified by the stimulatory effect of resistance exercise on SKAR (S6K1 Aly/REF-like substrate), a nuclear protein that facilitates the transport of mRNA to the cytosol, through the mTORC1 signal transduction pathway (to be explored in section 13.5).
4. **RNA degradation.** One of the ways through which exercise modifies the rate of RNA degradation is by changing the concentration of many miRNA, which bind to mRNA through base pairing, thus promoting mRNA degradation (section 4.18).
5. **Translation.** Changes in the rate of translation seem to be an important control point of gene expression by exercise. The amount of many muscle proteins increases with exercise without corresponding increases in mRNA, which points to an increase in their translation rate or a decrease in their degradation rate. As far as translation is concerned, initiation seems to be the step that is most affected by exercise. Initiation requires the presence of a group of proteins called **eukaryotic initiation factors** in addition to the components presented in sections 4.14, 4.16, and 4.17 (that is, mRNA, ribosomes, charged tRNAs, ATP, and GTP). Increased concentrations of eukaryotic initiation factors have been found after both endurance and resistance exercise. In addition, exercise boosts translation efficiency and polypeptide chain elongation, as discussed in section 13.5. Finally, exercise-induced changes in miRNA concentrations may affect translation as well as RNA degradation.

6. **Proteolysis.** As mentioned in section 12.4, exercise speeds up proteolysis in muscle. However, we do not know with certainty how this occurs—that is, whether it happens through activation of soluble cytosolic proteases, autophagy, or the ubiquitin proteasome system, all of which are described in section 12.3.
7. **Post-translational modification and protein targeting.** As far as post-translational modification is concerned, we will consider many protein phosphorylations and a case of protein acetylation as ways in which gene expression is influenced by both resistance and endurance exercise (sections 13.5 and 13.6). As for protein targeting, we will see that the exercise-induced post-translational modification of a protein that plays a key role in the adaptations to exercise (PGC1α) affects its targeting to the nucleus.

13.3 Kinetics of a Gene Product After Exercise

The fact that changes in the rates of protein synthesis and degradation are measurable hours or even days after exercise (section 12.3) implies that gene expression is modified not only during exercise but also in the recovery period. In fact, the changes that take place during recovery appear to account for the major part of adaptations to training.

We can monitor changes in gene expression during recovery from exercise by measuring the concentration of RNA and proteins in muscle or other tissues at different times after an exercise session—for example, during a 24-hour period. In this way, we determine the *kinetics* of the gene products. If we perform such measurements following repeated exercise sessions in a training program, we can monitor adaptations to training.

As you might expect, the kinetics of gene products after exercise differ from one RNA to another and from one protein to another. In general, changes in muscle RNA content are larger and more rapid than changes in protein content. Thus, an RNA may increase several fold a few hours after an exercise session, whereas a protein may take several sessions to exhibit a measurable increase.

When it comes to genes encoding proteins (which form most of the human genes, as presented in section 4.12), there is usually no agreement between the kinetics of a specific mRNA and the cognate protein. Studies reviewed by Mark Hargreaves have produced a potpourri of responses to exercise and training, including the following:

- A transient increase in mRNA after each exercise session, accompanied by a gradual increase in protein during the initial sessions of the training program and a later stabilization (figure 13.3*a*)
- A gradual increase in both mRNA and protein during the initial sessions of the training program, followed by a plateau in mRNA while the protein keeps rising (figure 13.3*b*)
- A gradual increase in both mRNA and protein during the initial sessions of the training program, followed by a drop in mRNA and a plateau in the protein (figure 13.3*c*);
- No change in mRNA throughout the training program, accompanied by an initial rise and subsequent plateau in the protein (figure 13.3*d*)
- A sustained rise in mRNA throughout the training program without any change in the protein (figure 13.3*e*).

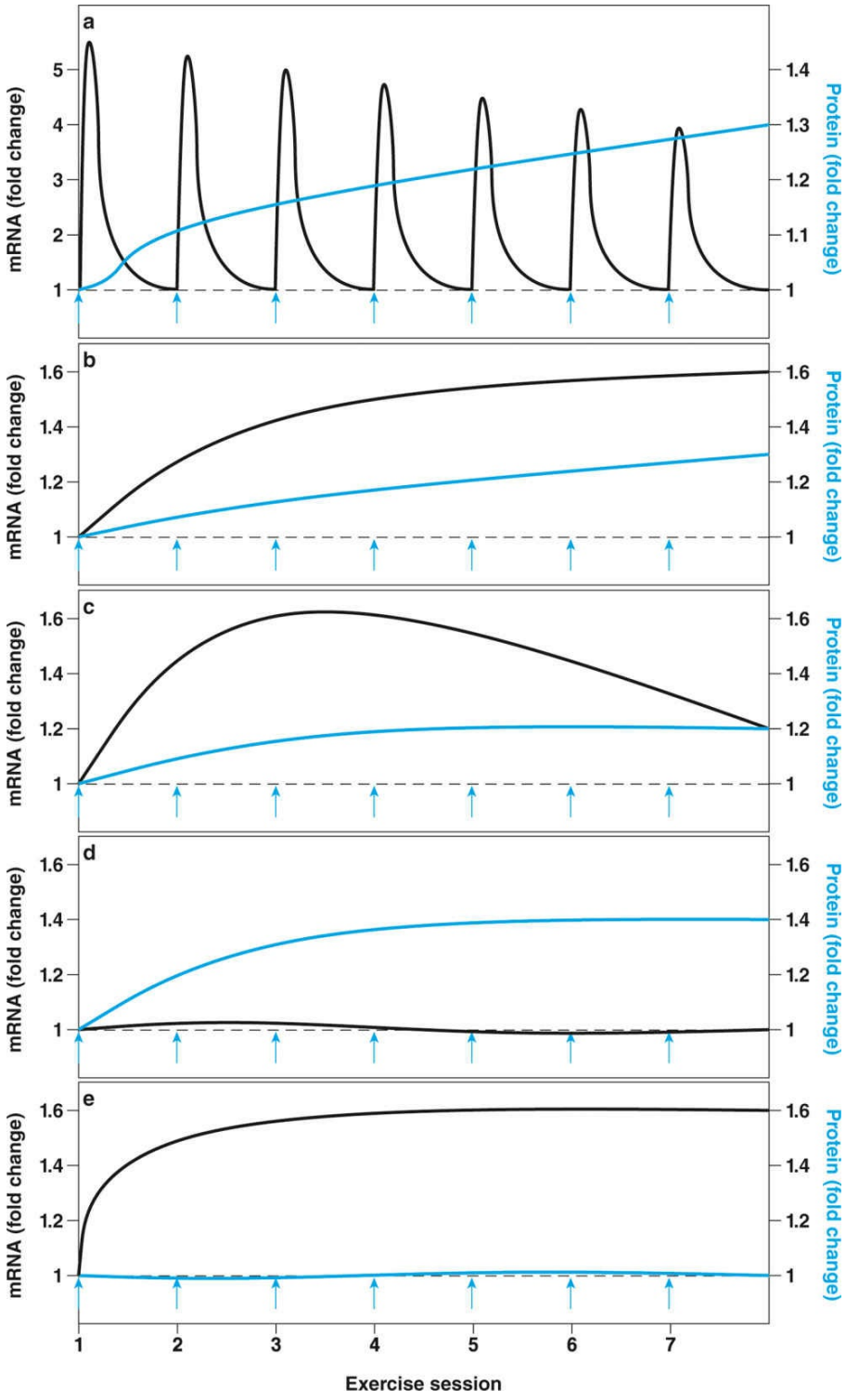


Figure 13.3 **Postexercise kinetics of gene products.** The muscle mRNA (black) and protein contents (color) can change in any of a variety of ways during a training program in which exercise sessions (indicated by arrows) are spaced one to three days apart. Some ways are shown here. *(a)* Messenger RNA rises transiently after each session and returns to baseline before the next one; protein rises gradually at first, then stabilizes. *(b)* mRNA rises

gradually after the initial sessions, then stabilizes; protein rises at a slower rate but does not stabilize. *(c)* Messenger RNA rises gradually after the initial sessions, then drops; protein rises at a slower rate, then stabilizes. *(d)* Messenger RNA does not change considerably; protein rises gradually at first, then stabilizes. *(e)* Messenger RNA remains elevated throughout the training program, but the protein does not change.

Such discordance between mRNA and protein kinetics shows the importance of all the stages in gene expression depicted in figure 13.2—not just transcription and translation—in determining adaptations to exercise. For example, the fact that a protein may keep rising in the face of a plateau in mRNA (as in the second pattern just listed) may be explained by a decrease in proteolytic rate. You may practice seeking explanations for other discordances in figure 13.3. (Also, problems 1 through 3 are related to explaining the mRNA and protein kinetics in figure 13.3.) The discordance in question also shows that it is not safe to deduce effects of exercise on muscle protein content from changes in mRNA content, although the latter may be easier to do in the laboratory. (See the highlight box for experimental techniques for measuring RNA and protein.)

Tools in the Study of Gene Expression

Several remarkable inventions in recent decades have greatly facilitated our study of gene expression and of the ways in which it is affected by various factors, such as diet, exercise, and disease. To begin with, the base sequence of large DNA molecules is now easily and quickly determined through the use of computerized instruments that rely partly on the pioneering work of British biochemist Frederick Sanger, who invented a method of DNA sequencing in 1977. Incidentally, Sanger had introduced the amino acid sequencing of proteins a quarter century earlier (in 1953) by deciphering the sequence of insulin. These discoveries earned him two Nobel Prizes in Chemistry, one in 1958 and one in 1980. In 1983, the invention of the **polymerase chain reaction (PCR)** by Kary Mullis (also a Nobel laureate in Chemistry, in 1993) enabled the amplification and characterization of tiny amounts of DNA (consisting of even a single molecule), thus opening new avenues in

genomics, diagnostics, forensics, archaeology, and paleontology.

Changes in the muscle content of RNA species or proteins of interest after acute or chronic exercise can be examined by first extracting a mixture of RNA or proteins from specimens obtained through muscle biopsy at various time points. RNA species are then separated by electrophoresis in a gel, transferred to a membrane by electrophoretic blotting, and allowed to base-pair (or **hybridize**, as we call it) with a probe, which is synthetic RNA or single-stranded DNA of complementary base sequence to part of the RNA of interest. The probe carries radioactive or light-emitting atoms, which enable detection by exposure to an X-ray film and measurement by densitometry. This technique is known as **Northern blot**.

A more sensitive and quantitative method of measuring RNA is **quantitative** (or **real-time**) **reverse transcription PCR**, which begins with the synthesis of DNA that has a base sequence complementary to the RNA of interest (hence termed **cDNA**). Researchers then amplify the cDNA by PCR and measure it during amplification (in real time) by using fluorescent DNA probes that emit light when the PCR product has been synthesized. This procedure allows measurement of the amount of RNA in the original specimen.

Proteins, the end of gene expression, can be measured in muscle extracts by a technique known as **Western blot**, or **protein immunoblot**. Similarly to the Northern blot, the proteins are separated by gel electrophoresis and transferred to a membrane by electrophoretic blotting. Then the protein of interest is stained with an antibody carrying a color-producing tag. Finally, color intensities are compared among samples to discover changes in the amount of the protein with acute or chronic exercise.

In addition to the Western blot, enzymes, in particular, can be measured through their catalytic activities. Doing so requires conditions that ensure maximal enzyme activity (*V*max, section 3.16), such as saturating substrate concentration, presence of necessary cofactors, optimal pH, and optimal ionic strength. (Temperature is almost invariably kept at 37 °C to mimic the human body temperature.) One

then measures the rate of production of a product or the rate of consumption of a substrate by employing a variety of techniques—spectrophotometric, fluorometric, radioactive, mass spectrometric, chromatographic, or electrophoretic—as described in section III.7. Under such conditions, enzyme activity corresponds to enzyme amount. Note, however, that maximal enzyme activity may differ from the actual activity in the tissue; if one is interested in the latter, one needs to observe the tissue's natural conditions.

An alternative way of discovering changes of muscle proteins in response to exercise is through **proteomics**, defined in section 3.3 as the large-scale, comprehensive study of the proteomes in terms of protein identity, quantity, and function. Proteomic investigations have employed an assortment of gel electrophoresis, liquid chromatography, and mass spectrometry techniques (section III.7) to identify and measure muscle proteins. Such investigations (reviewed by Ana Isabel Padrão and collaborators) have the potential to expand our understanding of protein changes in response to exercise.

Progress in **genomics** and **transcriptomics** (sections 4.6 and 4.10) has enabled the simultaneous examination of the expression of thousands of genes through **DNA microarray**, or **DNA chip**, or **gene chip**, technology. Short deoxyribonucleotide sequences, characteristic of the genes of interest, are deposited by robotic devices in minute amounts as dots on a plate measuring a couple of centimeters in each dimension (the chip). Alternatively, the sequences are synthesized directly on the chip. The RNA molecules extracted from a tissue are then converted into cDNA with fluorescently labeled deoxyribonucleotides, and these cDNA molecules are left to hybridize to complementary base sequences on the chip. The dots that fluoresce correspond to genes that are expressed.

It is even possible to treat the chip with a mixture of two cDNA sets, each deriving from the RNA complement of a tissue under two conditions that we want to compare—for example, muscle before and after exercise. In that case, each cDNA set is prepared with nucleotides that fluoresce in different colors. Dots on the chip emit one color (say

green) if expression of a gene increased with exercise, another (red) if expression decreased, and yet another (yellow) if expression did not change. Thus, DNA chips let us evaluate gene expression changes at the RNA level on a global scale.

An analogous technique, evaluating gene expression changes at the protein level, is **protein microarray**, or **protein chip**. Plates now contain antibodies or other substances that capture specific proteins rather than deoxyribonucleotide sequences. Use of protein microarrays is not as widespread as that of DNA microarrays, because handling proteins is technically more challenging than handling DNA.

13.4 Exercise-Induced Changes That May Modify Gene Expression

The modification of gene expression in a cell by an external or internal stimulus is triggered by one or more changes taking place somewhere in the cell. Which of the many changes caused by exercise signal the modification of gene expression in a muscle fiber? The answer is not known with certainty, but research has pointed toward several suspects. The main ones are as follows:

- The decrease in ATP concentration and the concomitant increase in the ADP and AMP concentrations
- The increase in the cytosolic Ca^{2+} concentration because of Ca^{2+} release from the sarcoplasmic reticulum upon excitation of the muscle fiber
- The decrease in the glycogen concentration
- The decrease in the oxygen concentration (**hypoxia**) due to increased O_2 consumption in the electron-transport chain
- Changes in the **redox state**, defined as the ratio of the concentrations of oxidized to reduced substances
- The decrease in pH caused by the anaerobic breakdown of carbohydrates
- The application of tension (stretching) on the fiber

- The increased binding of ligands to cell receptors

Some of these stimuli stand at the top of complex signal transduction pathways mediating the effects of resistance and endurance exercise on gene expression. These pathways are being gradually deciphered and are the subject of the next two sections.

13.5 Mechanisms of Exercise-Induced Muscle Hypertrophy

The uncertainties that I wrote about in the introduction to this chapter encompass the mechanisms of exercise-induced muscle hypertrophy. Of the several possible mechanisms, I have chosen to present two of the best characterized and, apparently, most important: the **mTORC1 pathway** and satellite cell activation.

The mTORC1 Pathway

A major signal transduction pathway mediating the effects of resistance exercise on gene expression involves the protein **mammalian target of rapamycin**, or **mechanistic target of rapamycin (mTOR)**. The protein owes its name to the fact that it is indirectly inhibited (that is, after binding to another cellular protein) by rapamycin, a compound of bacterial origin used medically to suppress the immune system.

Mammalian TOR is a protein kinase that phosphorylates proteins at serine residues (figure 10.10) or threonine residues (figure 10.16). It participates in the formation of two functionally distinct protein complexes—**mTOR complex 1 (mTORC1)** and **mTOR complex 2 (mTORC2)—**of which the former is known to be involved in exercise-induced muscle hypertrophy. Mammalian TORC1 is composed of five subunits, one of which is mTOR.

Mammalian TORC1 lies in the middle of a signal transduction pathway (figure 13.4), of which the lower part (“downstream” of mTORC1) is less ambiguous than the upper one (“upstream” of mTORC1). In other words, we know more about what resistance exercise does to mTORC1 and to the proteins that mTORC1 affects than we do about the path through which

resistance exercise comes to affect mTORC1. Thus, I will begin with the lower part, then move to the upper one.

A session of resistance exercise activates mTORC1 either through phosphorylation of mTOR or through an allosteric effect on the entire complex. The activated mTORC1 phosphorylates two proteins with the rather complex names **eukaryotic initiation factor 4E-binding protein 1 (4EBP1)** and **ribosomal protein S6 kinase 1 (S6K1)**. 4EBP1, in its unphosphorylated form, binds and, in doing so, inactivates **eukaryotic initiation factor 4E (eIF4E)**, which is one of several proteins needed for translation initiation in eukaryotic ribosomes. When phosphorylated by mTORC1, 4EBP1 is detached from eIF4E, which is then free to interact with other initiation factors and form a complex that is a prerequisite for translation initiation.

The other target of mTORC1, S6K1, is a protein kinase with multiple substrates, which hampers identification of the ones essential for the effect of mTORC1 on muscle protein synthesis. Ironically, the first substrate that was discovered, ribosomal protein S6 (and the one that gave the kinase its name), does not appear to be essential, as Brian Magnuson and associates have reviewed. Two other probable candidates are **S6K1 Aly/REF-like substrate (SKAR)** and **eukaryotic elongation factor 2 kinase (eEF2K)**. Let's see how they are involved in muscle protein synthesis.

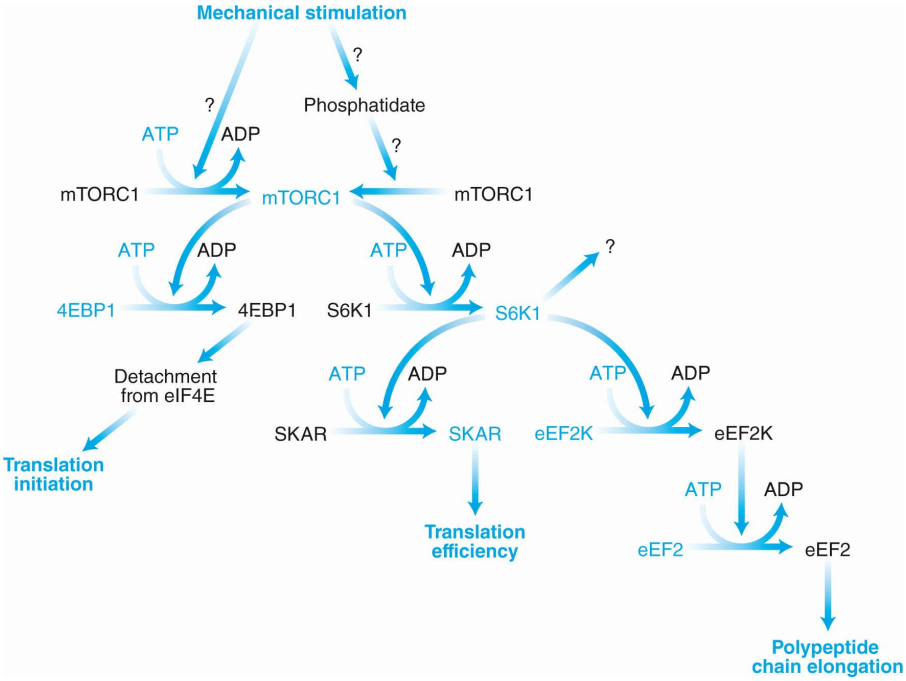


Figure 13.4 The mTORC1 signal transduction pathway. Mechanical stimulation of the muscle by resistance exercise leads to activation of mTORC1 through either phosphorylation or allosteric binding of phosphatidate. Mammalian TORC1 phosphorylates 4EBP1, thus promoting its detachment from eIF4E, which participates in translation initiation. In addition, mTORC1 phosphorylates and activates S6K1, which phosphorylates SKAR, eEF2K, and, possibly, other targets. SKAR phosphorylation promotes translational efficiency, whereas phosphorylation deactivates eEF2K, resulting in diminished phosphorylation of its substrate, eEF2. Because the unphosphorylated form of eEF2 is the active one, the net result is promotion of polypeptide chain elongation. Promotion of translation initiation, translation efficiency, and elongation all contribute to increased protein synthesis. As in the signal transduction pathways outlined in figures of previous chapters, the more active forms of proteins are depicted in color.

SKAR is a nuclear protein that binds to newly synthesized mRNA and facilitates its transport to the cytosol. Phosphorylated SKAR somehow enhances the translation efficiency (that is, how many protein molecules are synthesized per mRNA molecule before it is degraded) and, hence, the rate of protein synthesis. On the other hand, eEF2K, yet another protein kinase, phosphorylates eukaryotic elongation factor 2 (eEF2), which is a protein needed for the movement of the ribosome along mRNA (figure 4.28*c* and 4.28*d*). This movement allows the ribosome to read one codon after another and add one amino acid after another to a growing polypeptide chain. Phosphorylation inactivates both eEF2K and eEF2. Thus, S6K1 exerts a negative effect on a negative effect, which produces a positive effect on the elongation of the polypeptide chain being synthesized.

We add the term *eukaryotic* in front of initiation and elongation factors to distinguish them from bacterial initiation and elongation factors, which are different proteins.

Several studies (reviewed by Chris McGlory and colleagues) have shown increased phosphorylation of proteins in the mTORC1 signal transduction pathway after resistance exercise in humans. In addition, rapamycin intake blocked the rise in muscle protein synthesis after resistance exercise. These findings show that the mTORC1 pathway plays an important role in this response.

Research (again reviewed by McGlory and colleagues) has also shown that single resistance-exercise sessions—as well as the first week (and up to

six weeks, but not subsequent weeks) of resistance training—were accompanied by increases in the muscle content of some rRNA species and some ribosomal proteins. The increases apparently resulted from induction of the genes that encode these products. This finding suggests that resistance exercise may promote the generation of new ribosomes, a process termed **ribosomal biogenesis**, which would boost the capacity of muscle to synthesize new proteins. However, it is not clear whether this response involves the mTORC1 pathway or other pathways.

Let's move now to the upper part of the mTORC1 pathway and ask how resistance exercise activates mTORC1. Currently, the answer is not known with certainty, despite intense research efforts. It appears that the trigger is the mechanical stimulus delivered to muscle by resistance exercise; however, the particular biological signals created by this mechanical stimulation remain unknown. One possible candidate is **phosphatidate**, the parent glycerophospholipid that we met in section 5.9 and figure 5.14 and considered as an intermediate compound in triacylglycerol synthesis (section 11.4). However, it is unclear how mechanical stimulation of the muscle raises the muscle phosphatidate concentration or how phosphatidate activates mTORC1. The latter could be either the effect of direct binding to mTOR or an effect on the other mTORC1 subunits. In either case, mTORC1 activation by phosphatidate does not involve mTOR phosphorylation.

Satellite Cell Activation

Exercise-induced muscle hypertrophy in response to resistance training seems to result in part from an increase in the DNA content of muscle fibers. This increase appears to occur thanks to the addition of nuclei deriving from **satellite cells** to the multiple nuclei already present in each fiber; these additional nuclei are termed *myonuclei* for distinction. Satellite cells are small cells, belonging to the category of adult stem cells, which are located close to a muscle fiber (figure 13.5) and wrapped in the same **basal lamina**, a sheet of extracellular matrix between the sarcolemma and the endomysium that surrounds each fiber. They contain little more than a nucleus, a fact indicative of their mission.

Adult stem cells are present in the adult body but have not differentiated; they can differentiate into specialized cells.

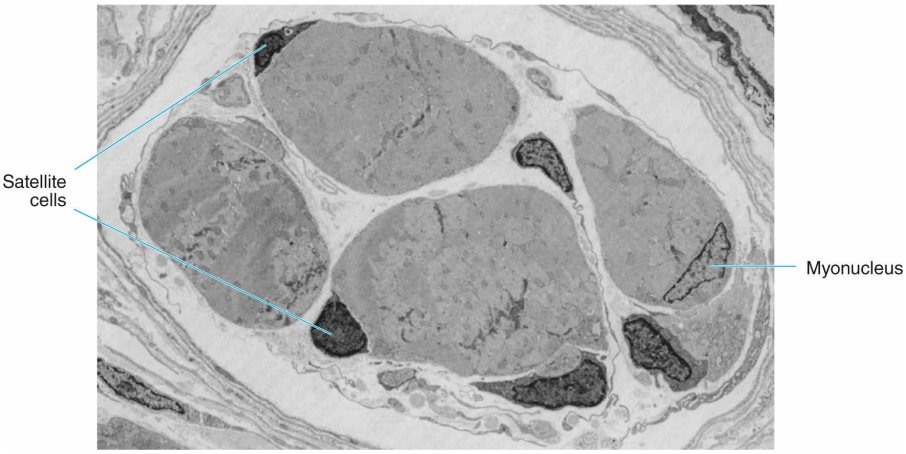


Figure 13.5 **Satellite cells.** An electron micrograph of four muscle fibers shows two satellite cells stuck to two of the fibers. The satellite cells are stained dark due to the presence of chromatin in their nuclei (cf. figure 1.9). The volume of a satellite cell is occupied almost entirely by the nucleus. A myonucleus, lying inside a muscle fiber and close to the plasma membrane, can be seen on the right.

Courtesy of Mikel H. Snow.

Satellite cells serve as a reserve of myogenic (that is, muscle-producing) cells and play an important role in muscle repair: When muscle fibers are damaged because of injury, satellite cells receive signals to proliferate. In fact, the number of satellite cells more than doubles three days after eccentric damaging exercise, as reviewed by Tim Snijders and coworkers. Some of the progeny remain in stock for future needs, and the rest differentiate into myoblasts, which fuse together to generate new fibers.

In addition to muscle fiber regeneration, satellite cells seem to play an important role in the hypertrophic response to resistance training. Indeed, nondamaging resistance exercise triggers proliferation of satellite cells, but the increase in number is lower than that caused by damaging exercise, peaking at about 40% after three days. As in the case of injury, some of the progeny return to their so-called quiescent state for future needs, and the rest differentiate into myoblasts. However, rather than fusing with each other, these myoblasts fuse with their adjacent muscle fibers, thus endowing them with additional nuclei (figure 13.6). The resulting increase in a muscle fiber's

DNA is believed to boost transcription and translation, thus contributing to muscle hypertrophy.

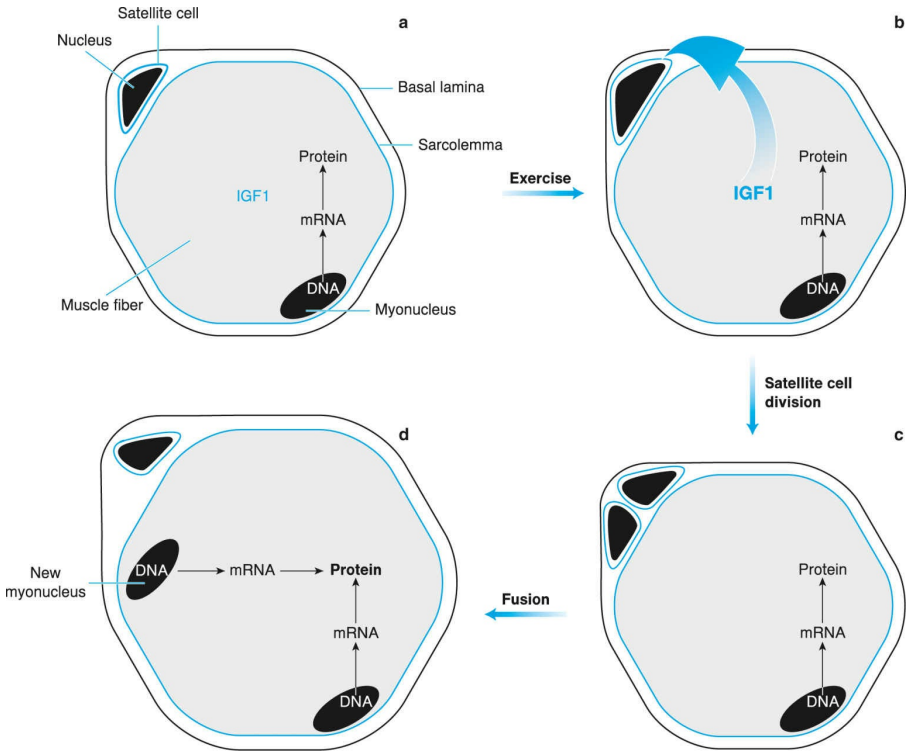


Figure 13.6 **Satellite cell activation contributes to muscle hypertrophy.** *(a)* Diagram of a cross-section of a muscle fiber depicts a myonucleus lying close to the sarcolemma and offering the information contained in its DNA for mRNA and protein synthesis. A satellite cell lies close to the muscle fiber; both are enclosed in the basal lamina. The muscle fiber has a low resting IGF1 concentration. *(b)* After resistance exercise, the IGF1 concentration rises and IGF1 binds to its receptor in the plasma membrane of the satellite cell, causing the cell to grow in preparation for mitosis. *(c)* The satellite cell divides. *(d)* One of the daughter cells fuses with the muscle fiber, thus providing a new nucleus and more DNA for mRNA and protein synthesis. Augmented protein synthesis, in turn, increases the cross-sectional area of the muscle fiber.

The biochemical events that lead from resistance exercise to satellite cell proliferation and fusion with muscle fibers are not known with certainty. Nevertheless, IGF1 seems to play a key role. It is produced by the liver in response to the secretion of **growth hormone** (also a peptide hormone) to the bloodstream by the anterior pituitary gland in the brain. In turn, IGF1 is released to the circulation and, acting on a variety of target organs (such as skeletal muscle, bone, the skin, and the liver itself) promotes their growth.

Thus, growth hormone and IGF1 serve the classical **endocrine** function of hormones, as described in section III.4—that is, synthesis in an endocrine gland, transport by blood, and alteration of the function of target tissues, organs, and cells. In addition, IGF1 is produced by skeletal muscle, where it serves **autocrine** and **paracrine** (rather than endocrine) functions: After its molecules are secreted from muscle fibers, they act either on the very same cells that synthesized them (autocrine function) or on contiguous cells (paracrine function).

The paracrine function of IGF1 comes into play in the case of satellite cell activation. IGF1 binds to the **IGF1 receptor** in the plasma membrane of satellite cells, triggering one or more possible signal transduction pathways, as reviewed by Hang Yin and collaborators. One such pathway is the PI3K cascade, which is also used by insulin (figure 10.48), because the IGF1 receptor is very similar to the insulin receptor (figure 10.46): Upon IGF1 binding, the receptor is activated by autophosphorylation and initiates a cascade of interactions leading to the phosphorylation and activation of PKB/Akt (figure 13.7).

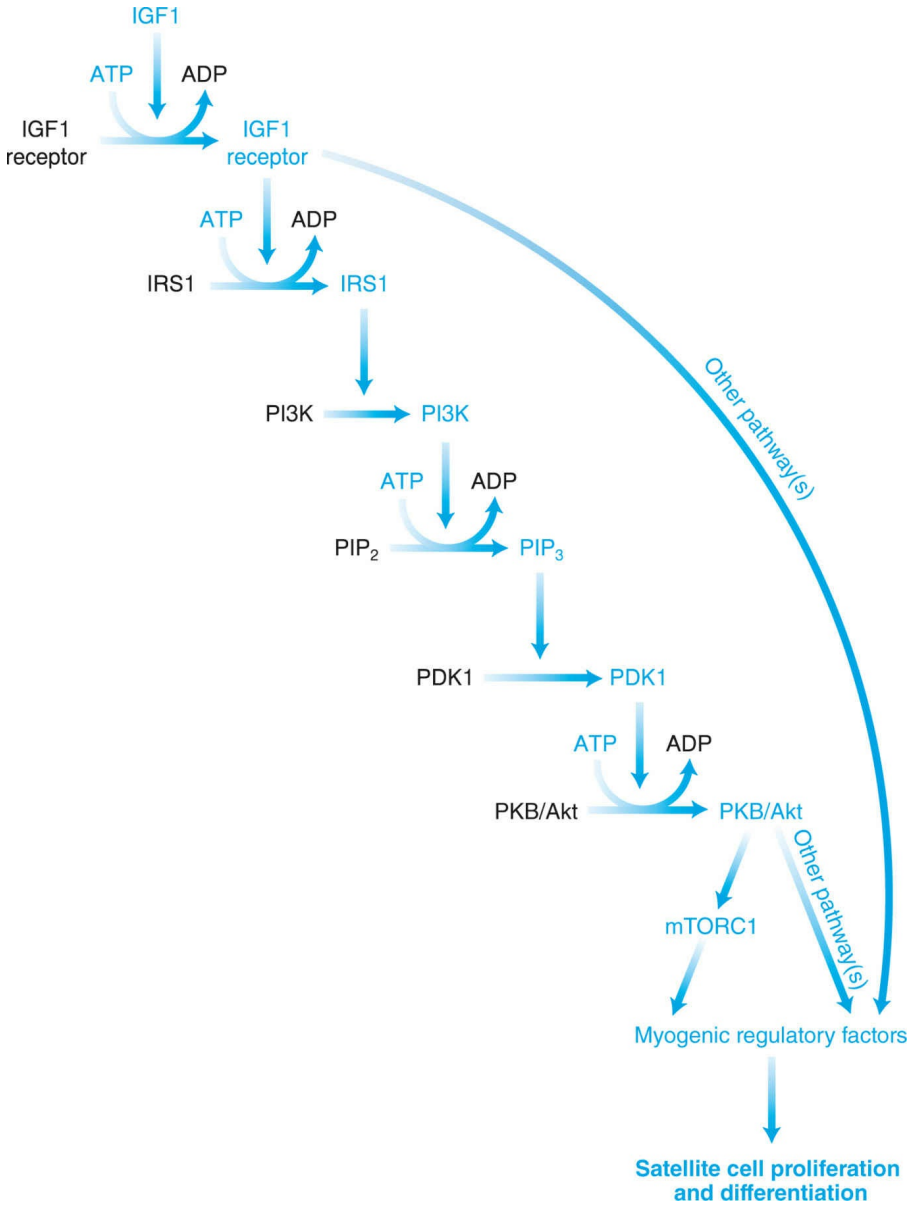


Figure 13.7 **How IGF1 induces satellite cell activation.** IGF1, secreted by muscle fibers, binds to and activates its receptor in the associated satellite cells. The IGF1 receptor phosphorylates IRS1, which activates PI3K, which phosphorylates PIP_2 to PIP_3, which activates PDK1, which phosphorylates and activates PKB/Akt. In turn, through a number of additional steps, possibly involving the mTORC1 pathway, PKB/Akt upregulates a number of myogenic regulatory factors, which promote satellite cell proliferation and differentiation. Other signal transduction pathways may also convey the signal of IGF1 for satellite cell activation (long curved arrow).

Next, the picture becomes fuzzy, as the targets to which PKB/Akt passes the torch of satellite cell activation are not known with certainty. One possibility is that PKB/Akt stimulates the mTORC1 pathway through a series

of complex interactions. However, the possibility also exists of stimulating other signal transduction pathways. One way or another, IGF1 and possibly other factors end up increasing the concentration of several transcription factors, termed **myogenic regulatory factors**, in the satellite cells. These factors induce genes that encode proteins regulating satellite cell proliferation and differentiation.

Growth Boosters as Doping Substances

Because of their potential to induce muscle hypertrophy, IGF1 and MGF may be used by athletes to boost performance in strength or sprint events. For this reason, their use is prohibited by the World Anti-Doping Agency (WADA), which regulates doping matters worldwide. WADA prohibits a substance or method if it meets any two of the following three criteria:

1. Scientific evidence or experience that the substance or method enhances or has the potential to enhance sport performance
2. Scientific evidence or experience that the use of the substance or method represents an actual or potential health risk to the athlete
3. WADA's determination that the use of the substance or method violates the spirit of sport

WADA also prohibits a substance or method if scientific evidence or experience indicates that the substance or method has the potential to mask the use of other prohibited substances or methods. WADA's list of prohibited substances includes IGF1, MGF, and growth hormone in the category of "peptide hormones, growth factors, related substances, and mimetics."

Another doping tool might be a group of drugs designed to block **myostatin**, a protein that inhibits muscle growth through yet-unidentified mechanisms and thus prevents the development of excessive muscle mass. The importance of myostatin is exemplified by a report (by Markus Schuelke and collaborators) showing that a

mutation in the myostatin gene resulted in inactive protein, gross muscle hypertrophy, and increased strength in a child. Thus, drugs that block myostatin action could act like lifting a brake on muscle growth, thereby permitting unusual muscle hypertrophy or even hyperplasia. Myostatin inhibitors are prohibited by WADA under the "hormone and metabolic mediators" category. Such compounds, and others that may reproduce effects of training, have been dubbed **exercise mimetics**, a rather ambitious term, since it seems highly improbable that a drug can ever duplicate the numerous effects of any kind of training on the human body.

A different approach for achieving muscle growth (and other performance-boosting outcomes, such as increased endurance) by illegal means would be the introduction of genes in order to modify the amount of a protein of interest. This approach brings us to the realm of **gene doping**, which is considered a prohibited method by WADA. In particular, WADA prohibits the use of nucleic acids or nucleic acid analogues, the use of gene-editing agents designed to alter genome sequences, the transcriptional or epigenetic (a term that we will discuss in section 13.7) regulation of gene expression, as well as the use of normal or genetically modified cells. There is no evidence for the use of gene doping in competitive sport so far.

13.6 Mechanisms of Exercise-Induced Increase in Muscle Mitochondrial Content

Increased mitochondrial content (through either mitochondrial biogenesis or mitochondrial hypertrophy, as discussed in section 12.12) is probably the most remarkable adaptation of skeletal muscle to endurance training. This effect results from increased synthesis of mitochondrial proteins, which are formed on the basis of genetic information contained not only in the nuclear DNA but also in the small circular mitochondrial DNA. Human mitochondrial DNA contains 37 genes, which encode 11 subunits of proteins of the electron-transport chain, 2 subunits of ATP synthase, 2 rRNA, and 22

tRNA.

The existence of mitochondrial DNA attests to the origin of the mitochondrion as an aerobic bacterium that was engulfed by a larger anaerobic cell about a billion years ago. In this mutually beneficial union, the bacterium found nutrients to live on and proliferate, and the larger cell found an energy-efficient machinery, because aerobic catabolism yields more ATP than does anaerobic catabolism. The bacterium evolved into a mitochondrion, and the anaerobic cell evolved into an aerobic cell.

The 37 mitochondrial genes constitute only a minor fraction of the genes needed for mitochondrial structure and function: The overwhelming majority (about 1,100 genes) are nuclear. Proteins encoded by the latter are synthesized in the cytosol or endoplasmic reticulum (as are all proteins encoded by nuclear genes) and then transported to become parts of the mitochondrial membranes or matrix. How the nuclear and mitochondrial genomes cooperate in producing the right mix of mitochondrial components is being gradually unraveled.

Synthesis of mitochondrial proteins is controlled primarily at the level of transcription by two classes of other proteins, **transcription factors** and **transcription coactivators**. The former bind to specific DNA base sequences in the promoters or enhancers of genes, thus serving as docking sites for RNA polymerase to initiate transcription (section 4.11). The latter bind to transcription factors rather than DNA and then recruit additional proteins needed for transcription initiation, thus coactivating, as we say, transcription.

Both transcription factors and transcription coactivators are synthesized in one place of a cell and exert their actions in another. This is so because protein synthesis takes place in the cytosol and endoplasmic reticulum (where the ribosomes needed for translation exist), whereas transcription takes place in the nucleus and mitochondria (where the DNA to be transcribed exists). Thus, after being synthesized, transcription factors and transcription coactivators translocate to the nucleus or mitochondria. This translocation is part of the protein targeting mentioned in sections 13.1 and 13.2 and figure 13.2. Let’s now consider these important regulators of gene expression.

Transcription factors that control transcription of nuclear genes encoding

mitochondrial proteins include **nuclear respiratory factors 1** and **2** (**NRF1** and **NRF2**). These factors bind to the promoters of genes encoding

- proteins that incorporate other proteins encoded by nuclear genes in the mitochondria;
- proteins of the electron-transport chain;
- proteins involved in heme biosynthesis (remember that several proteins of the electron-transport chain contain heme; section 10.14); and
- proteins involved in transcription and replication of mitochondrial DNA (including the mitochondrial transcription factors to be mentioned shortly), as well as processing of mitochondrial RNA.

Another group of transcription factors that control the synthesis of mitochondrial proteins encoded by nuclear genes are the **peroxisome proliferator-activated receptors**, or **PPAR**. These factors are activated when a ligand, such as a fatty acid or fatty acid derivative, binds to them. Three members of this family are known: PPARα, PPARβ (or PPARδ), and PPARγ. They control the transcription of hundreds of genes involved in nearly all pathways of lipid metabolism.

A different group of transcription factors are distinct in that, although they are encoded by nuclear genes, they act as transcription factors in the mitochondria; thus they are crucial for the synthesis of mitochondrial proteins. They include **mitochondrial transcription factors A**, **B1**, **and B2 (TFAM**, **TFB1M**, and **TFB2M)**. Their genes are controlled by NRF1 and NRF2, and they bind to mitochondrial promoter DNA and help RNA polymerase to initiate transcription of the mitochondrial genome. In addition, TFAM contributes to replication and repair of mitochondrial DNA.

A final transcription factor worth mentioning is **p53**, which owes its name to the fact that it was discovered as a protein (hence the letter *p*) of apparent (through electrophoresis) molecular mass of 53 kDa. It was later discovered that the actual mass is 44 kDa. This factor is encoded by a nuclear gene and serves primarily as a tumor suppressor, a role that we will explore in section 15.5. Among other functions, this versatile protein acts as a transcription factor in both the nucleus and mitochondria. In the nucleus, in particular, p53 upregulates genes encoding NRF1, TFAM, and proteins of the electron-

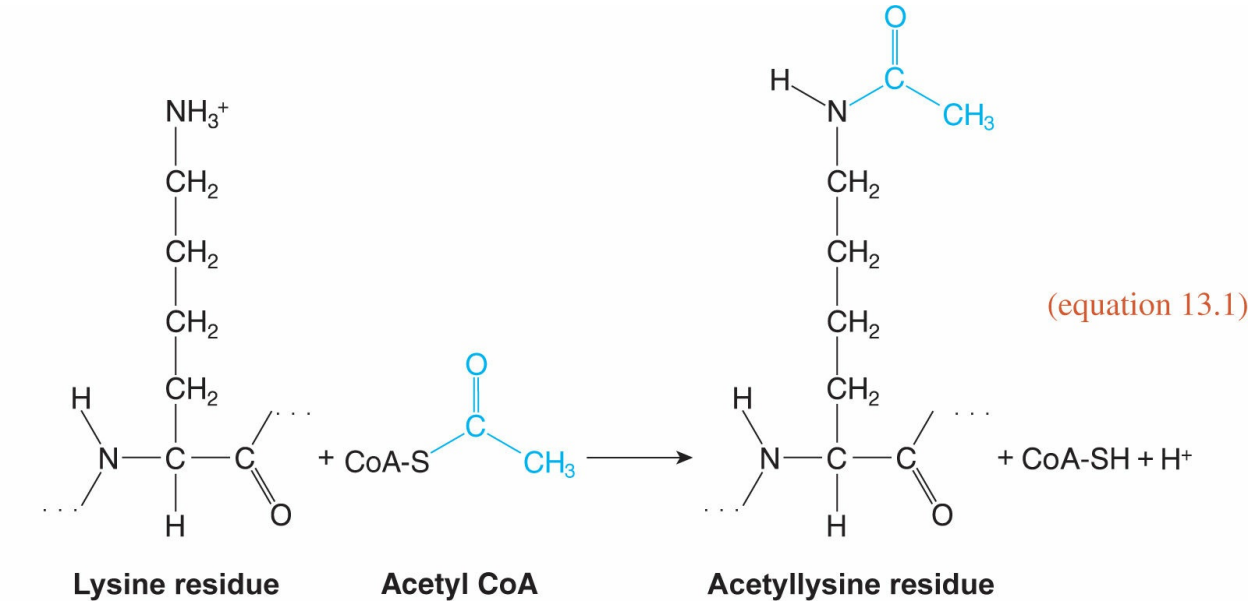
transport chain.

Let's move now to transcription coactivators. **PPARγ coactivator 1α (PGC1α)** is considered the master regulator of the increase in muscle mitochondrial content and, generally, of the changes in muscle phenotype induced by endurance training. PGC1α interacts not only with PPARγ (as the name suggests) but also with other PPAR, with NRF1, and with NRF2. In doing so, it potentiates the induction of the genes that these transcription factors control.

This potentiation includes the mitochondrial as well as nuclear genes. This is the case because, as mentioned earlier, NRF1 and NRF2 (two transcription factors acting in the nucleus) promote the synthesis of TFAM, TFB1M, and TFB2M—three transcription factors that travel to the mitochondria, where they induce mitochondrial genes. In this way, PGC1α coordinates the nuclear and mitochondrial genomes in achieving mitochondrial expansion.

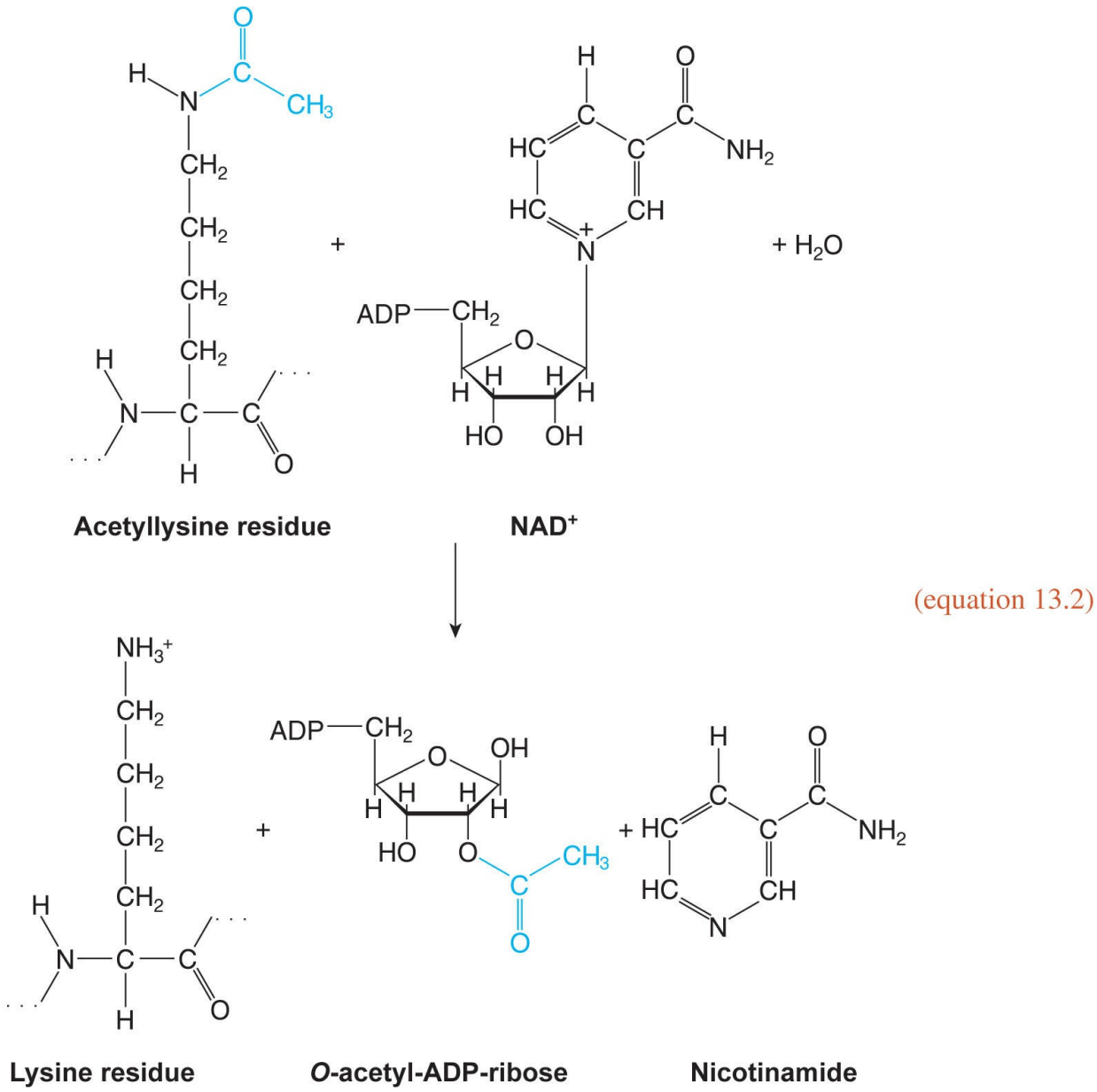
The ability of PGC1α to function as a transcription coactivator is influenced by two kinds of reversible post-translational modifications (as reviewed by Yuho Kim and colleagues): phosphorylation and acetylation. Phosphorylation takes place at serine and threonine residues through the catalytic action of **AMP-activated protein kinase (AMPK)**, introduced in section 11.15. Dephosphorylation is catalyzed by a protein phosphatase such as the one presented in section 10.5.

Acetylation, that is, addition of an acetyl group, takes place at a lysine residue of PGC1α and produces **acetyllysine** through the catalytic action of an **acetyltransferase** according to the reaction



(equation 13.1)

Acetyl CoA serves as the donor of the acetyl group as in many other cases (for example, in the first reaction of the citric acid cycle; section 10.12 and figure 10.27). PGC1α **deacetylation** (removal of the acetyl group from acetyllysine) involves NAD^+ and water as substrates in a reaction producing nicotinamide and *O*-acetyl-ADP-ribose.



(equation 13.2)

This important reaction for PGC1α activity (as we will see shortly) is catalyzed by **silent mating-type information regulation 2 homolog 1 (SIRT1)**, an enzyme characterized as NAD^+-dependent deacetylase for distinction from deacetylases that operate by simple hydrolysis, such as the one that we will encounter in the next section.

In its basal, inactive state, PGC1α is acetylated and unphosphorylated. This status changes with endurance exercise, resulting in the mobilization of the entire transcription regulatory apparatus described earlier to augment the synthesis of mitochondrial proteins. A key trigger for this change appears to

be the rise in the cytosolic AMP concentration due to ATP and ADP degradation during exercise. AMP activates AMPK in a way that deserves a fairly detailed presentation. The enzyme consists of three subunits, designated α, β, and γ. The α subunit hosts the enzyme's active site, and β and γ play regulatory roles.

AMPK activity is controlled in a rather complex way. To be fully active, the α subunit needs to be phosphorylated at threonine 172. In the basal, inactive state, this residue is being constantly phosphorylated and dephosphorylated by cellular protein kinases and phosphatases, respectively. However, binding of two AMP to the γ subunit causes a conformational change that exposes the enzyme's active site and, at the same time, prevents protein phosphatases from dephosphorylating Thr^{172}. As a result, enzyme activity rises more than 100 fold.

The activated AMPK phosphorylates PGC1α, which, in addition, is deacetylated by SIRT1. Deacetylation is favored by an increase in the NAD^+ concentration in the hours following endurance exercise through an uncertain mechanism.

Through the concerted action of AMPK and SIRT1, PGC1α reverts from an acetylated and unphosphorylated state to a deacetylated and phosphorylated state, which is the active one. PGC1α then translocates from the cytosol to the nucleus, where it

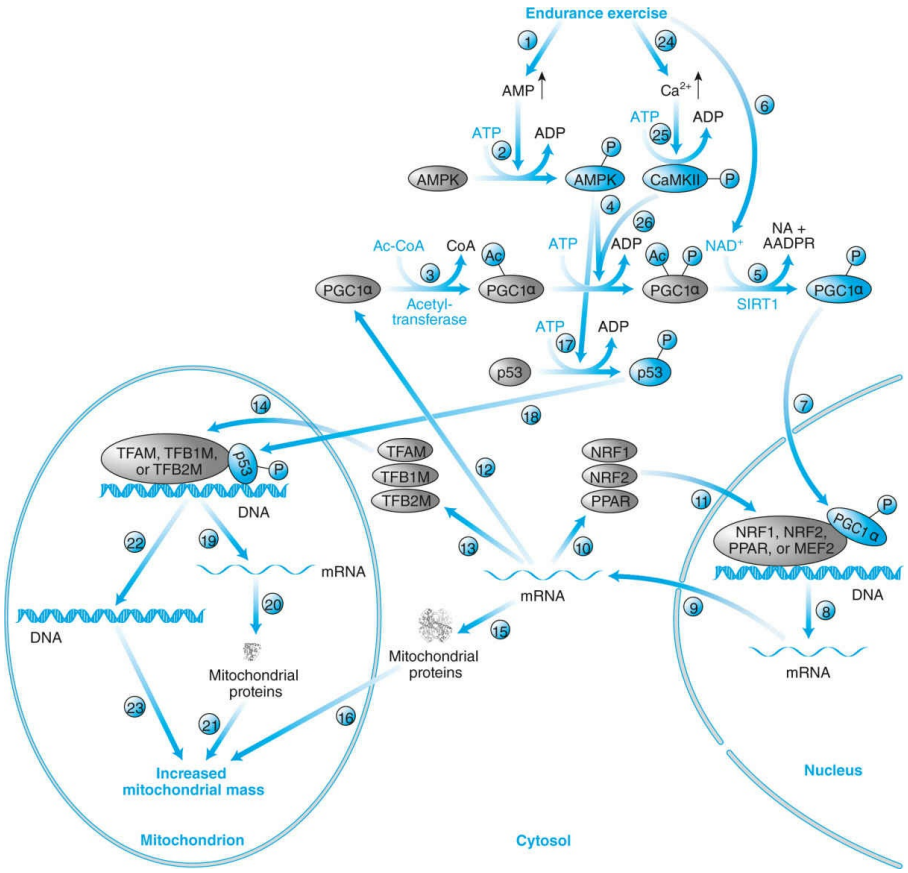


Figure 13.8 **How endurance exercise augments muscle mitochondrial content.** The signal transduction pathway that is best characterized regarding exercise-induced increase in muscle mitochondrial content (though with weak evidence for some of its steps in humans) begins with the rise in the cytosolic AMP concentration due to muscle activity *(1)*. AMP activates AMPK by exposing its active site and allowing it to remain phosphorylated *(2)*. AMPK targets PGC1α, which is inactive when acetylated (*Ac* stands for acetyl group) by an acetyltransferase *(3)*. AMPK phosphorylates PGC1α *(4)*, and SIRT1 deacetylates it *(5)* with the use of NAD^+, which is converted into nicotinamide (NA) and *O*-acetyl-ADP-ribose (AADPR). Exercise may increase NAD^+ *(6)*, thus promoting PGC1α deacetylation and activation. PGC1α travels to the nucleus *(7)*, where it coactivates several transcription factors in promoting transcription by RNA polymerase (omitted here for clarity) of many genes related to mitochondrial structure and function *(8)*. The mRNA species thus produced exit the nucleus *(9)* and are translated into the following: nuclear transcription factors *(10)*, which translocate to the nucleus to promote transcription *(11)*; PGC1α *(12)*; mitochondrial transcription factors *(13)*, which translocate to the mitochondria to promote transcription *(14)*; and many mitochondrial proteins *(15)*, which are targeted to the mitochondria *(16)*. AMPK phosphorylates and activates p53 *(17)*, which migrates to the mitochondria *(18)* to aid the transcription factors in transcribing mitochondrial genes *(19)*. Mitochondrial mRNA is translated *(20)*, and the proteins produced join the mitochondrial proteins encoded by nuclear genes to form new mitochondrial mass *(21)*. TFAM promotes mitochondrial DNA replication as well *(22)*, which is also needed for mitochondrial growth *(23)*. Finally, the exercise-induced rise in the cytosolic Ca^{2+} concentration *(24)* results in CaMKII activation and

autophosphorylation *(25)*. The activated CaMKII phosphorylates and activates PGC1α *(26)*. As in previous figures, the more active forms of proteins are depicted in color.

binds to NRF1, NRF2, and members of the PPAR group to coactivate gene expression. PGC1α even coactivates the expression of the very gene that encodes it (thus exerting positive feedback on its own production) by binding to the transcription factor **myocyte enhancer factor 2 (MEF2)**. As a result, endurance exercise causes an increase in both PGC1α activity and content in muscle.

Apart from PGC1α, AMPK activates p53 either directly, through phosphorylation, or indirectly, though other pathways. Phosphorylated p53 enters the mitochondria, where it associates with DNA and TFAM to promote transcription.

In describing the complex way in which endurance exercise increases muscle mitochondrial content, I have followed a bottom-up approach (from the genes that are induced up to the exercise stimulus). You may recapitulate the story of the most complex process described in this book through the reverse, top-down, approach presented in figure 13.8.

In addition to AMP, Ca^{2+} may be a signal for the increase in muscle mitochondrial content induced by endurance exercise. Ca^{2+} binds to **calmodulin**, a calcium sensor presented as a subunit of phosphorylase kinase in section 10.5. Calmodulin also exists in free form. Upon binding Ca^{2+}, calmodulin can associate with several proteins and modify their biological activity. One such protein is **Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)**, which is activated by Ca^{2+}-calmodulin.

CaMKII takes the form of a complex of several identical subunits (usually 12), and each activated subunit can phosphorylate a neighboring one, as reviewed by Margaret Stratton and coworkers. This autophosphorylation results in activation that outlives Ca^{2+} sequestration in the sarcoplasmic reticulum after the end of exercise (section 8.11). CaMKII phosphorylates and activates PGC1α in a way similar to that of AMPK. In this manner, the increase in the cytosolic Ca^{2+} concentration during exercise contributes to the increase in muscle mitochondrial content.

Synthetic compounds that may promote an increase in muscle mitochondrial content account for their fair share of the WADA Prohibited

List. Two groups of such compounds are activators of AMPK and PPARδ agonists. An example of the former is **5-aminoimidazole-4-carboxamide ribonucleotide**, or **AICAR**. An example of the latter bears the code name **GW1516**, or **GW501516**. Activators of AMPK and PPARδ agonists are listed as doping substances in WADA's "hormone and metabolic mediators" category.

An agonist is a (usually synthetic) compound that binds to a receptor and stimulates its biological action.

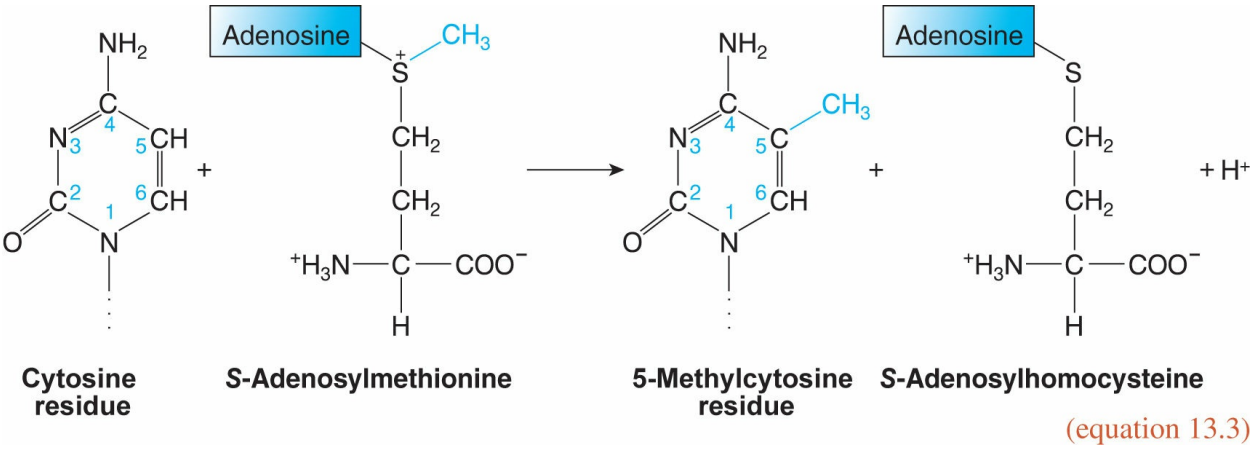
13.7 Exercise and Epigenetics

In recent years, researchers have discovered that certain phenotypic characteristics can be passed from one generation of cells or organisms to the next without being encoded in the base sequence of genes. This discovery has led to the development of a field called **epigenetics** (meaning "on genetics" in Greek) to distinguish it from classical **genetics** (the study of how characteristics are inherited through the base sequence of genes).

Although epigenetic characteristics do not reside in the DNA base sequence, they nevertheless reside in the chromosomes, taking two major forms: DNA methylation and histone modification. Let's explore these chromosomal changes.

Some researchers consider microRNA action (section 4.18) part of epigenetics. However, miRNA are encoded in the base sequence of DNA, so they are genetic, not epigenetic.

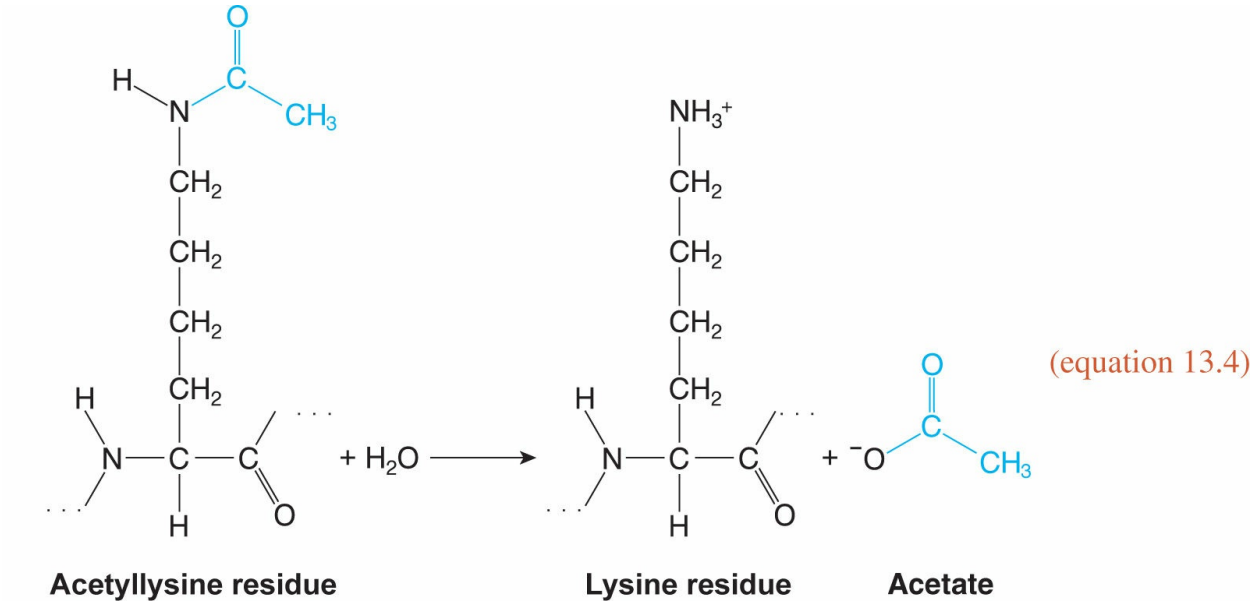
DNA methylation takes place at cytosine residues with the addition of a methyl group at C5.



(equation 13.3)

The methyl group comes from ***S*-adenosylmethionine**, introduced in section 6.1 (subsection on folate) as an important methyl donor in metabolic reactions. A **DNA methyltransferase** catalyzes the reaction. Removal of this epigenetic mark (a process termed **DNA demethylation**) requires excision of 5-methylcytosine (either directly or after conversion into another base) and replacement by cytosine.

Histone modification consists in the post-translational addition of groups —such as the acetyl, methyl, and phosphoryl—to side chains of amino acid residues in **histones**. Remember that histones are proteins around which DNA is wound in the nucleus (section 4.6 and figure 4.12). Histone acetylation, the modification most extensively studied, takes place at lysine residues according to equation 13.1. This epigenetic mark is removed through hydrolysis catalyzed by a **histone deacetylase**.



(equation 13.4)

Epigenetic changes leave their print on the phenotype by affecting transcription rate. DNA methylation in cytosine-rich regions of gene promoters slows down transcription, leading to gene repression. This effect happens because methylation attracts specific proteins that render DNA inaccessible to transcription factors.

In contrast to DNA methylation, histone acetylation speeds up transcription, leading to gene induction. Acetylation eliminates the positive charges from the lysine side chains and, hence, their electrostatic bonds with the negatively charged phosphate groups in DNA. This change weakens the coiling of DNA around the histones and makes it more accessible to transcription factors. Conversely, histone deacetylation represses a gene by making the DNA more tightly coiled around the histones and, hence, less accessible to transcription factors.

Some epigenetic marks are transmitted to the next generation by means of a variety of cellular mechanisms that are not known in their entirety. For example, it seems that DNA polymerase, with the help of other proteins, can retain methyl groups on DNA during replication. Also, the pattern of histone modification in the parent cell may be copied on the newly synthesized histones, and old histones are mixed with new ones in the process of packaging the DNA of the daughter cells.

Evidence is accumulating that both acute and chronic exercise can cause epigenetic changes. A meta-analysis by William Brown concluded that exercise reduced DNA methylation in 60% of the genetic elements tested in various human tissues. In addition, induction of several genes in muscle by acute endurance exercise was accompanied by transient hypomethylation (that is, decrease in methylation) in their promoters, with a stronger effect of hard rather than light exercise, as Romain Barrès and colleagues have found. This was also shown to be the case with acute and chronic resistance exercise by Robert Seaborne and coworkers, who, in addition, presented evidence for long-lasting changes suggestive of an epigenetic memory. How exercise reduces DNA methylation is not known. Research on whether exercise affects histone modification is limited and inconclusive.

In closing, much remains to be learned about the kinds and mechanisms of exercise-induced epigenetic changes. A bigger question is whether such

changes are transmitted from the parents to their offspring or whether regular moderate-intensity exercise during pregnancy (which is considered beneficial for mother and child alike) leaves epigenetic marks on the fetus. If so, this would mean in practice that a physically active parent would endow his or her children with some health benefits of exercise independent of genetic makeup.

Summary

Skeletal muscles adapt to training by exhibiting impressive plasticity in form and function. Adaptations are achieved through concerted changes in the expression of both nuclear and mitochondrial genes. Such changes can occur in transcription, RNA processing, transport of mature RNA from the nucleus to the cytosol, RNA degradation, translation, proteolysis, post-translational modification, and protein targeting. Evidence now exists that all of these processes can be modified by exercise.

Gene products (that is, RNA and proteins) in muscle undergo a variety of changes in quantity after exercise. Because gene expression involves many stages and steps, the kinetics of a specific mRNA and the protein it encodes are rarely in agreement. In general, mRNA changes are larger and faster than protein changes. Messenger RNA content is usually measured through quantitative (or real-time) reverse transcription polymerase chain reaction, whereas protein content is commonly measured through immunoblot.

The biochemical changes mediating the effects of exercise on gene expression in muscle are largely unknown but may be triggered by increases in AMP and Ca^{2+}, a decrease in glycogen, hypoxia, changes in the redox state, a drop in pH, mechanical tension, and increased binding of ligands to cell receptors. Different signal transduction pathways mediate adaptations to resistance and endurance training. Resistance training seems to elicit muscle hypertrophy mainly through the mTORC1 pathway

and satellite cell activation; in the latter, IGF1 plays an important role. Endurance training, on the other hand, seems to augment the muscle mitochondrial content mainly though AMPK and PGC1α activation. Important roles may also be played by epigenetic changes, such as cytosine methylation and histone modification.

Problems and Critical Thinking Questions

1. Offer one or more explanations for the mRNA and protein kinetics seen in figure 13.3*c*.
2. Answer the same question for figure 13.3*d*.
3. Answer the same question for figure 13.3*e*.
4. (Integrative problem) Based on the information presented in this chapter and the previous one, would you say that the two processes believed to be primarily responsible for exercise-induced muscle hypertrophy—that is, increased protein synthesis and satellite cell proliferation—coincide temporally? Explain your reasoning.
5. As mentioned in section 13.1, of all the stages in the control of gene expression, the two main determinants of protein content in a cell are thought to be transcription and translation. At which level, transcriptional or translational, do resistance training and endurance training exert their primary effects on gene expression?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

muscle plasticity

induction, upregulation

repression, downregulation

transcription
RNA processing
RNA degradation
translation
proteolysis
post-translational modification
protein targeting
glycosylation
insulin-like growth factor 1, IGF1
mechano growth factor, MGF
eukaryotic initiation factor
polymerase chain reaction, PCR
hybridize
Northern blot
quantitative reverse transcription PCR, real-time reverse transcription PCR
cDNA
Western blot, protein immunoblot
proteomics
genomics
transcriptomics
DNA microarray, DNA chip, gene chip
protein microarray, protein chip
hypoxia
redox state
mTORC1 pathway
mammalian target of rapamycin, mechanistic target of rapamycin, mTOR
mTOR complex 1, mTORC1
mTOR complex 2, mTORC2
eukaryotic initiation factor 4E-binding protein 1, 4EBP1

ribosomal protein S6 kinase 1, S6K1
eukaryotic initiation factor 4E, eIF4E
S6K1 Aly/REF-like substrate, SKAR
eukaryotic elongation factor 2 kinase, eEF2K
ribosomal biogenesis
phosphatidate
satellite cell
basal lamina
growth hormone
endocrine
autocrine
paracrine
IGF1 receptor
myogenic regulatory factor
myostatin
exercise mimetic
gene doping
transcription factor
transcription coactivator
nuclear respiratory factor 1, NRF1
nuclear respiratory factor 2, NRF2
peroxisome proliferator-activated receptor, PPAR
mitochondrial transcription factor A, TFAM
mitochondrial transcription factor B1, TFB1M
mitochondrial transcription factor B2, TFB2M
p53
PPARγ coactivator 1α, PGC1α
AMP-activated protein kinase, AMPK
acetylation
acetyllysine

acetyltransferase
deacetylation
silent mating-type information regulation 2 homolog 1, SIRT1
myocyte enhancer factor 2, MEF2
calmodulin
Ca^{2+}/calmodulin-dependent protein kinase II, CaMKII
5-aminoimidazole-4-carboxamide ribonucleotide, AICAR
GW1516, GW501516
epigenetics
genetics
DNA methylation
S-adenosylmethionine
DNA methyltransferase
DNA demethylation
histone modification
histone
histone deacetylase

References and Suggested Readings

Barrès R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, Caidahl K, Krook A, O'Gorman DJ, Zierath JR (2012). Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metabolism* 15: 405-411.

Bond P (2016). Regulation of mTORC1 by growth factors, energy status, amino acids, and mechanical stimuli at a glance. *Journal of the International Society of Sports Nutrition* 13: 8.

Brown WM (2015). Exercise-associated DNA methylation change in skeletal muscle and the importance of imprinted genes: A bioinformatics meta-analysis. *British Journal of Sports Medicine* 49: 1567-1578.

Hargreaves M (2015). Exercise and gene expression. *Progress in Molecular Biology and Translational Science* 135: 457-469.

Kim Y, Triolo M, Hood DA (2017). Impact of aging and exercise on mitochondrial quality control in skeletal muscle. *Oxidative Medicine and Cellular Longevity* 2017: 3165396.

Li JJ, Bickel PJ, Biggin MD (2014). System wide analyses have underestimated protein abundances and the importance of transcription in mammals. *PeerJ* 2: e270.

Magnuson B, Ekim B, Fingar DC (2012). Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochemical Journal* 441: 1-21.

McGlory C, Devries MC, Phillips SM (2017). Skeletal muscle and resistance exercise training; the role of protein synthesis in recovery and remodeling. *Journal of Applied Physiology* 122: 541-548.

Padrão AI, Ferreira R, Amado F, Vitorino R, Duarte JA (2016). Uncovering the exercise-related proteome signature in skeletal muscle. *Proteomics* 16: 816-830.

Schuelke M, Wagner KR, Stolz LE, Hübner C, Riebel T, Kömen W, Braun T, Tobin JF, Lee SJ (2004). Myostatin mutation associated with gross muscle hypertrophy in a child. *New England Journal of Medicine* 350: 2682-2688.

Seaborne RA, Strauss J, Cocks M, Shepherd S, O'Brien TD, van Someren KA, Bell PG, Murgatroyd C, Morton JP, Stewart CE, Sharples AP (2018). Human skeletal muscle possesses an epigenetic memory of hypertrophy. *Scientific Reports* 8: 1898.

Snijders T, Nederveen JP, McKay BR, Joanisse S, Verdijk LB, van Loon LJ, Parise G (2015). Satellite cells in human skeletal muscle plasticity. *Frontiers in Physiology* 6: 283.

Stratton MM, Chao LH, Schulman H, Kuriyan J (2013). Structural studies on the regulation of Ca^{2+}/calmodulin dependent protein kinase II. *Current Opinion in Structural Biology* 23: 292-301.

World Anti-Doping Agency. (2018, January 1). Prohibited list. www.wada-ama.org/en/resources/science-medicine/prohibited-list-documents.

Yin H, Price F, Rudnicki MA (2013). Satellite cells and the muscle stem cell niche. *Physiological Reviews* 93: 23-67.

CHAPTER 14

Integration of Exercise Metabolism

Learning Objectives

After reading this chapter, you should be able to do the following:

- Discuss the interconnection of metabolic pathways yielding ATP from the available energy sources in exercise and evaluate the energy sources in terms of quantity, ATP yield, rate of ATP resynthesis, and predominance in specific sports.
- Define the energy systems in terms of the energy sources and enzymes they include, list the factors that influence the choice of energy sources during exercise, describe what effects each factor has, and propose mechanisms through which each factor exerts its effects.
- Calculate the contribution of each energy system to an exercise based on available graphs.
- Compare the adaptations to different types of training.
- Predict changes in the respiratory exchange ratio when changes are made in various exercise parameters or exerciser characteristics.
- Discuss the roles played (or not played) in exercise by various

hormones.

- Explain how exercise influences the redox state and review the role of oxidative stress in exercise metabolism.
- Define fatigue and discuss its various causes in different exercises.
- Explain the major metabolic processes during recovery from exercise.
- Describe the effects of detraining on exercise-related parameters.

In previous chapters of part III, we have separately examined the effects of exercise on the metabolism of compounds of high phosphoryl-transfer potential, carbohydrates, lipids, and proteins. This approach was dictated by the need to study each class of energy sources in depth. Nevertheless, there were several instances in which the metabolism of one class intermingled with the metabolism of another. For example, we saw that glycogenolysis and glycolysis, the two main processes of carbohydrate breakdown, are accelerated by a rise in AMP, a product of the breakdown of compounds with high phosphoryl-transfer potential. We also saw that a lack of carbohydrates promotes the diversion of acetyl CoA, produced through β oxidation of fatty acids, from the citric acid cycle to ketogenesis. Finally, we saw that the breakdown of glucogenic amino acids yields glucose, whereas that of ketogenic amino acids yields ketone bodies.

In addition to the fact that metabolism of one class of energy sources crosses paths with that of another, there is hardly any kind of exercise that does not require a considerable contribution of energy from at least two classes, as pointed out in the introduction to part III. It is necessary, therefore, to integrate information about the use of each energy source in exercise and about the adaptations to training in a way that provides a complete picture of exercise metabolism. This integration is the subject of the present chapter, which covers topics such as the factors influencing the choice of energy sources during exercise, the metabolic specialization of different muscle fiber

types, the adaptations to different kinds of training, the reversal of adaptations when training is interrupted, the coordination of metabolism in different tissues and organs by hormones, the causes of fatigue, and the recovery of the energy state in the body after exercise.

14.1 Interconnection of Metabolic Pathways

To facilitate our integration of information about exercise metabolism, we can take a panoramic view of the metabolic pathways described in previous chapters. Such a view (figure 14.1) will convince you of how interrelated the fates of energy sources are.

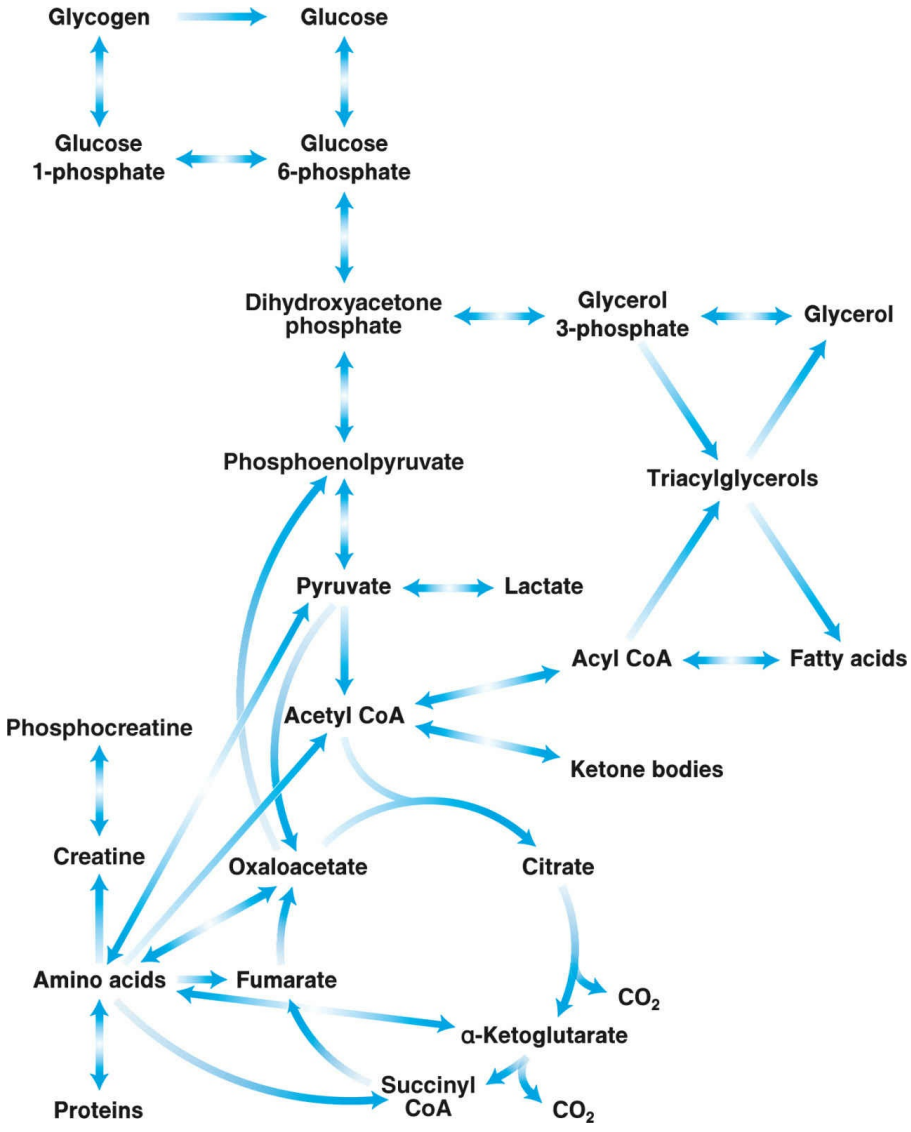


Figure 14.1 **Metabolic interconnections.** The metabolic pathways of compounds with high phosphoryl-transfer potential, carbohydrates, lipids, and proteins are connected by a multitude of intermediate compounds, the most important of which are presented.

Let’s begin with carbohydrates. Glucose entering a cell is rapidly phosphorylated to glucose 6-phosphate. This compound can proceed through the glycolytic pathway and end up in pyruvate while ATP is produced. The same fate awaits the glucosyl units of glycogen, most of which are converted initially into glucose 1-phosphate and then into glucose 6-phosphate. It is also possible to synthesize glycogen from glucose through the two sugar phosphates.

Pyruvate holds a central place in metabolism thanks to its ability to follow a variety of alternative routes. To begin with, it can be converted into lactate through a reversible reaction. In contrast, the conversion of pyruvate into acetyl CoA is irreversible and paves the road to its complete oxidation to carbon dioxide through the citric acid cycle. Electrons produced in the process drive the production of many ATP molecules thanks to the coupling of the electron-transport chain to oxidative phosphorylation. Pyruvate can also be converted into oxaloacetate, a compound of the citric acid cycle. This conversion is the first step in glucose synthesis through gluconeogenesis, which proceeds through phosphoenolpyruvate. Last, pyruvate can be converted into alanine (one of the amino acids) and vice versa. This interconversion forms but one of the bridges linking carbohydrate and protein metabolism.

Let’s move now to lipids. Triacylglycerols, the most abundant lipid category, are hydrolyzed to glycerol and fatty acids. Glycerol communicates with glycolysis and gluconeogenesis through glycerol 3-phosphate and dihydroxyacetone phosphate; glycerol 3-phosphate is also required for triacylglycerol synthesis. Fatty acids, on the other hand, are activated by conversion into acyl CoA, which can be either channeled back to triacylglycerol synthesis or degraded into acetyl CoA through β oxidation. Apart from yielding ATP by its oxidation, acetyl CoA (also deriving from carbohydrates) is used in fatty acid synthesis and ketone body formation (ketogenesis).

Proteins are hydrolyzed to amino acids, which follow various catabolic

routes leading to the incorporation of their amino groups into urea (through the urea cycle) and the conversion of their carbon skeletons into pyruvate, acetyl CoA, or compounds of the citric acid cycle. All of these compounds can be broken down to yield energy or can be used in the synthesis of glucose and fatty acids. Conversely, pyruvate and compounds of the citric acid cycle can produce amino acids, which are used in protein synthesis. Some amino acids are used in the synthesis of creatine, which is converted into phosphocreatine, a compound of high phosphoryl-transfer potential. The reverse conversion (of phosphocreatine into creatine) is accompanied by ATP production.

14.2 Energy Systems

We can classify the energy sources described in previous chapters into three energy systems. The systems include not only the sources themselves but also the enzymes that ensure ATP resynthesis from the degradation of these sources. Each system is characterized by two parameters of pivotal importance for its usefulness in exercise:

- The energy it can yield
- Its power, that is, the ratio of energy to the time within which it is produced (in simple terms, how fast the system yields energy)

The three energy systems are as follows:

1. The **ATP–phosphocreatine system**, which includes the following:
   - In terms of energy sources, ATP, ADP, and phosphocreatine
   - In terms of enzymes, creatine kinase and adenylate kinase, or myokinase

   The energy of the ATP–phosphocreatine system is low, but its power is high. Thus, the system predominates in maximal exercise bouts lasting up to about 7 s, such as weightlifting, jumps, throws, a 60 m dash, and explosive efforts in ball games or fight sports.
2. The **lactate system**, which includes the following:

- In terms of energy sources, carbohydrates
- In terms of enzymes, those of glycogenolysis (that is, phosphorylase and the debranching enzyme), those of glycolysis, and lactate dehydrogenase

The lactate system has intermediate energy and power. Thus, it predominates in maximal bouts lasting about 7 s to 1 min. Examples include the 100, 200, and 400 m runs; the 50 and 100 m swims; and the 200, 500, and 1,000 m cycling races.

3. The **oxygen system**, or **aerobic system**, which includes the following:
   - In terms of energy sources, carbohydrates, lipids, and proteins
   - In terms of enzymes, pyruvate dehydrogenase; the enzymes of lipolysis, of fatty acid degradation, of amino acid degradation, of the citric acid cycle, and of the electron-transport chain; and ATP synthase.

   The energy of the oxygen system is high, but its power is low. Thus, the system predominates in maximal exercise tasks exceeding 1 min in duration. Examples include the 800 m run and upward, the 200 m swim and upward, rowing, and cross-country skiing. The oxygen system also predominates at rest and in light, moderate-intensity, or hard exercises of any duration.

14.3 Energy Sources in Exercise

Tables 14.1 and 14.2 summarize the most appreciable energy sources of the three energy systems—in terms of quantity and rate of ATP resynthesis—in the bodies of an average man and an average woman. The tables indicate the quantity of each source at rest, the amount of ATP it yields, and the maximal rate of ATP resynthesis from the breakdown of the source itself or (in the case of liver glycogen and adipose tissue triacylglycerols) the source's breakdown products after blood has transported them to the exercising muscles.

The data presented in tables 14.1 and 14.2 are based on the following assumptions and facts, most of which I have already presented in chapters 9

through 11.

Table 14.1 Energy Sources Available to an Exercising Average Man

Source	Quantity (mmol)	ATP yield (mmol)		Maximal rate of ATP resynthesis (mmol · kg^{-1} · s^{-1})	
		Anaerobic	Aerobic	Anaerobic	Aerobic
Muscle ATP	180	180	—	—	—
Muscle phosphocreatine	600	600	—	2.6	—
Muscle glycogen	2,315[a]	6,945	71,765	1.5	0.5
Liver glycogen	525[a]	1,050	15,750	0.2	0.1
Myocellular triacylglycerols	174	—	62,640	—	0.3
Adipose tissue triacylglycerols	12,791	—	4,604,760	—	0.2

[a]As glucosyl units.

Table 14.2 Energy Sources Available to an Exercising Average Woman

Source	Quantity (mmol)	ATP yield (mmol)		Maximal rate of ATP resynthesis (mmol · kg^{-1} · s^{-1})	
		Anaerobic	Aerobic	Anaerobic	Aerobic
Muscle ATP	112	112	—	—	—
Muscle phosphocreatine	372	372	—	2.6	—
Muscle glycogen	1,435[a]	4,395	44,485	1.5	0.5
Liver glycogen	432[a]	864	12,960	0.2	0.1
Myocellular triacylglycerols	108	—	38,880	—	0.3
Adipose tissue triacylglycerols	16,279	—	5,860,440	—	0.2

[a]As glucosyl units.

- They refer to a model 75 kg man and a model 62 kg woman.
- The individuals' muscle masses are 30 kg (40% of body mass) and 18.6 kg (30% of body mass), respectively.
- Their livers weigh 1.7 and 1.4 kg, respectively.
- In both sexes, muscle contains 6 mmol · kg^{-1} of ATP, 20 mmol · kg^{-1} of phosphocreatine, 1.25% (12.5 g · kg^{-1}) of glycogen, and 0.5% (5 g · kg^{-1}) of triacylglycerols.
- The liver contains 5% (50 g · kg^{-1}) of glycogen.
- The man's adipose tissue contains 11 kg of triacylglycerols; the corresponding value for the woman is 14 kg.
- Each glucosyl unit of muscle glycogen yields 3 ATP anaerobically and 31 ATP aerobically.
- Each glucosyl unit of liver glycogen yields 2 ATP anaerobically and 30 ATP aerobically, since it is delivered to muscle as glucose.
- On the basis of a usual fatty acid composition, the average molecular mass of a triacylglycerol is approximately 860 Da, and the average

yield of the complete oxidation of its components (that is, 3 fatty acids and glycerol) is about 360 ATP.

Obviously, the values in the two tables will differ with different tissue masses and energy source contents. Therefore, you should use them with knowledge of the assumptions on which they are based; you should *not* treat them as constants.

I have not included the following energy sources in tables 14.1 and 14.2:

- Plasma glucose and fatty acids, because their amounts are negligible as compared with those of their major sources (liver glycogen and adipose tissue triacylglycerols, respectively), which are included in the tables
- Plasma triacylglycerols, because they do not participate substantially in energy provision during exercise
- Muscle ADP, glucose, and fatty acids, because their quantities are minimal
- The body's proteins, because their contribution to the energy expenditure of exercise is minor

Tables 14.1 and 14.2 show a general inverse relationship between the quantity of ATP that a source can yield and the maximal rate at which it can do so. Thus, phosphocreatine, the smallest source of ATP, is also the fastest, whereas adipose tissue triacylglycerols, the largest of all sources, are also the second slowest. This combination of an advantage and a disadvantage gives the various sources a *raison d' être*, since, if the largest source were also the fastest, then the body would enjoy no benefit from using the others. Therefore, thanks to these inverse gradients of quantity and power, each energy source has a place in some kind of exercise.

Tables 14.1 and 14.2 also show huge differences in energy yield among classes of energy sources, spanning several orders of magnitude: three-digit values for the ATP yield of compounds with high phosphoryl-transfer potential, five-digit values for carbohydrates, and seven-digit values for lipids. Because of this disparity, it makes good sense to try to maximize or spare the former two to increase performance. We will consider several ways to do so in this chapter.

14.4 Choice of Energy Sources During Exercise

As stated under the second principle of exercise metabolism (section III.2), and as discussed on several occasions throughout part III, exercise is almost always fueled not by a single energy source but by a combination of sources. What, then, is the contribution of each available source to the demands of exercise? The answer depends on several factors, which, in accordance with the fourth principle of exercise metabolism, can be grouped as follows:

- Exercise parameters, including intensity, duration, and program
- Characteristics of the exerciser, including sex, age, nutritional state, training state, and the genome
- Environmental factors, including ambient temperature and hypoxia

Knowing the factors that influence the body's choice of energy sources during exercise—and, more important, knowing in which direction these factors alter the mixture of energy sources—holds theoretical and practical value. Such knowledge allows us to modify the factors that can be modified in order to achieve a mixture that can promote performance, health, or both. Let's examine all of these factors.

14.5 Effect of Exercise Intensity on Choice of Energy Sources

Fatty acids deriving from the hydrolysis of adipose tissue triacylglycerols and, to a lesser degree, of myocellular triacylglycerols serve as the main fuels of the body at rest and during light exercise (figure 14.2). The low rate of ATP resynthesis from these sources is sufficient to balance the low rate of ATP breakdown. As exercise intensity rises, changes in the concentrations of substances that we explored in chapters 10 and 11 (that is, increase in P_i, ADP, AMP, Ca^{2+}, and epinephrine; and decrease in ATP) speed up carbohydrate and lipid degradation, thus counterbalancing the increased demand for ATP. The carbohydrates used are muscle glycogen and plasma

glucose; the latter emanates mainly from the breakdown of liver glycogen. The lipids used are the ones mentioned earlier.

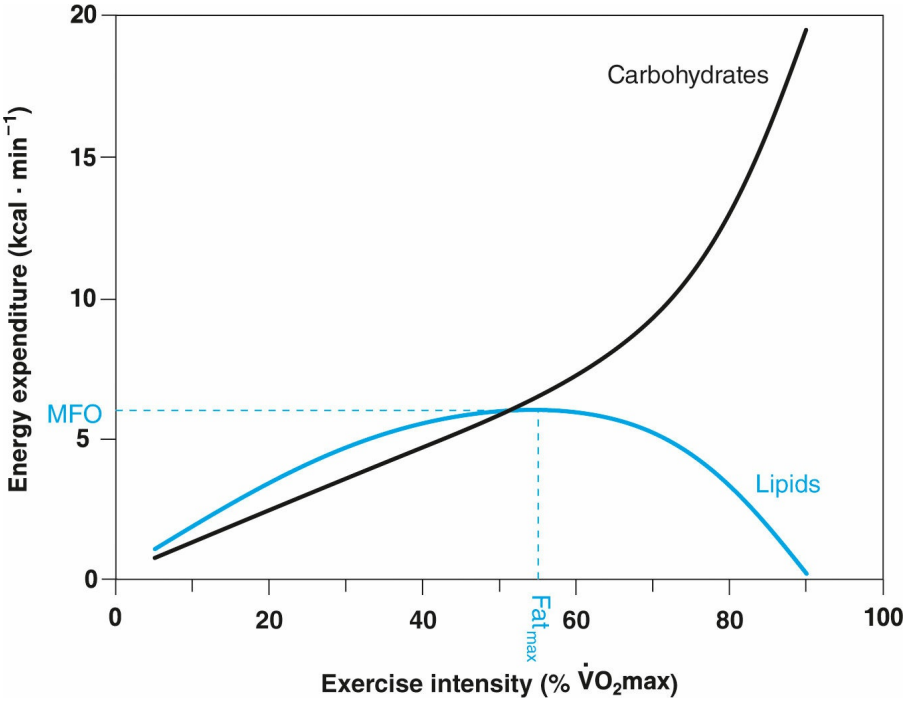


Figure 14.2 Energy sources at different exercise intensities. The amounts of carbohydrates and lipids fueling prolonged exercise depend on exercise intensity. At rest and during light exercise, most of the energy spent by the whole body comes from lipid breakdown. At around 50% of $\dot{V}O_2max$, the energy from carbohydrate breakdown catches up with that from lipid breakdown. At higher exercise intensities, carbohydrate breakdown rises sharply, whereas lipid breakdown drops. Thus, there is an exercise intensity (Fat_{max}) at which fat oxidation is maximal. The latter is usually expressed in g · min^{-1}, which we calculate by dividing kcal · min^{-1} by 9, since 1 g of lipids yields 9 kcal (section 2.6). Thus, a value of 6 kcal · min^{-1} in this example corresponds to 0.86 g · min^{-1}.

The rise in the amount of energy deriving from carbohydrates as intensity increases is larger than the rise in the amount of energy from lipids. Thus, the percentage contribution of carbohydrates to the energy expenditure of exercise increases, whereas that of lipids decreases. As a result, the two percentages become equal somewhere between 45% and 65% of $\dot{V}O_2max$, and the contribution of carbohydrates exceeds that of lipids at higher intensities. In fact, not only the percentage but also the absolute contribution of lipids (in terms of grams oxidized per minute) declines at higher intensities

and becomes negligible at about 90% of $\dot{V}O_2max$. This behavior of lipid oxidation in exercise introduces two interesting variables: the intensity at which fat oxidation peaks (usually symbolized as **Fat_{max}**) and the rate of **maximal fat oxidation** (usually abbreviated as **MFO**).

Fat_{max} ranges between 40% and 70% of $\dot{V}O_2max$, whereas MFO ranges between 0.4 and 0.7 $g \cdot min^{-1}$ for most healthy and lean individuals, although extreme values for both variables have been reported in the literature (as low as 20% and as high as 90% of $\dot{V}O_2max$ for Fat_{max} and as low as 0.2 and as high as 1.3 $g \cdot min^{-1}$ for MFO). It is desirable to have both variables as high as possible for aerobic endurance performance and health. In terms of performance, a high MFO at high Fat_{max} would mean that an endurance athlete could burn a lot of fat at high exercise intensity, thus sparing carbohydrates. Luckily, this condition can be achieved through proper training, as we will see in section 14.14. In terms of health, a high MFO is considered protective against metabolic perturbations that we will consider in the next chapter. However, since training raises MFO, it is not safe to attribute health benefits to an increased MFO and not to other effects of training on the body.

A common misconception is that fat burning promotes weight loss, fat loss, or both. In reality, weight and fat loss result from a negative energy balance regardless of whether fat or carbohydrate is burned during exercise.

The reason for the reduction in lipid oxidation as exercise intensity increases above Fat_{max} is not known with certainty. Some authors attribute it to the fact that carbohydrates yield more energy than do lipids for a certain amount of oxygen. Indeed, if we go back to the reaction describing the oxidation of glucose (reaction 10.16) we see that it yields 30 ATP when oxidized by 6 O_2. Thus, glucose yields 5 ATP per O_2. In comparison, if we go to the reaction describing the oxidation of a fatty acid (in particular, palmitate, reaction 11.19), we see that it yields 106 ATP when oxidized by 23 O_2. Thus, palmitate yields 4.6 ATP per O_2. Consequently, the supporters of the hypothesis maintain, the use of oxygen to burn carbohydrates is more energy efficient, and this efficiency becomes critical during hard exercise,

when the availability of oxygen is limited.

The main problem with this hypothesis is the underlying assumption that the limiting factor of aerobic energy production is oxygen availability inside the mitochondria. As discussed in section 10.21, oxygen suffices to support maximal ATP resynthesis in the mitochondria even during maximal exercise (not to mention moderate-intensity or hard exercise, which is the case for exercise at Fat_{max} or above). Rather, the limiting factor of aerobic ATP resynthesis seems to be the rate of acetyl CoA oxidation through the citric acid cycle, which, however, is common to both carbohydrate and lipid oxidation.

According to the existing evidence, the most likely reason for the drop in the rate of lipid oxidation at high exercise intensities is the reduction in the muscle concentration of carnitine in its free, not acylated, form, as discussed by Anne-Marie Lundsgaard and collaborators. Remember that carnitine is needed for the entry of long-chain fatty acids into the mitochondria (section 11.9). The reduction in carnitine is due to its consumption by acetyl CoA according to the reaction

$$\text{Acetyl CoA} + \text{carnitine} \rightleftharpoons \text{acetylcarnitine} + \text{CoA} \qquad \Delta G^{\circ\prime} = -0.3 \text{ kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 14.1)}$$

The reaction is catalyzed by **carnitine acetyltransferase**, an enzyme located in the mitochondrial matrix, and is a variation of reaction 11.15, with the acetyl group in place of the acyl group.

The rise in the muscle acetyl CoA concentration during exercise as a result of both carbohydrate and lipid oxidation drives reaction 14.1 to the right, thus depleting carnitine and limiting its availability for the synthesis of acylcarnitine according to reaction 11.15. As a result, there is a decrease in the rate of fatty acid entry into the mitochondria and, consequently, a decrease in the rate of fatty acid oxidation. There is also evidence that the drop in the cytosolic pH during hard exercise inhibits carnitine acyltransferase I (the enzyme catalyzing reaction 11.15).

On the other hand, reaction 14.1 curbs the rise in the [acetyl CoA]/[CoA] ratio and the subsequent activation of pyruvate dehydrogenase kinase, which, as discussed in section 10.11, phosphorylates and inhibits pyruvate dehydrogenase, in effect slowing down carbohydrate oxidation. Thus,

reaction 14.1 facilitates carbohydrate oxidation during exercise.

Carbohydrates are basically broken down aerobically. However, as exercise intensity rises, so does the portion of carbohydrates that are broken down anaerobically to support the demand for high power output. Finally, at efforts of maximal intensity (which are necessarily very short) there is a substantial contribution of energy from phosphocreatine, which exhibits the highest rate of ATP resynthesis among energy sources.

14.6 Effect of Exercise Duration on Choice of Energy Sources

The proportion of energy sources does not remain stable during exercise even if intensity is stable, due primarily to the limited quantity of most sources. Generally speaking, *during exercise of constant intensity the contribution of the smaller energy sources decreases in favor of the larger sources.* Let's apply this principle to two time frames.

First, we examine the proportion of muscle energy sources during exercise of constant maximal intensity (which drives an athlete to exhaustion within a few minutes) by using an example from a review by Paul Gastin (figure 14.3). The rate of phosphocreatine breakdown peaks almost instantaneously, then drops rapidly and zeroes at 25 s. The acceleration of glycogenolysis and glycolysis is a bit slower but is still impressive and rapid enough to allow the maintenance of a high power output as phosphocreatine falls. At first, most of the glycogen that is broken down ends up in lactate. However, as seconds go by, more and more of the pyruvate produced through glycolysis follows the route of oxidation to CO_2. Upon completion of one half minute of exercise, the curves of glycolysis and oxidative phosphorylation intersect. From that point on, most of the ATP is produced by oxidative phosphorylation. The power of the oxygen system gradually increases, whereas that of the lactate system decreases. When the latter plunges to zero (after about 3.5 min), the athlete is unable to maintain the required power output and gives up.

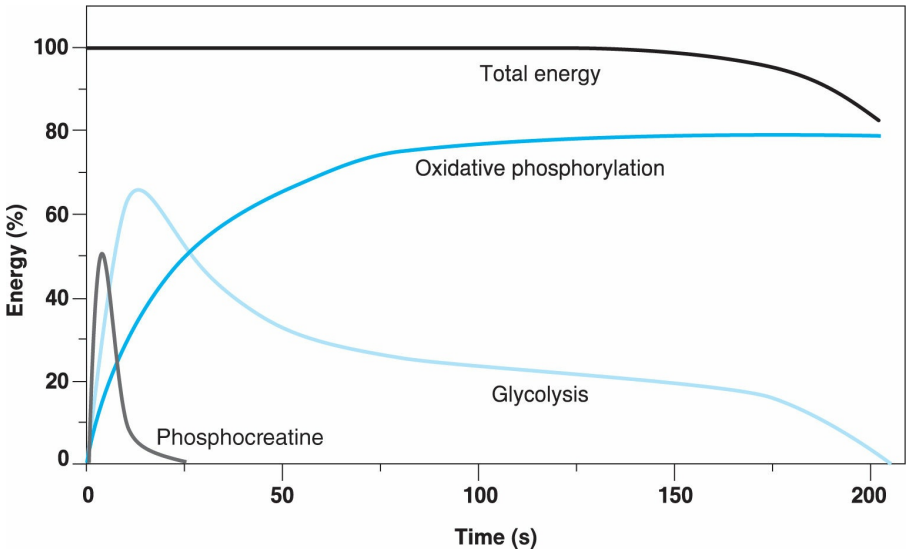


Figure 14.3 Energy sources during maximal exercise. Phosphocreatine is the major source for ATP resynthesis in muscle during the first seconds of maximal exercise (in a cycle ergometer). Glycolysis takes the lead at 7 s, only to give way to oxidative phosphorylation at 30 s. Energy supply is constant for 2.5 min and then decreases. Exhaustion of the athlete ensues a minute later. Note how similar the curves of glycolysis and total energy are during the last half minute. This graph lets you find the percentage contribution of each source to total energy at any time point along the horizontal axis. Here's how: Draw a vertical line upward from the time point of interest and, from the points where it intersects the curves of the three energy sources, draw horizontal lines to the left. The point where each line meets the vertical axis indicates the percentage contribution of the corresponding energy source. The sum of the three percentages is 100% at any time, with two exceptions: the first few seconds, when it is short of 100% by the percentage contribution of ATP itself, and the last half minute, when total energy falls below 100%.

Adapted by permission from P.B. Gastin, 2001, "Energy System Interaction and Relative Contribution During Maximal Exercise," *Sports Medicine* 31(10): 725-741.

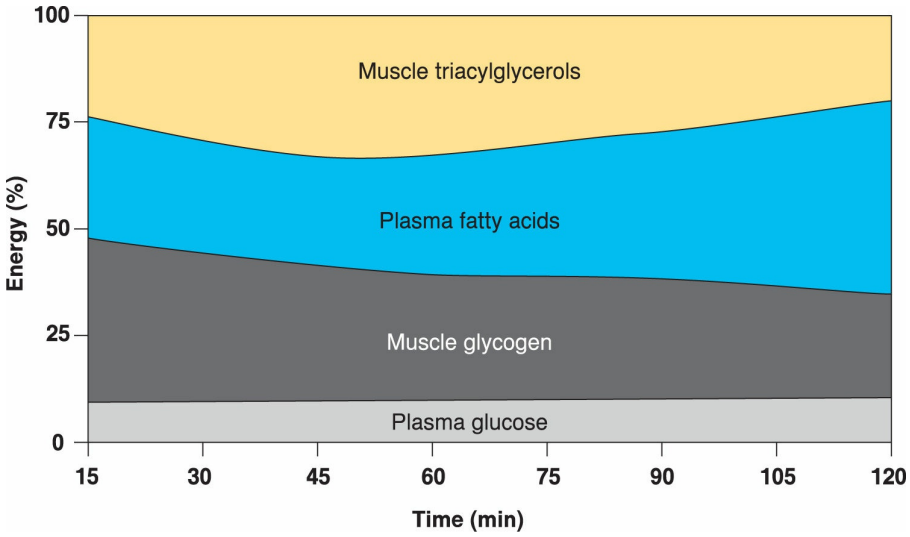


Figure 14.4 Energy sources during prolonged exercise. The percentage contribution of carbohydrates to whole-body energy supply drops gradually during prolonged efforts. The data are from endurance cyclists who exercised at 65% of $\dot{V}O_2$max. The horizontal axis starts at 15 min because it was not possible to make reliable estimates of the contribution of each energy source during the initial minutes of exercise. As in figure 14.3, you can find the percentage contribution of each source to total energy at any time point along the horizontal axis by drawing a vertical line and then horizontal lines from the points where the vertical line intersects the curves of the four energy sources.

From J.A. Romijn et al., "Regulation of Endogenous Fat and Carbohydrate Metabolism in Relation to Exercise Intensity and Duration," *American Journal of Physiology* 265 (1993): E380-E391. Adapted by permission from The American Physiological Society.

Let's move now to the proportion of energy sources during constant exercise of moderate or high intensity, which can be maintained for hours, being fed almost exclusively by the aerobic breakdown of carbohydrates and lipids (figure 14.4). The proportion of these sources does not stay constant but shifts gradually to lipids as muscle and liver glycogen are depleted. Thus, the rate of glycogen degradation falls, whereas that of fatty acid degradation rises.

14.7 Interplay of Duration and Intensity: Energy Sources in Running and Swimming

The events of running and swimming offer a first-rate opportunity to study the interplay of the two major factors affecting the proportion of energy sources during exercise: duration and intensity. The reason is that running and swimming events comprise a wide variety of distances, which, in turn, impose a variety of speeds, because, no matter how hard they try, athletes cannot run or swim a long distance at the speed they run or swim a short one.

What, then, is the proportion of energy sources in running and swimming events? Our guide to answering the question will be figure 14.5, which presents the percentage contribution of each energy system to energy supply depending on the duration of a maximal exercise. The graph, constructed by Gastin on the basis of more than 40 studies, differs from similar graphs in several other sources in that the curves of the three energy systems are shifted

to the left. This shift happens because, as mentioned in section 10.22, the experimental data of recent years have upgraded the capacity of the aerobic system and degraded the capacity of the two anaerobic systems.

The graph in figure 14.5 differs from the one in figure 14.3 in that the horizontal axis represents total exercise duration rather than time points. For example, whereas figure 14.3 shows that 50% of muscle energy at the 30th second of maximal exercise derives from the lactate system and another 50% from the oxygen system, figure 14.5 shows that 23% of the energy for a maximal exercise bout lasting 30 s derives from the ATP–phosphocreatine system, 52% from the lactate system, and 25% from the oxygen system. These percentages are reasonable, because, although the contribution of the ATP–phosphocreatine system at the 30th second is null, that system offered a considerable amount of energy at the beginning of exercise, resulting in an overall contribution of 23%. On the other hand, although the contribution of the aerobic system at the 30th second is 50%, the system offered only a minor amount of energy at the beginning of exercise, resulting in an overall contribution of 25%. For other comparisons of the two graphs, see problems 1 and 2.

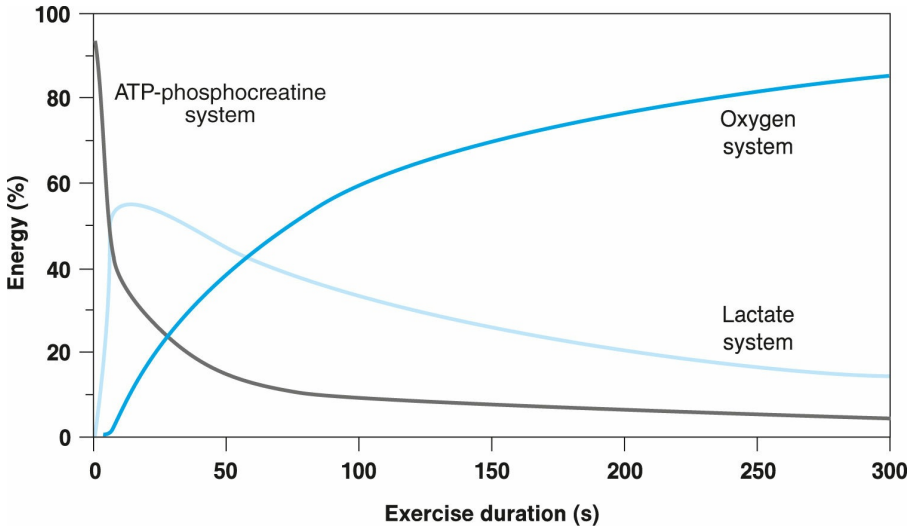


Figure 14.5 Percentage contribution of the energy systems to total energy supply in muscle depending on the duration of maximal exercise. The graph lets you find the contribution of each energy system for any duration of maximal exercise up to 5 min in the same way as described in the caption for figure 14.3. However, whereas figure 14.3 shows instantaneous contributions of energy sources at any time point of choice, the present figure shows cumulative contributions of the energy systems up to the time point of choice. The

ATP–phosphocreatine system dominates in efforts lasting up to 7 s, whereas the lactate system dominates in efforts lasting 7 to 60 s. Together, the two anaerobic systems contribute most of the energy used in exercises lasting up to 75 s, when (after an initial lag) the gradually rising curve of the aerobic system reaches 50%.

Adapted by permission from P.B. Gastin, 2001, "Energy System Interaction and Relative Contribution During Maximal Exercise," *Sports Medicine* 31(10): 725-741.

Having clarified how the two graphs differ, let's explore figure 14.5. In maximal exercise tasks lasting a few seconds, most of the energy comes from the ATP–phosphocreatine system. However, its curve displays such a rapid decline that it intersects the rapidly rising curve of the lactate system at just 7 s. Thus the lactate system reigns in efforts of longer duration, but, after a fast peak in efforts lasting 10 to 15 s, it gives way to the oxygen system; the curves of the two systems cross at 60 s. If we consider the anaerobic ATP–phosphocreatine and lactate systems together, then we can say that anaerobic metabolism overshadows aerobic metabolism in maximal exercise tasks lasting up to 75 s.

Now we can examine the proportion of energy sources for muscle in running and swimming, as promised at the beginning of this section. Table 14.3 presents data on eight running events. As expected, speed (therefore, intensity) decreases as distance increases. The lactate system dominates in the 100, 200, and 400 m runs, followed by either the ATP–phosphocreatine system (in the 100 and 200 m runs) or the oxygen system (in the 400 m run). In longer runs (800, 1,500, 5,000, and 10,000 m, as well as the marathon), the oxygen system prevails, followed by the lactate system. The percentage contribution of each system may vary slightly from one athlete to another.

Table 14.3 Energy Sources in Running Events

			Contribution of energy systems (%)[b]		
Distance (m)	Time (s)[a]	Speed (m · s^{-1})	ATP–phosphocreatine	Lactate	Oxygen
100	9.58	10.44	39	56	5
200	19.19	10.42	30	55	15
400	43.03	9.30	17	48	35
800	100.91	7.93	9	33	58
1,500	206	7.28	4	20	76
5,000	757.35	6.60	1	6	93
10,000	1,577.53	6.34	1	3	96
42,195	7,377	5.72	0	1	99

[a]Men's outdoor world record, or all-time record in the case of the marathon run (last line).

[b]Calculated on the basis of figure 14.5.

Table 14.4 presents the corresponding data on six swimming events. The lactate system provides most of the energy in the 50 and 100 m swims, followed by either the ATP–phosphocreatine system (in the 50 m swim) or the oxygen system (in the 100 m swim). In the 200, 400, 800, and 1,500 m swims, the oxygen system provides most of the energy, followed by the lactate system.

Table 14.4 Energy Sources in Freestyle Swimming Events

			Contribution of energy systems (%)[b]		
Distance (m)	Time (s)[a]	Speed (m · s^{-1})	ATP–phosphocreatine	Lactate	Oxygen
50	20.91	2.39	28	54	18
100	46.91	2.13	16	46	38
200	102	1.96	9	33	58
400	220.07	1.82	4	18	78
800	452.12	1.77	2	9	89
1,500	871.02	1.72	1	5	94

[a]Men's long-course world record.
[b]Calculated on the basis of figure 14.5.

The fact that anaerobic metabolism dominates in maximal exercises lasting up to 75 s, in combination with the fact that the blood lactate concentration after such exercises exceeds 10 mmol · L^{-1} or even 20 mmol · L^{-1}, shows how erroneous it is to believe that the so-called "anaerobic threshold" (at or around 4 mmol · L^{-1}) marks the transition from mostly aerobic to mostly anaerobic metabolism. As pointed out also in section 10.33, exercise metabolism remains predominantly aerobic at intensities well above the supposed threshold.

14.8 Effect of Exercise Program on Choice of Energy Sources

So far, we have discussed the factors affecting the selection of energy sources during continuous exercise bouts of more-or-less constant intensity. Nevertheless, many sporting activities (such as ball games) are characterized by periods of hard or maximal activity interspersed with intervals of rest, light exercise, or moderate-intensity exercise. In such cases, the proportion of energy sources exhibits great fluctuation within very short periods.

In tennis, for example, the athlete exercises hard or maximally for

intermittent periods of a few seconds, followed by periods of rest that last, on average, three times as long. During the hard and maximal efforts, phosphocreatine and the anaerobic breakdown of carbohydrates serve as major muscle ATP sources. In contrast, during the periods of rest, the aerobic breakdown of carbohydrates and lipids becomes the dominant energy source.

Intermittent exercise is also used extensively during training. In fact, **interval training** has drawn much attention in recent years, thanks to the adaptations it elicits and to the benefits it elicits for performance and health, many of which we will consider in this chapter and the following one. Interval training involves repeated bouts of hard, maximal, or supramaximal efforts performed within a single training session and separated by periods of recovery, which usually range from half to several times the duration of the exercise bout.

The proportion of energy sources during interval exercise changes from one bout to the next. Because intervals are usually not sufficient to fully restore the energy state of muscle (see section 14.26), the contribution of the ATP–phosphocreatine system gradually declines in favor of the lactate system, and the contribution of the latter gradually declines in favor of the oxygen system. In fact, the shorter the interval, the more prominent these shifts are. Thus, although an initial or a few initial near-maximal, maximal, or supramaximal bouts may be primarily anaerobic, an interval exercise session consisting of several bouts will almost certainly be aerobic as a whole.

14.9 Sex Differences in Choice of Energy Sources During Exercise

Sexes differ in the proportion of carbohydrates to lipids used during prolonged exercise of low, moderate, or high intensity. Studies in recent years (reviewed by Michaela Devries) have confirmed that, as compared with men, eumenorrheic women (that is, women who have a normal menstrual cycle and menstruation) oxidize more lipids and fewer carbohydrates and proteins when exercising at the same relative intensity (for example, at the same percentage of $\dot{V}O_2max$). I note the latter because women usually have lower $\dot{V}O_2max$ than men. Let’s explore this disparity before we proceed.

I use the term *sex* rather than the related one, *gender*, because *sex* refers to the biological attributes stemming primarily from the sex chromosomes (section 4.6). *Gender*, on the other hand, refers to socially and culturally established distinctions or to personal identification.

The lower $\dot{V}O_2$max of women as compared with men results primarily from three factors. First, on average, women have a smaller heart and, specifically, a smaller left ventricle (the chamber that pumps blood to the tissues) relative to body mass or fat-free body mass. Consequently, they have a lower stroke volume, that is, volume of oxygen-rich blood pumped to the body with each heartbeat. Second, women have a lower blood hemoglobin concentration (section 16.1), resulting in less oxygen being delivered per unit volume of blood. Third, women have a lower percentage of muscle mass (section 10.2) and fat-free body mass (section 11.3), which is counterbalanced by a higher percentage fat mass. Since muscles and other lean tissues are metabolically more active than adipose tissue, a woman's body takes up and consumes a smaller portion of the oxygen that blood delivers.

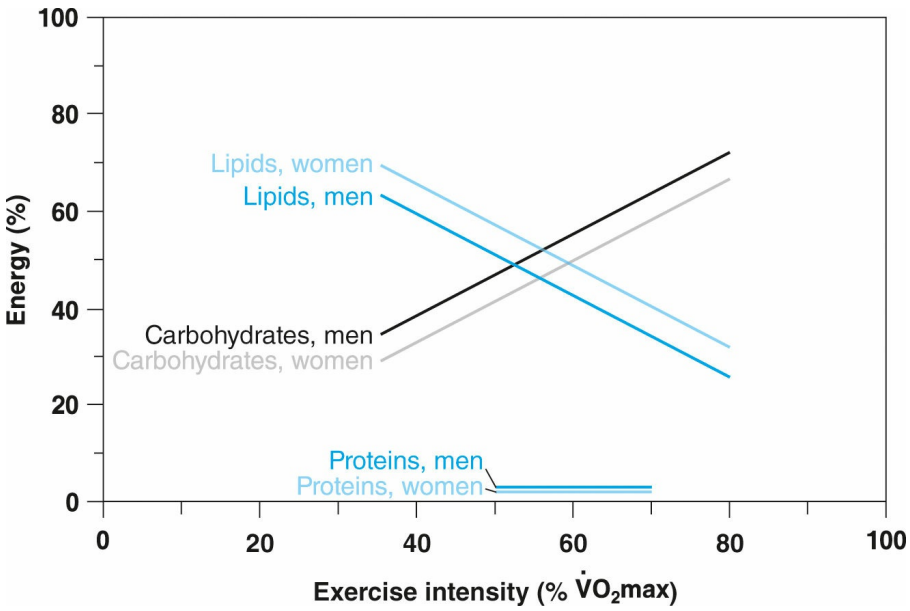


Figure 14.6 Choice of energy sources as a function of exercise intensity in men and eumenorrheic women. In both sexes, the percentage contribution of carbohydrates to whole-body energy expenditure rises, while that of lipids declines, as exercise intensity increases. In women, however, the contribution of carbohydrates is lower (by 6% to 7%) than in men, and

that of lipids is higher (by 7% to 8%). The point at which the two solid lines (representing men) intersect and the point at which the two faint lines (representing women) intersect mark the intensities at which the contributions of carbohydrates and lipids become equal. Like carbohydrates, proteins contribute less (by about 1%) to the energy expenditure of exercise in women, at least in the range of 50% to 65% of $\dot{V}O_2max$, in which studies have been conducted.

You can see now why it is important to define the terms of the comparison between the sexes. If, for example, a man has a $\dot{V}O_2max$ of 45 mL · kg^{-1} · min^{-1} and a woman has a $\dot{V}O_2max$ of 38 mL · kg^{-1} · min^{-1} (a typical difference), then exercising at 60% of each one's $\dot{V}O_2max$ would elicit respective oxygen uptakes of 27 and 22.8 mL · kg^{-1} · min^{-1}. Hence, the same relative intensity translates into a lower absolute intensity for the woman. Comparing the two sexes in relative, rather than absolute, terms is fairer and makes more sense biologically. For other comparisons, see problem 5.

Figure 14.6 presents typical lines of carbohydrate, lipid, and protein oxidation in the two sexes as a function of exercise intensity. Because women use more lipids, the intensity at which the contribution of carbohydrates reaches that of lipids is higher in women than in men. Research (reviewed by Devries) has shown that the lower reliance of eumenorrheic women on carbohydrates during exercise results from lower contribution of glucose by the liver to the active muscles and, possibly, lower use of muscle glycogen. On the other hand, it is not clear which fat source accounts for their higher reliance on lipids, that is, whether it is adipose tissue triacylglycerols, myocellular triacylglycerols, or both.

14.10 How Sex Influences Choice of Energy Sources During Exercise

What is the reason for the differences in exercise metabolism between sexes described in the preceding section? Researchers have explored the roles of three sex hormones—**testosterone**, **estradiol**, and **progesterone** (figure 14.7)—all three of which are **steroid hormones**. This family of compounds encompasses tens of members, which derive from cholesterol and are divided into five categories on the basis of structure and biological function:

- **Glucocorticoids**, acting primarily to increase the blood glucose concentration and represented by cortisol
- **Mineralocorticoids**, involved in water and salt homeostasis in the body and represented by aldosterone
- **Androgens**, the main male hormones, represented by testosterone
- **Estrogens**, the main female hormones, represented by estradiol
- **Progestogens**, or **progestagens**, aiding in the initiation and maintenance of pregnancy and represented by progesterone.

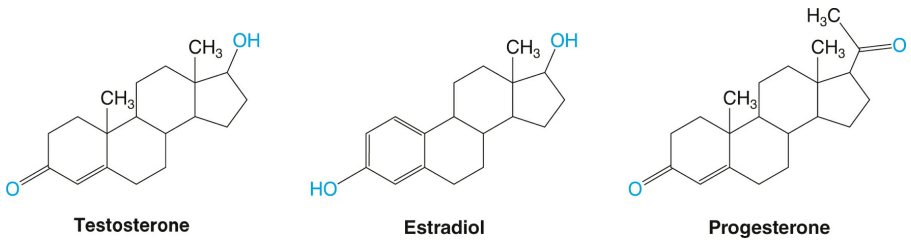


Figure 14.7 Sex hormones. Testosterone, estradiol, and progesterone are major sex hormones that control sex-linked characteristics and reproduction. Their molecules bear the characteristic 4 rings of cholesterol (figure 5.17) but have fewer than its 27 carbons: 19, 18, and 21, respectively. All three compounds are amphipathic. Their hydrophilic ends are shown in color, and the hydrophobic parts are shown in black.

Testosterone, the major male sex hormone, is synthesized and secreted mainly by the testes. Estradiol and progesterone, the major female sex hormones, are synthesized and secreted mainly by the ovaries. As such, the three hormones are prime candidates for the role of mediating the effects of sex on the choice of energy sources during exercise. Studies (reviewed by Devries) have addressed the role of each hormone by pharmacologically raising or suppressing its plasma concentration and then observing any effect on exercise metabolism. The results suggest that estradiol promotes lipid use while demoting carbohydrate and protein use in exercise. Testosterone does not seem to have an effect, and the role of progesterone remains poorly explored.

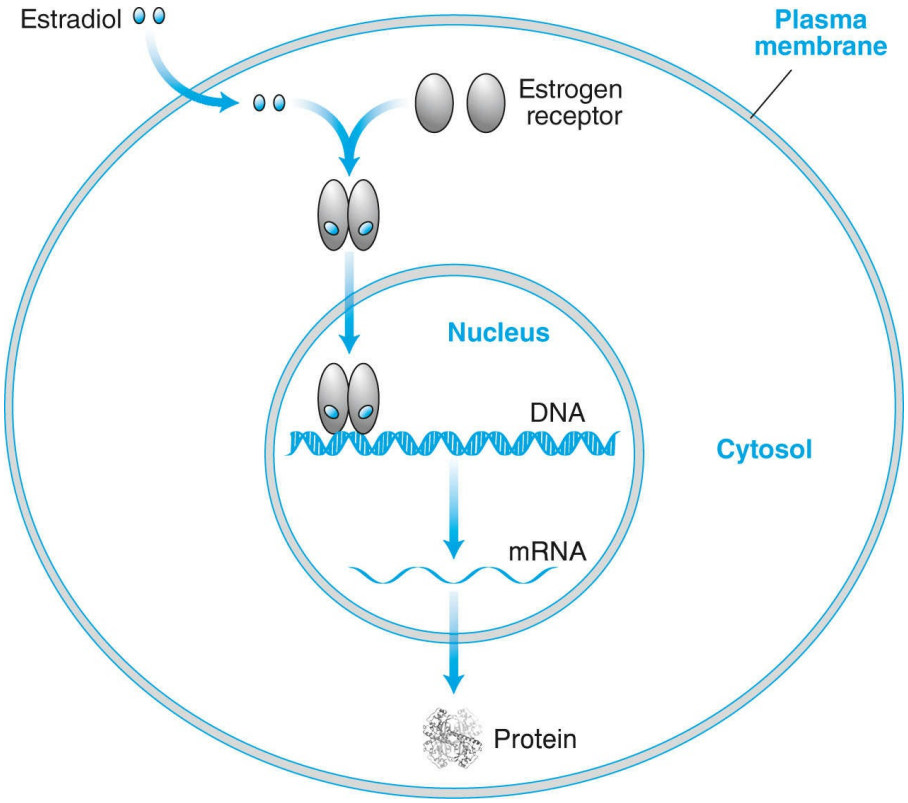


Figure 14.8 **Estradiol action.** Estradiol crosses the plasma membrane of target cells to meet its receptor in the cytosol. Binding of the hormone to the receptor changes the receptor's shape, causing it to dimerize and translocate to the nucleus. The activated receptor binds to DNA and induces specific genes, resulting in increased mRNA and protein synthesis.

How, then, does estradiol exert its effects on exercise metabolism? Like steroid hormones in general, estradiol differs from the hormones that we have encountered thus far in that, rather than binding to a receptor at the plasma membrane of cells in target tissues, it crosses the membrane thanks to the amphipathic character it shares with cholesterol. Target tissues include the ovaries, uterus, and breasts. There is also evidence that skeletal muscle is a target for estradiol.

Once in the cytosol of a target cell, estradiol binds to the **estrogen receptor** (figure 14.8). This binding causes a conformational change that elicits receptor dimerization (that is, association of two receptor molecules) and migration to the nucleus, where the activated receptor acts as a transcription factor: It binds to specific DNA sequences and serves as docking site for RNA polymerase, which initiates transcription, thus inducing specific genes. Induction of these genes results in the development of the so-

called female secondary sex characteristics such as breasts. However, it is not known which estradiol-induced genes affect fuel selection in exercise.

Secondary sex characteristics are so named for distinction from the primary ones, which are the reproductive organs (such as ovaries and uterus in the female sex).

The role of estradiol and the possible role of progesterone in fuel selection during exercise raise another question: Does the proportion of energy sources during exercise differ in different phases of the menstrual cycle? The basis for this question is the fact that the second half of the cycle (the so-called **luteal phase**), which follows ovulation at mid-cycle, is characterized by higher estradiol and progesterone concentrations than is the first half (the **follicular phase**). Considerable disagreement exists among studies on this matter. It seems that differences between menstrual cycle phases, if any, are small. A frequent finding is a lower use of muscle glycogen during exercise in the luteal phase as compared with the follicular phase, although this difference has no effect on the overall ratio of carbohydrates to lipids oxidized, which does not differ between phases. What is more, exercise performance does not differ between phases.

14.11 Effect of Age on Choice of Energy Sources During Exercise

Age influences the proportion of energy sources during exercise. Studies reviewed by Neil Armstrong and associates, as well as by Michael Riddell, have shown that children (before the onset of puberty) oxidize proportionally more lipids and fewer carbohydrates than do adults of the same sex in prolonged exercise at a given relative intensity. Moreover, maximal fat oxidation relative to fat-free body mass is higher in children than adults. In addition, children produce less lactate and suffer a smaller drop in muscle pH than do adults during exercise at a given relative intensity. Thus, as compared with adults, children catabolize fewer carbohydrates, both aerobically and anaerobically, during exercise.

Proposed reasons for the differences in exercise metabolism between

children and adults include the lower glycogen and lactate dehydrogenase contents of children's muscles. The former would result in a lower carbohydrate breakdown and the latter in a lower lactate production. Adolescents stand between children and adults with respect to these differences.

No changes in the proportion of energy sources during exercise seem to occur throughout adulthood, including old age, in men. In women, however, a change occurs after menopause, since the concentrations of sex hormones, including estradiol, gradually drop. As a result, the proportion of carbohydrates to lipids used during exercise becomes similar to that in men.

14.12 Effect of Carbohydrate Intake on Choice of Energy Sources During Exercise

As we have seen on several occasions, the amount of an energy source affects its contribution to meeting the energy demands of exercise. The amount of an energy source can be modified by nutrition, and the most convincing example is that of taking in surplus carbohydrates. Moderate-intensity or hard exercise exceeding one hour in duration, whether continuous or intermittent, can reduce muscle glycogen to such an extent that the rate of ATP regeneration drops substantially and aerobic endurance performance is compromised. These detrimental effects can be ameliorated by excess intake of carbohydrates before such exercise, during it, or both. Let's examine the details of properly increasing carbohydrate intake.

Excess carbohydrate intake during the days preceding a prolonged event has been aptly termed **carbohydrate loading**. Successful carbohydrate loading requires a careful combination of dieting and training. A typical protocol may begin six days before the event with moderate carbohydrate intake (providing 40% to 50% of daily energy) and regular training for three days. This combination is aimed at lowering the muscle glycogen content to induce the glycogen synthase gene. Then, in the final three days before the event, carbohydrate intake is increased to about 70% of daily energy in order to provide ample substrate for the upregulated muscle glycogen synthase. In

addition, training load is tapered off to curb glycogen breakdown.

In men, carbohydrate loading doubles the muscle and liver glycogen contents, resulting in the use of more glycogen and fewer lipids during exercise. It also usually increases performance in events lasting more than 90 min. However, women fail to increase their muscle glycogen content with carbohydrate loading unless they consume impractically high amounts of carbohydrates. Even then, they do not seem to enjoy performance benefits. Thus, carbohydrate loading does not appear to be an effective approach for increasing aerobic endurance performance in women. The reason for this difference from men is not clear.

Carbohydrates taken as supplements *during* prolonged moderate-intensity or hard exercise also affect the proportion of fuels used, and this effect is seen in both sexes. The continuous entry of glucose from the small intestine into blood during exercise results in its increased uptake by the active muscles and a spectacular rise in its contribution to ATP resynthesis (figure 14.9). Equally spectacular is the boost to performance, especially in events exceeding two hours in duration. Carbohydrate supplementation during exercise also serves to maintain euglycemia, which is threatened by prolonged moderate-intensity or hard exercise (section 10.29).

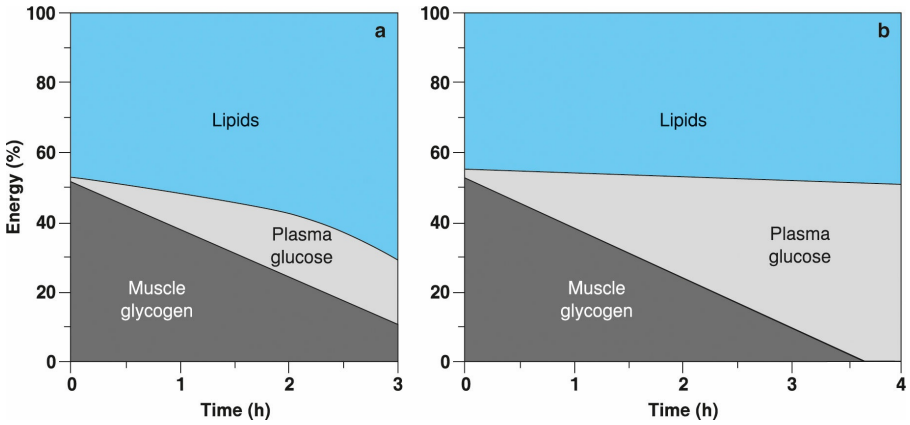


Figure 14.9 **Carbohydrate supplementation during exercise.** *(a)* Cycling at 70% to 75% of $\dot{V}O_2$max drove experienced athletes to exhaustion after approximately 3 h, at which time the contribution of carbohydrates to whole-body energy production had fallen below 30%. *(b)* When the athletes took carbohydrates during exercise, they could cycle for an additional hour, at the end of which carbohydrates still contributed half of the total energy. The difference was made by plasma glucose deriving primarily from the ingested carbohydrates. No sparing of muscle glycogen (that is, slowing down of glycogen utilization) was evident in response to

carbohydrate supplementation in this experiment or in similar ones employing cycling, although glycogen sparing has been found in studies employing running.

From E.F. Coyle, “Muscle Glycogen Utilization During Prolonged Strenuous Exercise When Fed Carbohydrate,” *Journal of Applied Physiology* 61 (1986): 165-172. By permission of The American Physiological Society.

Given the proven ergogenic effect of carbohydrate supplementation during prolonged exercise, a considerable amount of research has been devoted to establishing the optimal type and dosage of carbohydrates. The available evidence, reviewed by Asker Jeukendrup, shows that the most effective approach is to ingest about 60 g of glucose or glucose polymers (which are hydrolyzed to glucose in the small intestine; section 10.1) and 30 g of fructose per hour. These values correspond to the maximal amounts that can be absorbed through two different transporters (one for glucose and one for fructose) in the small intestine.

Such a combination of glucose and fructose maximizes the amount of exogenous carbohydrate that can be oxidized during exercise and appears to be more appropriate for ultra-endurance events (those exceeding 2.5 hours in duration). Curiously, ingesting sucrose (which is split into glucose and fructose) does not have the same maximal effect; it is only as effective as ingesting an equal amount of glucose alone. Events of shorter duration require lower amounts of exogenous carbohydrates (around 30 to 60 g per hour), which can be ingested in the form of a single substance (usually sucrose or glucose).

Athletes take carbohydrates during exercise in a variety of forms, including the so-called “sport drinks,” gels, and bars. Sport drinks usually contain 6% to 8% of carbohydrates and provide optimal hydration as well as energy. Gels and bars serve as more condensed sources of energy and require additional intake of water to prevent exercise-induced dehydration, especially in hot weather. There are no differences in the ergogenic effects of drinks, gels, and bars when equal amounts and types of carbohydrates are ingested.

14.13 Effect of Fat Intake on Choice of Energy Sources During Exercise

Let's consider now whether excess intake of fat, the other major fuel in prolonged moderate-intensity or hard exercise, influences the choice of energy sources. By analogy to carbohydrate loading, researchers have tested **fat loading**, also termed **low-carbohydrate high-fat diet** or **ketogenic diet**. This approach consists in obtaining 60% to 80% of daily energy from fat for one or more weeks, which is considerably longer than the period for carbohydrate loading.

Such long duration is needed to elicit adaptations that contribute to the use of more lipids and fewer carbohydrates in the whole body and in the muscles during exercise. Adaptations include an increase in myocellular triacylglycerols, increased uptake of plasma fatty acids by the active muscles, and use of plasma triacylglycerols for energy production. As a result, Fat_{max} and MFO (as defined in section 14.5) increase, reaching, on average, 80% of $\dot{V}O_2max$ and 1.6 g · min^{-1}, respectively. The plasma concentration of ketone bodies rises (hence the name *ketogenic diet*), and the muscle and liver glycogen stores drop or do not change.

The shift of exercise metabolism toward lipid utilization after fat loading persists even if we apply carbohydrate loading in the final one to three days before an event, aiming at replenishment of glycogen stores and maximal performance. However, fat loading followed by carbohydrate loading does not seem to be more ergogenic than carbohydrate loading alone. In addition, when endurance athletes trained for three weeks while consuming a low-carbohydrate high-fat diet, their performance did not improve, whereas it did improve among athletes on a high-carbohydrate diet, as found by Louise Burke and coworkers.

What about fat intake *during* exercise? Efforts to mimic the ergogenic effect of carbohydrate supplementation have employed an unusual form of fat, **medium-chain triacylglycerols**, or **MCT**. These triacylglycerols differ from those of regular food in that their acyl chains are shorter, typically containing 8 to 12 carbons, as opposed to 14 or more in most **long-chain triacylglycerols (LCT)**. MCT are absorbed faster than LCT, and when they deliver their acyl groups as fatty acids to cells for degradation, these fatty acids enter the mitochondria without the need for carnitine (section 11.9).

Medium-chain triacylglycerols (MCT) happen to be abbreviated just like the monocarboxylate transporters introduced in section 10.23 but are unrelated to them.

Based on these particularities, researchers have hypothesized that MCT supplementation provides fast additional energy during exercise, thus enhancing aerobic endurance performance. However, research has shown that, whether taken alone or with carbohydrates, MCT contribute little to the total energy of prolonged moderate-intensity exercise, partly because one cannot ingest a high amount of them without experiencing gastrointestinal distress. Moreover, MCT intake (alone or with carbohydrates) does not increase aerobic endurance performance as compared with the intake of equal energy solely from carbohydrates.

14.14 Adaptations to Endurance Training in the Proportion of Energy Sources Used During Exercise

The training state affects the proportion of energy sources during exercise thanks to training-induced adaptations. In fact, training-induced adaptations are so diverse and so important for performance and health that I will devote this and the following three sections to them, thus elaborating on the latter two principles of exercise metabolism outlined in section III.2. The most spectacular adaptations of the proportion of energy sources accompany **endurance training**. In a nutshell, *endurance training increases the ratio of lipids to carbohydrates used during prolonged exercise at both the muscle and whole-body levels.*

Endurance training is commonly employed with two major aims: to increase aerobic endurance performance and to increase what is termed **cardiorespiratory fitness**. The former primarily interests endurance athletes, whereas the latter is related to the health of the general population. The hallmark of both is the increase in $\dot{V}O_2max$.

Considerable debate surrounds the specific characteristics of an endurance training program that effectively increases $\dot{V}O_2max$. The American College

of Sports Medicine offers the following alternatives:

- Moderate-intensity exercise for at least 30 min per day on at least five days per week for a total of at least 150 min per week
- Vigorous exercise for at least 20 min per day on at least three days per week for a total of at least 75 min per week
- A combination of moderate-intensity and vigorous exercise to achieve an energy expenditure of at least 500 MET · min per week

Consult table III.1 (p. 163) for definitions of moderate-intensity and vigorous exercise.

Any continuous and rhythmic activity that engages major muscle groups is suitable, such as running, cycling, swimming, and aerobics. Exercise may be performed either in one session per day or in multiple sessions of at least 10 min to accumulate the recommended duration.

Although these guidelines seem to work well for most people, they are not adequate for everybody. Rather, it is becoming increasingly appreciated that large differences exist among individuals in the response to a given endurance training program or in the dose of training needed to elicit a significant response. The frequent division of individuals into “responders” and “nonresponders” has been challenged by studies, such as one by David Montero and Carsten Lundby, showing that increasing the training dose turns an ostensible nonresponder into a responder. Thus, it may be preferable to speak of “high responders” and “low responders,” although the effective training dose for a low responder may be so high that he or she can be practically considered a nonresponder.

Training dose, or training load, is a function of exercise intensity, duration, and frequency.

Let’s return now to adaptations to endurance training. Changes in the proportion of energy sources during exercise can be detected as early as one week from the beginning of an endurance training program, provided that the training load is substantial. Adaptations culminate after several months of training.

How are adaptations to training detected in the proportion of energy sources? In an interventional study, a person must perform two exercise tests (one at the beginning and the other at the end of a training program) of the same mode (for example, treadmill running), duration, and intensity. In each test, we measure the fuels used by employing a variety of laboratory techniques, such as those described in section III.7 and later in this chapter (section 14.22). As far as duplicating the test parameters is concerned, duration presents no problem (all you need is a timer). Duplicating intensity would also be straightforward (for example, set the treadmill at the same speed in the two tests) if it were not for the capacity of endurance training to increase $\dot{V}O_2max$. This factor forces us to distinguish absolute from relative intensity, as in sections 14.9 and 14.11, although now we refer to the same individual, not different ones (as when we compared men with women, and children with adults).

As an example, if you begin a three-month endurance training program with a $\dot{V}O_2max$ of 45 mL · kg^{-1} · min^{-1}, it can rise to 52 mL · kg^{-1} · min^{-1} in the end. The pre- and post-training tests may then be performed at the same *absolute* intensity, say, a treadmill speed eliciting an oxygen uptake of 27 mL · kg^{-1} · min^{-1}, or 60% of baseline $\dot{V}O_2max$. They also may well be performed at the same *relative* intensity, that is, 60% of $\dot{V}O_2max$ at the time of each test, which is 27 mL · kg^{-1} · min^{-1} before training and 31.2 mL · kg^{-1} · min^{-1} after training.

When the two tests are matched for absolute intensity, there is general agreement among researchers that the lipid-to-carbohydrate ratio is higher after training (figure 14.10). However, when the tests are performed at the same relative intensity, the ratio does not differ significantly. Nevertheless, cross-sectional studies comparing untrained individuals and endurance athletes who exercised at a fixed percentage of each participant's $\dot{V}O_2max$ have shown that endurance athletes have a higher lipid-to-carbohydrate ratio.

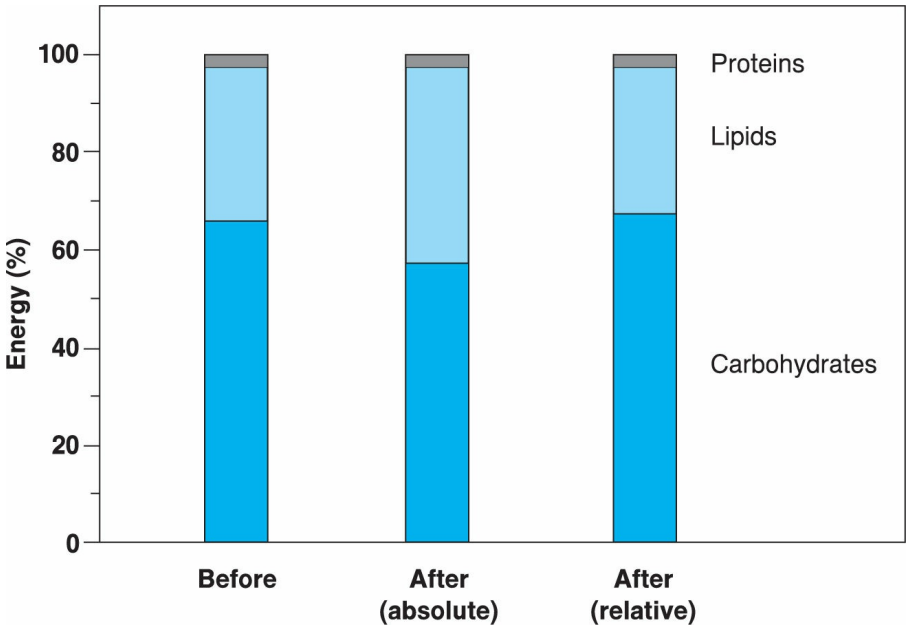


Figure 14.10 Effect of endurance training on the proportion of energy sources during exercise. When untrained men and women underwent endurance training for seven weeks, the percentage contribution of lipids to whole-body energy expenditure during prolonged moderate-intensity exercise at a fixed absolute intensity (cycling for 90 min at 60% of baseline $\dot{V}O_2max$) increased significantly (from 31% to 41%). However, when the post-training test was conducted at the same relative intensity as the pre-training test (60% of the new $\dot{V}O_2max$, which was higher than baseline by 20%), the percentages of lipids and carbohydrates did not change. In all three tests, proteins offered 3% to 4% of total energy.

From S.L. Carter, C. Rennie, and M.A. Tarnopolsky, "Substrate Utilization During Endurance Exercise in Men and Women After Endurance Training" *American Journal of Physiology: Endocrinology and Metabolism* 280 (2001): E898-E907. By permission of The American Physiological Society.

This discrepancy between interventional and observational studies may result from genetic differences between athletes and nonathletes, as I will describe in section 14.19. Alternatively, it may be that the weekly training loads usually employed in interventional studies are lower than the training loads of competitive athletes and that they do not suffice to raise the lipid-to-carbohydrate ratio during exercise at a given relative intensity. A third explanation may be that the athletes tested in the observational studies have a training background of years, whereas few interventional studies last more than a few months.

The benefit from the increase in the ratio of lipids to carbohydrates used during exercise at a fixed absolute intensity after endurance training is the

sparing of carbohydrates (the smaller and faster energy source of the two). Carbohydrate sparing may prolong exercise. Looking at it another way, one could say that the benefit is the ability to exercise at a higher intensity while spending the same amount of carbohydrate. This efficiency allows an endurance athlete to complete an exercise task earlier, thus increasing performance.

An additional factor that contributes to increased aerobic endurance performance after endurance training is the rise in the muscle glycogen content at rest, although the liver glycogen content does not appear to change, as reviewed by Javier Gonzalez and colleagues. The rise in the muscle glycogen content with endurance training ranges from 20% to 66% and may occur because exercise augments the sensitivity of muscle to insulin action (see section 15.7). Thus, if one consumes adequate carbohydrates during recovery from exercise (an issue explored in section 14.26 under the Glycogen Replenishment subheading), insulin will be secreted and will promote the synthesis of more glycogen thanks to the increased insulin sensitivity of muscle. Remember that insulin promotes not only plasma glucose uptake by muscle for glycogenesis but also glycogenesis per se by activating glycogen synthase (section 10.29 and figure 10.48).

14.15 How Endurance Training Modifies the Proportion of Energy Sources Used During Exercise

What makes a muscle shift toward lipid utilization and away from carbohydrate utilization during moderate-intensity exercise after endurance training? This shift results primarily from the following changes in the exercising muscles:

- *The muscle mitochondrial content increases.* As discussed in section 12.12, endurance training augments the mitochondrial content of muscles. This adaptation is mediated by complex signal transduction pathways that induce nuclear and mitochondrial genes encoding mitochondrial proteins (section 13.6 and figure 13.8). The increase in

muscle mitochondrial content boosts the capacity of the muscles to resynthesize ATP from the aerobic breakdown of carbohydrates, lipids, and proteins.

- *Glucose uptake decreases.* Although endurance training increases the muscle GLUT4 content, it seems to suppress the translocation of GLUT4 from its intracellular reservoir to the plasma membrane during exercise at a given absolute moderate intensity, as reviewed by Richter and Hargreaves. As a result, trained muscles take up less glucose from plasma during moderate-intensity exercise than do untrained muscles.
- *The rate of muscle glycogenolysis decreases.* Although endurance training augments the muscle glycogen content (as mentioned in the preceding section), it lowers the glycogenolytic rate during exercise at a given absolute moderate intensity, as shown by several researchers, including Paul LeBlanc and coworkers. This reduction appears to result from lower cytosolic concentrations of P_i and AMP, two of the compounds that speed up glycogenolysis (section 10.5). These lower concentrations, in turn, result from the better ATP homeostasis thanks to increased aerobic resynthesis in the mitochondria, an effect that prevents the accumulation of ATP degradation products such as P_i and AMP.
- *Fatty acid uptake increases.* This increase results primarily from a training-induced increase in **capillary density** (that is, the number of capillaries per unit of muscle cross-sectional area), an effect that we will discuss in section 15.4. That increase allows the delivery of more blood-borne fatty acids to muscle fibers. Endurance training also upregulates lipoprotein lipase in muscle, which may facilitate the hydrolysis of lipoprotein-bound triacylglycerols and increase their small contribution to the energy demands of exercise.

The combination of these adaptations boosts the oxidative capacity of the muscles and their capacity to catabolize lipids, while dampening their capacity to catabolize carbohydrates during moderate-intensity exercise. The latter effect includes both types of carbohydrate breakdown, that is, aerobic and anaerobic. As a result, endurance training diminishes lactate production

during exercise at a given absolute moderate intensity, an effect that is exploited in training practice, as we will discuss in section 17.4.

Training-induced adaptations in our capacity to mobilize the two major lipid sources during exercise—adipose tissue triacylglycerols and myocellular triacylglycerols—either do not take place or are equivocal. Specifically, endurance training does not seem to affect the lipolytic rate in adipose tissue either at rest or during exercise, as reviewed by Thomas Tsiloulis and Matthew Watt. In addition, it is unclear whether endurance training modifies lipolysis of myocellular triacylglycerols during exercise, both because this possibility has not been directly assessed and because of the following controversial findings, discussed by Thomas Alsted and collaborators. First, endurance training increased the muscle ATGL content but did not affect other protagonists of lipolysis presented in sections 11.6 and 11.7. Second, in a variety of studies, endurance training has either increased, decreased, or not changed the myocellular triacylglycerol content. Third, endurance athletes have higher myocellular triacylglycerol content that do nonathletes or sprint athletes, as shown by, among others, Yoshinao Nakagawa and Masaaki Hattori.

Although the change in the proportion of energy sources during exercise caused by endurance training derives from skeletal muscle adaptations, the increase in $\dot{V}O_2max$ results primarily from circulatory adaptations. Specifically, endurance training increases the total number of erythrocytes and, hence, the total amount of hemoglobin, which is the main determinant of $\dot{V}O_2max$ (see the highlight box titled Hemoglobin and Aerobic Capacity in chapter 3 on page 51). In addition, endurance training augments stroke volume and, hence, maximal cardiac output, that is, the blood volume that the heart pumps per minute of maximal effort. Thus, the increase in $\dot{V}O_2max$ is due mainly to enhanced oxygen delivery to the active muscles.

14.16 Adaptations of Exercise Metabolism to Resistance and Sprint Training

Resistance, or strength, training and sprint training (both of which rely heavily on anaerobic energy production) do not modulate the proportion of

energy sources during exercise to any considerable degree. Their effects are limited to some enzymes involved in energy production.

Resistance training with the characteristics described in section 12.12 exerts little, if any, effect on energy metabolism, as reviewed by Michael Deschenes and William Kraemer, despite its spectacular effect on muscle hypertrophy. Neither the muscle content of key energy sources (such as ATP, phosphocreatine, and glycogen) nor the muscle content of enzymes of the ATP–phosphocreatine system (that is, creatine kinase and adenylate kinase) or the lactate system (such as phosphorylase and phosphofructokinase) seem to change, according to most original research in this area. The muscle content of enzymes of the oxygen system (such as citrate synthase and succinate dehydrogenase) does not change or may be diminished along with the mitochondrial content in relative terms (that is, percentage of total muscle volume). The probable reason is that muscle hypertrophy is not accompanied by an increase in mitochondrial mass; thus it leads to a dilution of the resident mitochondria. Finally, $\dot{V}O_2max$ does not change with resistance training.

Sprint training is characterized by brief (typically 3 to 30 s) all-out or nearly all-out bouts, followed by sufficient time (typically several minutes) for the complete or almost complete resynthesis of muscle phosphocreatine and the recovery of sprinting ability. Such training results in increased sprint performance. Adaptations to sprint training differ from those to resistance training, probably because, despite being maximally activated in sprint training, the muscles do not work against high resistance. Like resistance training, sprint training does not change the concentrations of energy sources, but it does appear to upregulate enzymes of all three energy systems, as reviewed by Angus Ross and Michael Leveritt. Thus, muscle creatine kinase, adenylate kinase, phosphorylase, phosphofructokinase, lactate dehydrogenase, and mitochondrial enzymes have been reported to increase in several studies. These changes depended greatly on the parameters of the training program, such as sprint duration, interval duration, and training frequency. This fact brings us to the topic of the next section.

14.17 Adaptations of Exercise Metabolism to

Interval Training

Interval training elicits several of the favorable adaptations connected with endurance training or, to be precise, **moderate-intensity continuous training (MICT)**. These adaptations include increases in muscle mitochondrial content, $\dot{V}O_2max$, ratio of lipids to carbohydrates oxidized during exercise at a fixed absolute moderate intensity, resting muscle glycogen content, and aerobic endurance performance. In addition, muscle glycogenolysis and lactate production decrease during such exercise. One can achieve these adaptations by spending less total time (including intervals), and less net exercise time, than is spent in MICT. Thus, interval training is considered a time-efficient alternative to MICT, both for athletes and for the general population, though it is more physically demanding than MICT because of the high exercise intensities it employs.

Since an interval exercise session is defined not only by the duration and intensity of its exercise bouts (which suffice when one needs to define MICT) but also by the number of repetitions and the duration and intensity of the intervals, the variety of interval training programs exceeds that of endurance training programs. This variety makes it difficult to generalize the effects of interval training. In an effort to be more specific, researchers in the field discern two categories, to which I will refer when necessary. One is **high-intensity interval training (HIIT)**, which involves submaximal efforts eliciting at least 80% of HRmax. The other is **sprint interval training (SIT)**, which involves all-out efforts or an intensity corresponding to at least 100% of the power corresponding to $\dot{V}O_2max$.

This is where the preceding section of the discussion comes into play. You may have noticed that the term *sprint training* differs only slightly from the term *sprint interval training*. Their main distinction lies in the duration of the interval. As mentioned in the preceding section, sprint training requires sufficient time after each sprint to allow complete or near-complete recovery of sprinting ability. This is not the case with SIT (or with interval training in general), where intervals are incomplete. As a result, whereas each sprint in sprint training taxes primarily the two anaerobic energy systems, successive sprints in SIT glide toward the inexhaustible oxygen system.

Because the body of research supporting interval training is smaller than that supporting MICT, recommendations for the former are not as firmly established as those for the latter. Thus, the following are only examples meant to give you an idea of what is usually employed. Frequently used—and effective—HIIT protocols involve four bouts of 4 min at 85% to 95% of HRmax, separated by 3 to 4 min of passive recovery. Frequently used—and also effective—SIT protocols involve four to six all-out bouts of 30 s, separated by 4 min of active or passive recovery. Nevertheless, Niels Vollaard and Richard Metcalfe have proposed that SIT sessions with fewer (two or three) and shorter (10 to 20 s) sprints may be as effective or even more effective. In all cases, the recommended training frequency is three times per week.

How does interval training elicit adaptations that resemble those of MICT when the two have such different characteristics? The key appears to be the activation of PGC1α, the same protein that orchestrates the changes in gene expression in response to endurance training. Martin Gibala and John Hawley propose that HIIT and, even more, SIT elicit spectacular rises in the cytosolic concentrations of Ca^{2+} (because of release from the sarcoplasmic reticulum) and AMP (because of ATP degradation), which lead to PGC1α activation. PGC1α then translocates to the nucleus, where it coactivates the expression of many genes that encode mitochondrial proteins and the transcription factors activating the expression of these genes (section 13.6 and figure 13.8).

In addition, interval training perturbs other facets of muscle fiber homeostasis (such as the phosphocreatine concentration and pH) more than MICT does. Moreover, it does so in a larger portion of muscle mass, because the high intensity causes the recruitment of type II fibers, which are minimally recruited at moderate intensity because of their high excitation thresholds (section 8.9). This perturbation may compensate for the lower work performed by the active muscles in interval training as compared with MICT. A role may also be played by strong perturbation of homeostasis in other systems, such as the cardiovascular, respiratory, neural, and hormonal.

The highlight box titled Adaptations to Different Types of Training summarizes what we have addressed in sections 14.14 to the present one, as well as section 12.12.

Adaptations to Different Types of Training

Endurance (Moderate-Intensity Continuous) Training

- Increase in $\dot{V}O_2max$
- Increase in total body hemoglobin
- Increase in stroke volume and, hence, maximal cardiac output
- Increase in the proportion of lipids to carbohydrates used during prolonged exercise at a given absolute intensity
- Increase in muscle mitochondrial content and, hence, mitochondrial enzymes
- Increase in muscle GLUT4
- Decrease in muscle GLUT4 translocation to the plasma membrane during exercise at a given absolute moderate intensity
- Increase in resting muscle glycogen
- Decrease in muscle glycogenolysis during exercise at a given absolute moderate intensity
- Decrease in lactate production during exercise at a given absolute moderate intensity
- Increase in fatty acid uptake by muscle
- Increase in muscle capillary density
- Increase in muscle lipoprotein lipase
- IIX → IIA → I^a
- Increase in aerobic endurance performance

Resistance (Strength) Training

- Muscle hypertrophy
- Possible decrease of muscle mitochondrial content
- IIX → IIA^a
- Increase in maximal strength

Sprint Training

- Increase in muscle adenylate kinase and creatine kinase
- Increase in muscle phosphorylase, phosphofructokinase, and lactate dehydrogenase
- Increase in muscle mitochondrial enzymes
- Increase in sprint performance

Interval Training

- Increase in $\dot{V}O_2max$
- Increase in the proportion of lipids to carbohydrates used during prolonged exercise at a given absolute moderate intensity
- Increase in muscle mitochondrial content and, hence, mitochondrial enzymes
- Increase in resting muscle glycogen
- Decrease in muscle glycogenolysis during exercise at a given absolute intensity
- Decrease in lactate production during exercise at a given absolute intensity
- Increase in aerobic endurance performance

[a]Fiber-type transitions will be discussed in section 14.19.

14.18 Adaptations of Exercise Metabolism to Concurrent Training

Because the different types of training discussed in the preceding four sections elicit different desirable adaptations, athletes and exercisers often engage in more than one type of training. For example, team-sport players desire both high aerobic endurance and strength, since performance during matches depends on the contribution of both. In addition, members of the general population, as well as those with chronic diseases (as we will see in chapter 15), benefit from both endurance and resistance training. As a result, people often combine endurance and resistance exercises in a single session

or perform endurance exercise in one session and resistance exercise in another, in the context of a periodized training program. We describe these practices as **concurrent training**.

The question arises, then, as to whether concurrent training allows one to reap the benefits of both types of training. In recent decades, several researchers have found that mixing endurance and resistance exercises in a training session interferes with strength development and muscle hypertrophy (although it does not interfere with the development of aerobic endurance). This hindrance has been termed the **interference effect**, or **concurrent effect**. Evidence from rodents suggests that it may result from interference in a signal transduction pathway that mediates adaptations to resistance training by a signal transduction pathway that mediates adaptations to endurance training.

The former is the mTORC1 pathway, a major mediator of exercise-induced muscle hypertrophy (section 13.5 and figure 13.4). The latter is the pathway initiated by AMPK activation and believed to be a major mediator of the exercise-induced increase in muscle mitochondrial content (section 13.6 and figure 13.8). Researchers propose that when AMPK is activated as a result of endurance exercise, it phosphorylates proteins that inhibit mTORC1 activation. It is also possible that activated AMPK promotes proteolysis. Thus, the hypothesis goes, endurance exercise performed close to resistance exercise mitigates the effect of the latter on muscle hypertrophy.

However, not all researchers find an interference of concurrent training on adaptations induced by resistance training. As discussed by Vernon Coffey and John Hawley, and also by Jackson Fyfe and colleagues, several studies have demonstrated that concurrent exercise and AMPK activation do not compromise mTORC1 signaling or rates of muscle protein synthesis. They can even potentiate these responses, as compared with resistance exercise performed alone. Experts have pointed to several factors that may be responsible for these different findings. The factors fall under two of the categories presented in section 14.4: exercise parameters and characteristics of the exerciser.

Regarding exercise parameters, it seems that the concurrent effect can be mitigated or even reversed (that is, endurance training can facilitate muscle

hypertrophy induced by resistance exercise) by decreasing the work of MICT, as reviewed by Kevin Murach and James Bagley. One can achieve this facilitation by decreasing duration, intensity, frequency, or some combination of these factors. Alternatively, one can replace MICT with interval or sprint training involving less total work. Cycling appears to interfere less with muscle hypertrophy than running.

Another exercise parameter that may modify the concurrent effect is the order in which endurance and resistance exercise are performed. In a meta-analysis, Zsolt Murlasits and collaborators concluded that performing resistance exercise before endurance exercise in a training session resulted in superior gains in lower-body strength as compared with performing the exercises in the reverse order (the order of training did not affect the development of aerobic endurance). The authors hypothesize that fatigue caused by endurance exercise limits performance during subsequent resistance exercise, thus impeding adaptations. The concurrent effect fades away when endurance and resistance exercise are separated by six or more hours (which means that they are performed in separate training sessions).

As far as the characteristics of the exerciser are concerned, the training state seems to be important. Studies with untrained participants do not show a concurrent effect, whereas studies with trained ones do. The difference may be due to a given training stimulus eliciting a larger perturbation of homeostasis in untrained muscle than in trained muscle and bringing about adaptations that are not totally specific to exercise type, at both the signaling and the functional levels.

This partial lack of specificity means that, in novices, endurance training (specifically, cycling but not running) may cause some muscle hypertrophy through signal transduction pathways that respond primarily to resistance training. Conversely, resistance training may cause some increase in muscle mitochondrial content through signal transduction pathways that respond primarily to endurance training. Thus, an untrained muscle, being unable to fully distinguish between the components of concurrent training, responds in a generic fashion, experiencing a general improvement in function, with no interference effect. However, after a period of training, these responses become specific to exercise mode and may suffer an interference effect.

14.19 Effect of the Genome on Choice of Energy Sources in Exercise

The genome affects the choice of energy sources in exercise primarily by determining the proportion of muscle fiber types. In section 8.9, we saw that human muscles contain three fiber types—I, IIA, and IIX—of different contractile properties and excitation thresholds. Then, in chapters 9 through 11, I pinpointed several metabolic differences between fiber types. It is now time to gather, expand, and integrate that knowledge. As you will see, *metabolic differences serve the functional specification of fiber types.*

Type I Fibers

Type I fibers are made to contract repeatedly for prolonged periods; thus they are economical and resistant to fatigue. They are recruited at rest and at low exercise intensities thanks to their low excitation threshold. Their myosin ATPase activity and maximal shortening velocity are low. They have low ATP and phosphocreatine concentrations at rest, but, as compared with type II fibers, they suffer lower losses of these biomolecules during maximal exercise. As a result, they end up with higher ATP and phosphocreatine concentrations after exercise.

Type I fibers produce ATP primarily through the oxygen system and are thus known as **oxidative**. To meet their high oxygen demands, they are nourished by many capillaries and have a high myoglobin concentration. They also have a high mitochondrial content and, hence, a high concentration of mitochondrial enzymes. Researchers measure the activities of these enzymes as markers of the oxidative capacity of a muscle and its fiber type composition. Some of the most frequently measured enzymes are citrate synthase of the citric acid cycle, succinate dehydrogenase of the citric acid cycle and electron-transport chain, cytochrome *c* oxidase of the electron-transport chain, carnitine palmitoyltransferase I, and hydroxyacyl CoA dehydrogenase of β oxidation.

In addition, type I fibers have high contents of the glucose transporter GLUT4 and the fatty acid transporters FABP-PM, FAT/CD36, and FATP.

Finally, they have high triacylglycerol concentration and contain ATGL.

Type IIX Fibers

Type IIX fibers are the antipodes of type I: Made to perform fast contractions, they are uneconomical and vulnerable to fatigue. They are recruited at high exercise intensities because of their high excitation threshold. They have high myosin ATPase activity and maximal shortening velocity. Their resting ATP and phosphocreatine concentrations are high, but they suffer higher losses during maximal exercise as compared with type I fibers. As a result, they end up with lower ATP and phosphocreatine concentrations after exercise.

In addition, type IIX fibers have high contents of glycolytic enzymes and lactate dehydrogenase. When activated, they regenerate ATP mainly through the two anaerobic energy systems and produce considerable lactate; thus they are characterized as **glycolytic**. They have low aerobic capacity, due primarily to their low blood supply, myoglobin concentration, mitochondrial content, and triacylglycerol concentration.

Type IIA Fibers

Type IIA fibers lie between type I and type IIX fibers, though closer to the latter. They feature intermediate contractile properties and resemble type IIX fibers in the characteristics of the ATP–phosphocreatine and lactate systems. Nevertheless, they also display a substantial oxidative capacity because of intermediate blood supply, mitochondrial content, and triacylglycerol concentrations. Thus, they are characterized as **oxidative–glycolytic**.

Table 14.5 summarizes the characteristics of the three muscle fiber types.

Table 14.5 Properties of the Main Muscle Fiber Types in Humans

	Muscle fiber type		
Property	**I**	**IIA**	**IIX**
Excitation threshold	Lower	Higher	Higher
Myosin ATPase activity	Lower	Intermediate	Higher

Maximal shortening velocity	Lower	Intermediate	Higher
Economy	Higher	Intermediate	Lower
Resistance to fatigue	Higher	Intermediate	Lower
ATP concentration at rest	Lower	Higher	Higher
Phosphocreatine concentration at rest	Lower	Higher	Higher
ATP decrease with maximal exercise	Lower	Higher	Higher
Phosphocreatine decrease with maximal exercise	Lower	Higher	Higher
ATP concentration after maximal exercise	Higher	Lower	Lower
Phosphocreatine concentration after maximal exercise	Higher	Lower	Lower
Metabolic character	Oxidative	Oxidative–glycolytic	Glycolytic
Glycolytic enzyme activity	Lower	Higher	Higher
Lactate dehydrogenase activity	Lower	Higher	Higher
Blood supply (capillary-to-fiber ratio)	Higher	Intermediate	Lower
Myoglobin concentration	Higher	Lower	Lower
Mitochondrial content	Higher	Intermediate	Lower
Mitochondrial enzyme activity	Higher	Intermediate	Lower
GLUT4 content	Higher	Lower	Lower
FABP-PM, FAT/CD36, and FATP content	Higher	Lower	Lower
Triacylglycerol concentration	Higher	Lower	Lower
ATGL presence	Yes	No	No

Choice of Energy Sources in Exercise Depending on Fiber Type Composition

Human muscles contain a mixture of the three fiber types in proportions differing from one muscle to another and from one individual to another. These proportions, which are primarily determined genetically, affect the selection of energy sources during exercise. The more type I fibers a muscle has, the more energy the oxygen system will supply in a given exercise and the more the muscle will depend on lipid and carbohydrate oxidation. Conversely, the more type II fibers a muscle has, the more energy the ATP–phosphocreatine and lactate systems will provide and the more the muscle will depend on phosphocreatine and the anaerobic breakdown of carbohydrates.

Fiber type composition determines, to a great extent, whether a person can excel as an athlete in certain sports. For example, type I fibers prevail in the leg muscles of endurance athletes, whereas type II fibers prevail in the leg muscles of sprinters. In particular, the type I fiber content of the vastus

lateralis muscle has been found to be about 80% in marathon runners but only 30% in sprinters.

14.20 Muscle Fiber Type Transitions

Can a muscle fiber switch from one type to another? Yes, if the functional demands placed on the muscle change. This capacity is part of muscle plasticity, mentioned at the beginning of chapter 13. Muscle fibers change types in a thorough manner: Along with the isoforms of myosin heavy chain (the trademark of fiber type, as presented in section 8.9), the other properties of a fiber type also change. Thus, each fiber type appears to have specific gene expression programs. What is more, when one program is activated, the other is inhibited. How this happens is unknown. Changes are small-scale (by a few percentage units) and take months to appear.

The transition from one fiber type to another follows the sequence dictated by the gradient of the properties—that is, from type I to IIA or vice versa and from type IIA to IIX or vice versa—not directly from type I to IIX or vice versa. Two isoforms of myosin heavy chain may coexist in a muscle fiber during the transition state; they will be either I and IIa, or IIa and IIx.

What causes conversions of muscle fiber type? The transition from oxidative toward glycolytic fibers is brought about by the decrease in neuromuscular activity as a result of, for example, muscle denervation or decreased endurance training. In contrast, the transition from glycolytic toward oxidative fibers is brought about by the increase in neuromuscular activity as a result of, for instance, chronic electrical stimulation of a muscle or endurance training. These conversions are summarized in figure 14.11.

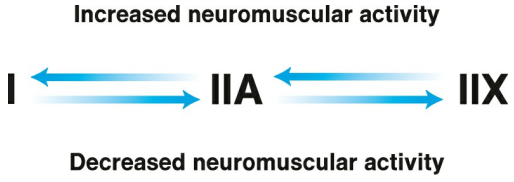


Figure 14.11 **Conversions of muscle fiber types and their causes.**

You may have noticed that I have mentioned only endurance training, not

other types of training, as a stimulus that elicits fiber type transitions. The reason is that fiber type transitions in response to other training types are either limited or undocumented. “Limited” here applies to resistance training, which elicits a transition from type IIX to IIA, as first mentioned in section 12.12. “Not documented” applies to sprint training and sprint interval training.

14.21 Effects of Environmental Factors on Choice of Energy Sources in Exercise

Research is rather limited on the effects of environmental factors, such as ambient temperature and hypoxia, on the proportion of energy sources during exercise. To begin with **ambient temperature**, there are findings (for example, by Mark Febbraio and collaborators) that, as compared with prolonged moderate-intensity exercise at neutral temperature (at around 20 °C), exercise in the heat (at around 40 °C) increases the proportion of carbohydrate to lipid oxidation. Studies on exercise in the cold (at around 3 °C) have shown this proportion to be either higher or lower, as compared with exercise at neutral temperature, as discussed by Dominique Gagnon and associates. On the other hand, there is evidence (for example, by Parkin and coworkers) that exercising in the heat or in the cold does not affect the choice of energy sources. In view of this controversy, one cannot draw a safe conclusion about whether or how ambient temperature affects the proportion of energy sources during exercise.

Hypoxia (that is, oxygen deficiency) occurs naturally in the whole body as a result of ascending to high altitude, because the atmospheric pressure and, hence, the oxygen concentration in the air decreases. Consequently, $\dot{V}O_2max$ also decreases with increasing altitude. These effects must be considered by researchers who wish to compare exercise metabolism between at high altitude and at sea level (characterized by **normoxia**). As in the case of comparing exercise metabolism before and after a training program (section 14.14), two choices exist. One is to compare exercises of the same absolute intensity (such as power at a cycle ergometer), in which case relative intensity (that is, percentage of $\dot{V}O_2max$) will be higher in hypoxia. The other

is to compare exercises of the same relative intensity, in which case absolute intensity will be lower in hypoxia.

The response of exercise metabolism to hypoxia seems to differ between sexes (as reviewed by Barry Braun) in a way reminiscent of women's higher reliance on lipids, as described in section 14.9. According to most studies in men, when moderate-intensity exercise is performed at the same absolute intensity at sea level and at an altitude of some thousand meters, the proportion of oxidized carbohydrates to lipids is higher at altitude. This is also the case at rest. When exercise is performed at the same *relative* intensity, the proportion of carbohydrates to lipids does not differ considerably between altitude and sea level. Thus, if hypoxia favors the use of an energy source in men, that source is carbohydrate.

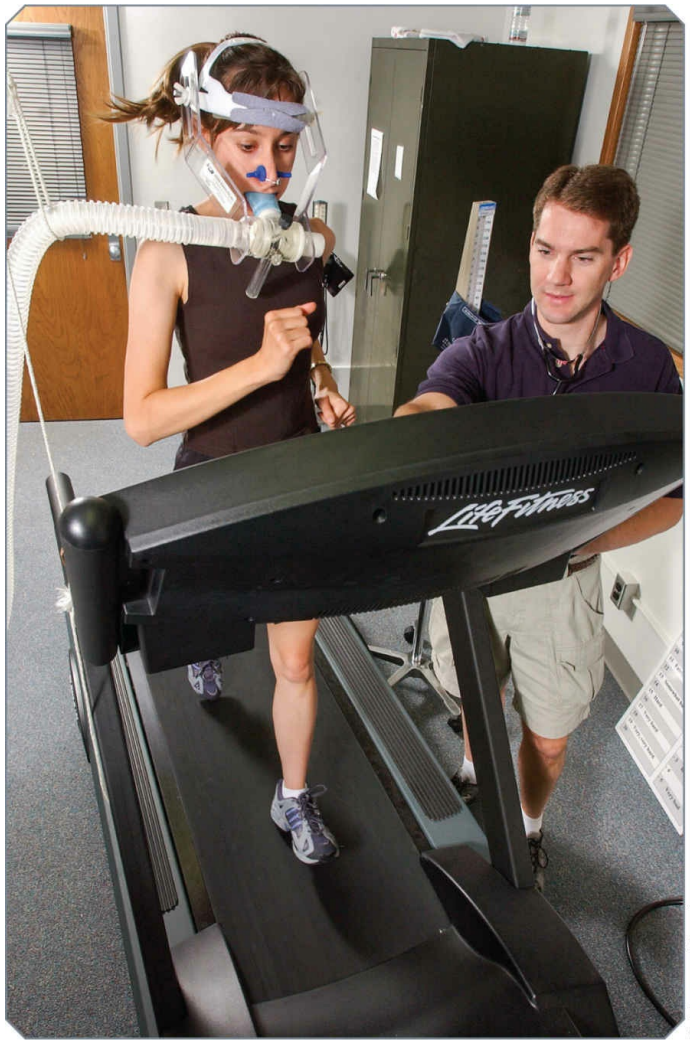


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Figure 14.12 **Respiratory gas analysis.** Wearing a mask that guides expired air through a volume sensor and gas analyzer while exercising allows the measurement of O_2 consumed and CO_2 produced. These measurements, in turn, allow assessment of the proportion of carbohydrates and lipids burned during exercise. Oxygen consumption alone is used to measure $\dot{V}O_2max$ (in a maximal exercise test) and estimate energy expenditure by multiplying the liters of O_2 consumed by about 5 kcal.

When women perform moderate-intensity exercise at high altitude, the proportion of oxidized carbohydrates to lipids is similar to that at the same absolute intensity at sea level. When exercise is performed at the same *relative* intensity, the proportion of carbohydrates to lipids is lower at high altitude. This is also the case at rest. Thus, if hypoxia favors the use of an energy source in women, that source is lipids. The reason is unclear for this difference between sexes in the effect of hypoxia on the choice of energy sources during exercise.

14.22 The Proportion of Fuels Can Be Measured Bloodlessly

We can assess the contribution of carbohydrates and lipids to the energy expenditure of the whole body during prolonged exercise in a bloodless and relatively easy manner through the **respiratory exchange ratio (RER)**. The RER is the ratio of the volume of expired CO_2 to the volume of O_2 consumed. We determine it by measuring the two gases in the inspired and expired air through a setup identical with the one used to determine $\dot{V}O_2max$ or, in general, oxygen uptake (figure 14.12). The ratio serves as an estimate of the **respiratory quotient (RQ)**, which is the ratio of the volume of CO_2 released to the volume of O_2 taken up at the cellular level.

Determining the proportion of fuels by measuring the RER is based on their different chemical composition. Specifically, carbohydrates have approximately one oxygen atom per carbon atom, whereas lipids are more reduced (hydrogenated) and contain only a few oxygens. As a result, carbohydrates need less O_2 than lipids per carbon atom and, hence, per molecule of CO_2 produced to be burned completely. You may convince yourself of this fact by examining reactions 10.16 and 11.19, the very ones I

invoked when discussing the ATP yield per O_2 in section 14.5. According to these reactions, the molar ratio—and volume ratio when it comes to gases—of CO_2 to O_2 is 6/6, or 1, for glucose and 16/23, or 0.7, for palmitate.

One mole of any gaseous element or compound occupies a standard volume at a given temperature and pressure.

Based on this difference, we can calculate the share of carbohydrates and lipids in the energy expenditure of exercise. An RER value close to 0.7 indicates a high share of lipids, whereas an RER value close to 1 indicates a high share of carbohydrates (figure 14.13). Protein oxidation can be ignored without seriously affecting the accuracy of the calculations, because it is minimal; alternatively, it can be determined though other techniques.

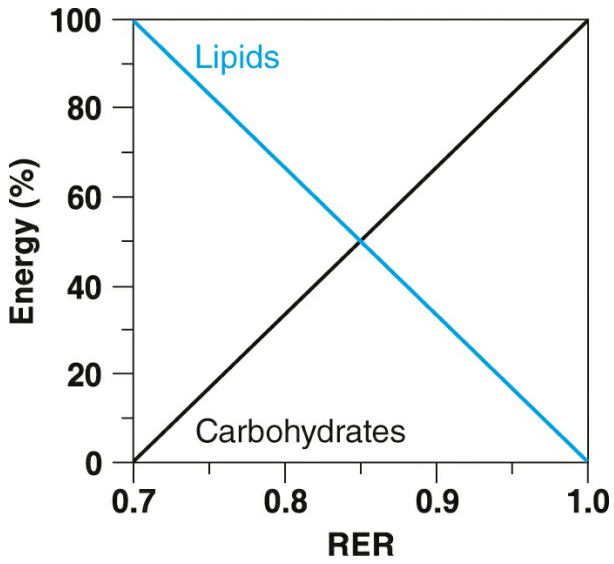

Figure 14.13 **A fuel breath test.** By measuring the RER, we can estimate the contribution of carbohydrates and lipids to energy expenditure: the higher the RER value, the higher the contribution of carbohydrates.

Can the RER obtain a value outside the 0.7 to 1 range? Surprisingly, although it cannot get below 0.7, it can get above 1, because near-maximal or maximal exercise augments the anaerobic carbohydrate breakdown, causing a rise in the plasma H^+ concentration, as described in section 10.23. This rise perturbs the bicarbonate system (section 3.12) and shifts reaction 3.3 to the

left, resulting in the release and expiration of additional CO_2, which in turn raises the RER above 1. Thus, an RER value above 1 indicates considerable contribution of the lactate system to the energy expenditure of exercise. The fact that part of the CO_2 measured does not stem from the oxidation of fuels means that one cannot calculate the proportion of fuels based on the RER during near-maximal or maximal exercise.

14.23 Hormonal Effects on Exercise Metabolism

Throughout part III, we have considered the important roles of hormones in controlling exercise metabolism. This section integrates that knowledge and completes the picture by presenting the effects of training on circulating hormone concentrations.

Epinephrine

Epinephrine mediates many of the effects of exercise on metabolism. Secretion of this catecholamine by the adrenal glands increases in response to neural signals that prepare the body to face danger or apply muscular effort. Epinephrine has been aptly named a **fight-or-flight hormone**, because it helps animals get food or avoid becoming food. Its secretion also increases when the plasma glucose concentration drops. Figure 14.14 shows the metabolic effects of epinephrine on its target tissues and organs.

In muscle, epinephrine speeds up glycogenolysis (section 10.6), thus elevating ATP resynthesis from carbohydrates. In the liver, epinephrine possibly speeds up glycogenolysis and gluconeogenesis (sections 10.28 and 10.26, respectively), thus augmenting glucose supply to blood and, from there, to muscle. In adipose tissue, epinephrine stimulates lipolysis (section 11.6), thus boosting fatty acid supply to blood and, from there, to muscle. In addition, the enhanced release of epinephrine and, more so, norepinephrine from sympathetic nerve endings in the pancreas stimulates glucagon secretion and inhibits insulin secretion. These changes boost glucose supply to blood, given the hyperglycemic action of glucagon and hypoglycemic action of

insulin.

Because of the profound and versatile effects of epinephrine and norepinephrine on metabolism and, in general, bodily functions, a wide variety of drugs have been developed that potentiate or mimic their actions. These drugs, termed **stimulants**, include amphetamine, cocaine, and ephedrine. WADA has included these substances under the namesake category and prohibits their use in sport.

The effects of training on the plasma epinephrine concentration have been reviewed by Hassane Zouhal and associates. In men, several (though not all) interventional studies have shown that endurance training mitigates the rise in epinephrine after exercise at a given intensity—absolute or relative, moderate or high. However, cross-sectional studies that compared endurance athletes and nonathletes have shown the opposite, that is, higher epinephrine in the athletes after exercise at a given relative—moderate or high—intensity. As in the case of a similar discrepancy between interventional and observational studies described in section 14.14, researchers attribute these conflicting findings to genetic differences between athletes and nonathletes, the higher training loads of athletes, or longer engagement by athletes in training. In women, there is no evidence for an effect of endurance training on the plasma epinephrine concentration.

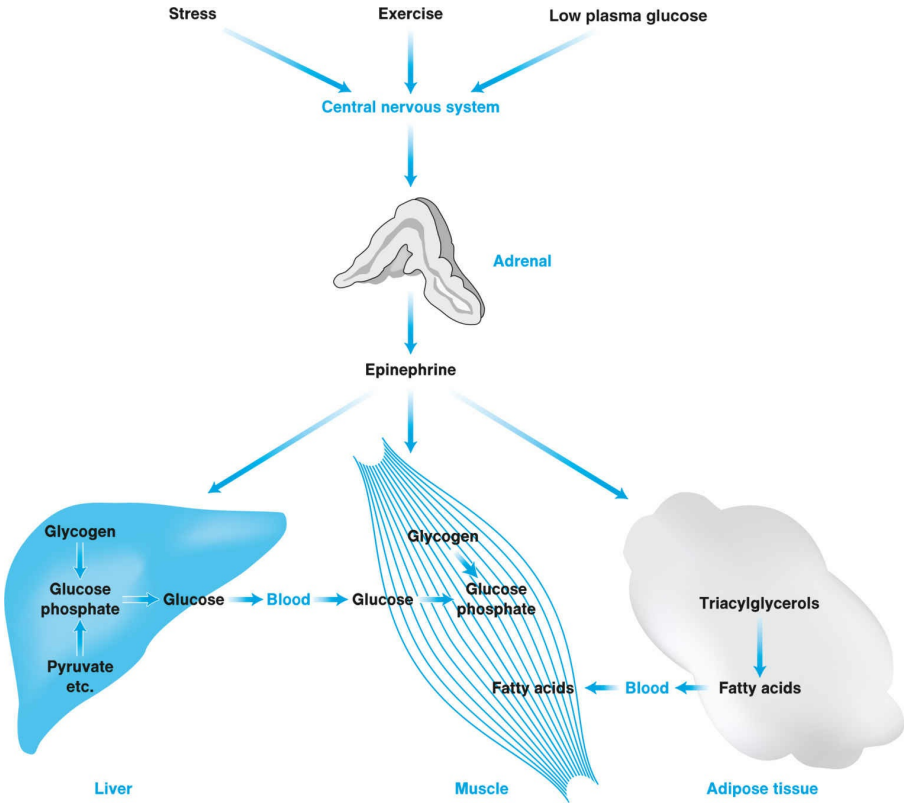

Figure 14.14 **A hormone for hard times.** Stress, exercise, and low plasma glucose act on the central nervous system to increase epinephrine secretion by the adrenal glands. Epinephrine stimulates glycogen breakdown into glucose 1-phosphate in muscle and, possibly during hard exercise, in the liver. Glucose 1-phosphate is used as an energy source in muscle. In contrast, in the liver, where epinephrine also possibly stimulates gluconeogenesis from pyruvate and other compounds, glucose 1-phosphate is converted into free glucose, which is exported. In adipose tissue, epinephrine stimulates lipolysis, driving fatty acids into the circulation.

Similar findings have been obtained with regard to resistance training and sprint training: Interventional studies have failed to show an effect of training, whereas cross-sectional studies have shown higher epinephrine responses to exercise at a given relative high intensity in male strength athletes or sprinters as compared with nonathletes. In women, again, there is no evidence for a training effect. Finally, there is no evidence for an effect of interval training on the plasma epinephrine concentration.

Glucagon

Glucagon acts solely in the liver (figure 14.15). This peptide hormone speeds

up glycogenolysis and gluconeogenesis while slowing down glycogenesis and glycolysis (sections 10.26 and 10.28). All of these effects raise the glucose concentration in the hepatocytes and then in blood. The rise in the plasma glucagon concentration in response to a drop in the plasma glucose concentration and to exercise contributes to glucose homeostasis. Endurance training mitigates the rise in glucagon caused by prolonged hard exercise.

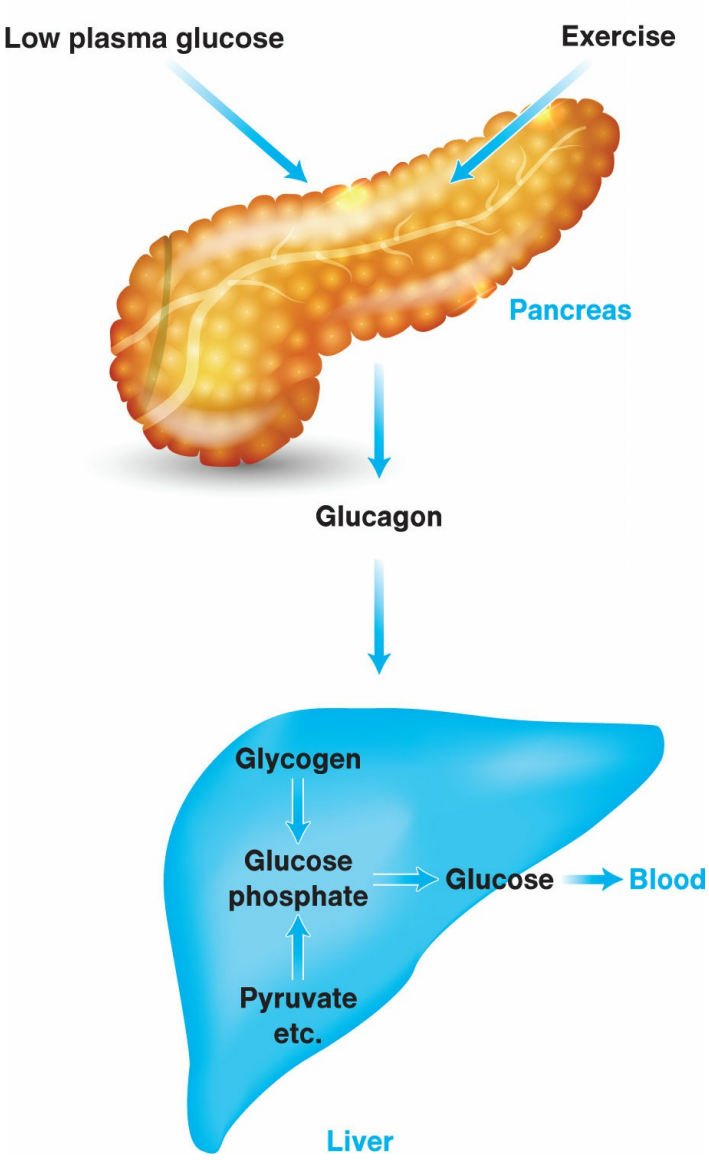

Figure 14.15 **A particularly picky hormone.** Glucagon has the liver as its sole target organ. In response to low blood glucose or exercise, the pancreas secretes more glucagon, which stimulates glycogenolysis and gluconeogenesis. The two processes cooperate to increase glucose production and raise blood glucose.

Pancreas © Guniita/Dreamstime.com

Insulin

Insulin, another peptide hormone, exerts opposite effects to those of epinephrine and glucagon. It signals the fed state by promoting carbohydrate storage, fat storage, and protein synthesis. Starting with carbohydrates, insulin facilitates the entry of glucose into muscle by enhancing the translocation of GLUT4 from intracellular vesicles to the plasma membrane (figure 10.20). As discussed in section 10.29, the hormone speeds up glycogenesis and slows down glycogenolysis in muscle and the liver. It also speeds up glycolysis and slows down gluconeogenesis in the liver. All of these effects converge in the disappearance of (free) glucose.

Insulin promotes triacylglycerol storage in adipose tissue, as explained in section 11.4. Finally, insulin promotes protein synthesis while inhibiting proteolysis. Promotion of protein synthesis is believed to pass through the indirect activation of mTORC1 by the protein kinase PKB/Akt, as mentioned in section 13.5 and shown in figure 13.7. Remember that PKB/Akt is activated by insulin through the PI3K cascade (section 10.29 and figure 10.48) and that mTORC1 promotes protein synthesis through the namesake pathway (section 13.5 and figure 13.4). How insulin inhibits proteolysis is unclear.

Because insulin promotes a positive protein balance and glycogenesis in muscle, athletes purportedly abuse it. WADA considers it a doping substance and prohibits it under the “hormone and metabolic mediators” category. Let me point out, however, that administration of insulin to healthy athletes has no documented beneficial effects on muscle mass, strength, or aerobic endurance. In any case, one should bear in mind the risk of developing severe acute hypoglycemia after an insulin injection, which can lead to coma.

As discussed in section 10.29, the plasma insulin concentration does not change in one direction during exercise; rather, it goes down when intensity is moderate and goes in either direction when intensity is high or maximal. Changes, if any, are usually small and do not seem to substantially affect metabolism during exercise. Trained persons experience even smaller perturbations in insulin with exercise. In contrast, exercise exerts a profound

effect on insulin action, a topic that I will address in section 15.7.

Other Hormones

The plasma concentration of several other hormones is influenced by exercise. However, these changes do not seem to affect exercise metabolism to any considerable degree. Here is a brief presentation of such hormones.

Growth hormone (first mentioned in section 13.5) is a peptide hormone synthesized and secreted by the anterior pituitary gland. It targets the liver, where it promotes the synthesis of **IGF1**, which, in turn, promotes the growth of muscle, bone, skin, and many internal organs. Through these actions, growth hormone plays a pivotal role in human development. Its plasma concentration exhibits spectacular increases with practically all types of exercise. The plasma concentration of IGF1 may increase or not change with exercise.

Of the steroid hormones listed in section 14.10, cortisol and testosterone will be dealt with in sections 18.6 and 18.7. **Aldosterone**, synthesized and secreted by the **adrenal cortex** (the outer layer of the adrenal glands), acts on the kidneys to promote Na^+ and water retention while increasing K^+ excretion in urine. Through these actions, aldosterone raises blood pressure. Exercise increases the plasma aldosterone concentration, as well as the plasma estradiol and progesterone concentrations.

Angiotensin is a peptide hormone produced in plasma from a precursor protein, **angiotensinogen**, which is synthesized and released to the circulation by the liver. Two proteases then act in sequence on angiotensinogen to produce active angiotensin, whose main action is to elicit vasoconstriction, resulting in a rise in blood pressure. In addition, angiotensin stimulates aldosterone production in the adrenal cortex, which also results in a rise in blood pressure. Finally, it promotes the release of **vasopressin**, or **antidiuretic hormone**, from the posterior pituitary gland. As its first name suggests, this is yet another hormone that raises blood pressure. It does so primarily by promoting water retention in the kidneys, as its second name suggests (it prevents diuresis). Exercise raises the plasma angiotensin concentration, and usually the plasma vasopressin concentration as well.

Two other hormones whose plasma concentrations increase with exercise

are **triiodothyronine** and **thyroxine** (for their biological role, refer to section 6.3 under the Iodine subheading). Finally, exercise increases **β-endorphin**, a peptide hormone produced in the central and peripheral nervous systems and involved in reducing bodily stress, suppressing pain, and bringing about euphoria.

For a detailed discussion of the effect of exercise on the endocrine system, refer to the review by Anthony Hackney and Amy Lane.

14.24 Redox State and Exercise Metabolism

In section 13.4, I cited changes in the **redox state** as a means by which exercise may modify gene expression. The rest of chapter 13 contained no other mention of the redox state, since the molecular mechanisms through which it influences gene expression are largely unknown. Nevertheless, exercise-induced perturbations in the redox state seem to play an important role in exercise metabolism, and the evidence for this role deserves to be presented.

As you should have realized by reading chapter 6 and chapters 9 through 12, **redox reactions** are widespread in biological systems and heavily involved in exercise metabolism. As a result, there are numerous **redox pairs** (apart from the NAD^+–NADH, $NADP^+$–NADPH, and FAD–$FADH_2$ pairs introduced in section 2.5), which contribute to the redox state and change with exercise. One example is pyruvate–lactate (see reaction 10.18). Nevertheless, one can single out a few pairs that serve as the major determinants of the redox state in a tissue or biological fluid. One such pair is formed by the reduced and oxidized forms of **glutathione**.

Reduced glutathione is a tripeptide of glutamate, cysteine, and glycine, with the unusual feature that the γ-carboxyl group of Glu is the one that is linked with Cys (figure 14.16) instead of the α-carboxyl group, which usually participates in peptide bond formation. The sulfhydryl group of Cys serves as the active group of the compound; hence, reduced glutathione is abbreviated as **GSH**. It acts as a **reducing agent**, or **antioxidant**, in reactions in which two of its molecules donate the hydrogen atoms of their sulfhydryl groups to a substrate. Thus, they become **oxidized glutathione**, or **glutathione**

disulfide, in which two glutathione residues are linked through a **disulfide bond** (section 3.5). Hence, oxidized glutathione is abbreviated as **GSSG**.

One of the reactions in which the GSH-GSSG redox pair participates is the elimination of a potentially harmful compound, **hydrogen peroxide** (H_2O_2). Before seeing how this is done, we need to make a detour. Hydrogen peroxide belongs to the family of **reactive**

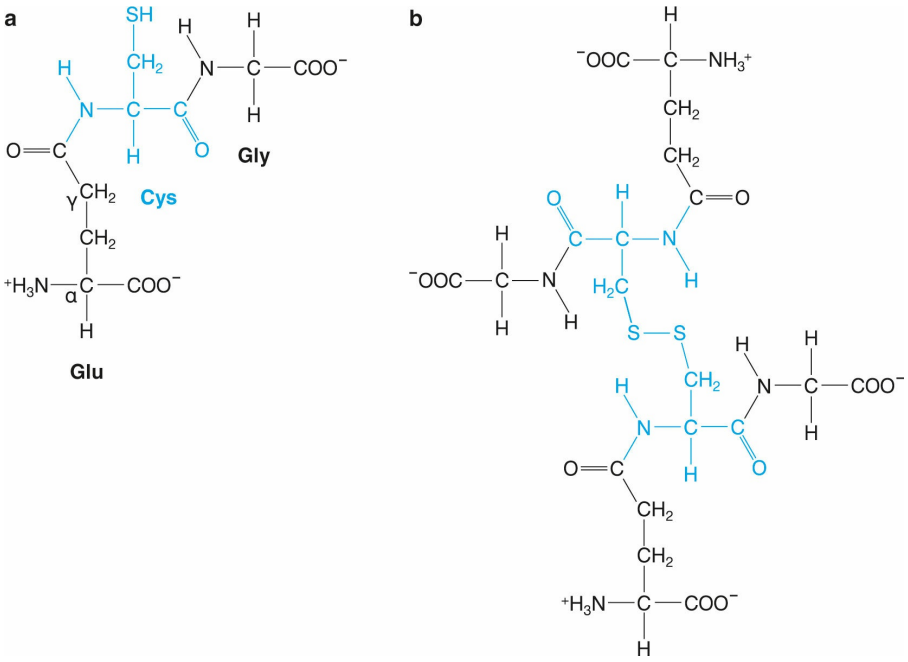

Figure 14.16 **Glutathione.** This important participant in redox reactions exists in two forms, one reduced and one oxidized. The reduced form (GSH, *a*) is a tripeptide with an unusual peptide bond, as described in the text. The oxidized form (GSSG, *b*) results when two GSH molecules shed the H of the sulfhydryl groups, letting their S atoms join in a disulfide bond.

oxygen and nitrogen species, or **RONS**, to which nitrogen oxide, the hydroxyl radical, and the superoxide radical (all introduced in section 1.5) also belong. As Michael Reid notes, these chemical entities are extremely fleeting and scarce in biological systems. Hydrogen peroxide forms in the process of eliminating the **superoxide** radical:

$$2\,O_2^{\bullet -} + 2\,H^+ \rightarrow H_2O_2 + O_2 \qquad \text{(equation 14.2)}$$

The reaction either takes place without a catalyst or is catalyzed by **superoxide dismutase (SOD)**, an important antioxidant enzyme. We met one form of it, residing in the mitochondrial matrix and abbreviated as MnSOD

(because it contains Mn^{2+} as cofactor), in figure 3.11 as an example of a protein with quaternary structure. Another form, residing in the cytosol, is abbreviated as CuZnSOD because it contains Cu^{2+} and Zn^{2+}.

The enzyme is so named because it catalyzes the **dismutation** of superoxide. Dismutation is a reaction in which a chemical entity is converted into a reduced and an oxidized product at the same time. Indeed, one $O_2^{\cdot -}$ in reaction 14.2 is reduced to H_2O_2, while another is oxidized to O_2. (Remember the definitions of *reduction* and *oxidation* in section 2.5.)

Hydrogen peroxide can be broken down in two ways. One is through the action of **catalase**, another important antioxidant enzyme, which catalyzes the reaction

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \qquad \Delta G^{\circ\prime} = -46.2\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 14.3)}$$

The name conveys no information about the substrates or the kind of reaction catalyzed by the enzyme; it just shows that it is a catalyst.

The other way brings us back to the GSH-GSSG pair, which participates in the reaction

$$2\ GSH + H_2O_2 \rightarrow\ GSSG + 2\ H_2O \qquad \Delta G^{\circ\prime} = -74.1\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 14.4)}$$

The reaction is catalyzed by **glutathione peroxidase**, a third important antioxidant enzyme, which is one of the selenoproteins mentioned in section 6.4 (under the Selenium subheading). Finally, **glutathione reductase** regenerates GSH at the expense of NADH or NADPH.

$$GSSG + NADH + H^+ \rightleftharpoons\ 2\ GSH + NAD^+ \qquad \Delta G^{\circ\prime} = -3.9\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 14.5)}$$

$$GSSG + NADPH + H^+ \rightleftharpoons\ 2\ GSH + NADP^+ \qquad \Delta G^{\circ\prime} = -4.2\ \text{kcal}\cdot\text{mol}^{-1} \qquad \text{(equation 14.6)}$$

In a healthy cell, a high concentration of GSH is always available for antioxidant protection. Other potent antioxidants include ascorbate (vitamin C), β-carotene (vitamin A), and α-tocopherol (vitamin E), presented in sections 6.1 and 6.2. These antioxidants are converted into their oxidized forms when used in redox reactions. Note that we humans cannot synthesize the three vitamins (although we can synthesize GSH); therefore, we need adequate intake of the vitamins for proper antioxidant protection.

Prolonged moderate-intensity or short hard exercise increases $O_2^{\cdot -}$ production in muscle as a result of increased hypoxanthine and xanthine oxidation (reactions 9.8 and 9.9). An additional source of $O_2^{\cdot -}$ is thought to be the reaction

$$NADPH + 2\ O_2 \rightleftharpoons NADP^+ + 2\ O_2^{\cdot -} + H^+ \qquad \text{(equation 14.7)}$$

The reaction is catalyzed by **NADPH oxidase**, an integral protein of the sarcolemma and transverse tubule membrane.

The increased production of $O_2^{\cdot -}$ and other RONS may perturb the balance between antioxidants and oxidants in favor of the latter, creating what has been termed **oxidative stress**. High levels of RONS decrease the force of contraction, thus leading to fatigue, a topic to which I will return in the next section.

In addition, RONS tend to oxidize DNA, proteins, and lipids. In DNA, guanine can be converted into 8-oxoguanine (figure 14.17*a*). In proteins, carbonyl groups can be attached to the side chains of amino acid residues (figure 14.17*b*). In lipids, fatty acids can be hydroxylated (figure 14.17*c*). Other modifications are also possible.

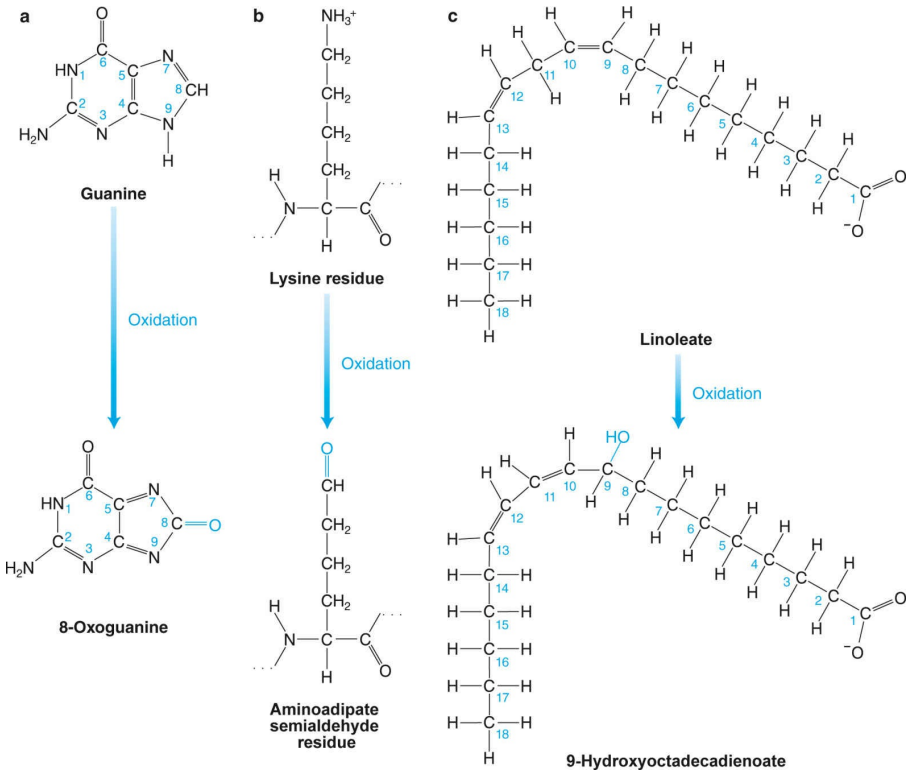

Figure 14. 17 Examples of oxidative damage to biomolecules. Oxidative stress can modify biological compounds, rendering them nonfunctional. *(a)* Guanine in DNA is converted into 8-oxoguanine. *(b)* A Lys residue in a protein is converted into an aminoadipate semialdehyde residue. *(c)* Linoleate is converted into 9-hydroxyoctadecadienoate.

Oxidized biomolecules may lose their biological activities, leading to impaired function or even diseases, such as atherosclerosis, asthma, and cancer. However, RONS are also important mediators in a variety of processes, including signal transduction, gene expression, immune function, cell proliferation, and enzyme activation. As far as exercise is concerned, evidence (reviewed by Scott Powers and colleagues) suggests that moderate RONS production during exercise plays an important role in the appearance of changes in muscle that are characteristic of adaptations to endurance training. These changes include the upregulation of PGC1α, a rise in antioxidant enzymes, and the increase in $\dot{V}O_2max$.

One way in which RONS may achieve these adaptations is by reversible oxidation of key cysteine residues to cystine (section 3.5 and figure 3.9) in signal proteins. This action changes the tertiary and quaternary structure of proteins and, hence, their biological activity. As an example of the role of RONS in adaptations to training, Nikos Margaritelis and associates found that men who experienced higher exercise-induced oxidative stress also exhibited larger adaptations to endurance training than did men who experienced lower exercise-induced oxidative stress.

For this reason, blunting RONS production through supplementation with antioxidant vitamins or other nutritional antioxidants has no ergogenic effect. In fact, chronic supplementation with such substances hampers adaptations to training and delays recovery of muscle function and muscle repair after exercise. Thus, it appears that low levels of RONS are needed for training-induced adaptations promoting performance and health. This view fits well with the principle of **hormesis** (meaning “spurt” in Greek), wherein a low dose of an agent may result in a beneficial adaptation but a high dose may exert a toxic effect.

14.25 Causes of Fatigue

In section 7.8, I cited Enoka's and Duchateau's definition of **fatigue** as a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability. Subsequent chapters contained sporadic references to fatigue. It is now time to address it in a comprehensive way.

Let's start by clarifying the two terms appearing at the end of the definition of fatigue. According to Enoka and Duchateau, performance fatigability refers to the decline in an objective measure of performance over a discrete period—for example, running speed during a race or peak power produced by a muscle during sustained activity. Performance fatigability depends on the contractile capability of the active muscles and the ability of the nervous system to provide an adequate activation signal.

Perceived fatigability, on the other hand, refers to changes in the sensations that regulate the integrity of the performer, such as the **rating of perceived exertion (RPE)**, introduced in section III.3. This factor, in turn, depends on the initial value and the rate of change in sensations that regulate the integrity of the exerciser on the basis of the maintenance of his or her homeostasis and psychological state.

A corollary of the modern, holistic definition of fatigue is that the traditional division between central and peripheral fatigue becomes difficult, if not impossible, to maintain. Specifically, it is not feasible to separate the decline in muscle force (considered peripheral fatigue) from sensations related to fatigue (considered central fatigue), especially during prolonged exercise. Thus, it is preferable to discuss the changes that may be responsible for the emergence of fatigue rather than the location of the changes.

What, then, are these changes? Several—such as changes in arousal, expectations, mood, and motivation, all within the domain of perceived fatigability—belong to the realm of psychology and, hence, fall outside the scope of this book. Thus, I will limit the discussion to changes that are related to exercise biochemistry.

The causes of fatigue that I will outline do not each apply to every exercise. Instead, they depend on factors, such as the ones listed in section 14.4, that influence the choice of energy sources during exercise—that is, exercise parameters, characteristics of the exerciser, and environmental

factors. Table 14.6 summarizes the most probable causes

Table 14.6 Probable Causes of Fatigue During Exercise Depending on Dominant Energy System

ATP–phosphocreatine system	Lactate system	Oxygen system
• Decreased motor unit firing rate • Phosphocreatine depletion	• Decreased motor unit firing rate • Decreased muscle excitation • P_i and H^+ accumulation	• Hypoglycemia • Dehydration • Hyperthermia • Decreased muscle excitation • Glycogen depletion • P_i and H^+ accumulation • RONS accumulation

of fatigue depending on the dominant energy system; it is meant to guide you through the discussion that follows.

Hypoglycemia

A probable cause of perceived fatigability is the **hypoglycemia** that appears during moderate-intensity or hard exercise tasks lasting more than an hour. Hypoglycemia results from the depletion of liver glycogen and the absence or insufficiency of carbohydrate intake during exercise. Glucose is the almost-exclusive fuel of neurons under normal conditions; thus, hypoglycemia compromises brain function and leads to fatigue. Hypoglycemia is often experienced by marathon runners. The condition is reversed quickly with oral or intravenous glucose administration.

Dehydration and Hyperthermia

Two of the changes that contribute to perceived fatigability are **dehydration** (loss of body water) and **hyperthermia** (high body temperature). They usually go hand in hand when one exercises for prolonged periods in the heat without ingesting adequate amounts of water or other fluid. Exercise at a high ambient temperature causes excessive sweating, which tends to remove water

from blood, the extracellular space, and the cytoplasm.

If water intake during exercise does not compensate for the loss, body temperature rises, because there is not enough water to dissipate the heat produced. Hyperthermia compromises mental functions (including information processing, cognition, and memory), which may lead to fatigue. Hyperthermia and dehydration may also impair muscle function. Both can be prevented via consumption of water, sport drinks, or similar fluids during exercise in quantities dictated by the sensation of thirst or slightly more.

Decreased Motor Unit Firing Rate

During prolonged (particularly, maximal isometric) exercise tasks, electromyography often detects a decrease in **motor unit firing rate** (section 7.8), which can lead to loss of force and, hence, fatigue. This decrease may result from several factors, which have been summarized by Janet Taylor and associates. One factor is that repetitive activation (that is, repeated firing) of motor neurons makes them less excitable; why this happens remains unknown. Another factor is that sensory neurons known as **muscle afferents**, which transmit feedback signals from the muscles to the central nervous system, modify their firing rates in response to mechanical and chemical changes generated within the active muscles, resulting in inhibition of what is termed *central drive* to motor neurons. These changes, and possibly others, inside the spinal cord and motor cortex contribute to both perceived and performance fatigability.

Decreased Muscle Excitation

The event that triggers contraction at the muscular level is the appearance of action potentials in the sarcolemma. In hardworking muscles, a large increase occurs in the extracellular [K^+] because of the multitude of action potentials generated and the inability of the muscle fibers to rapidly restore the [K^+] gradient across the sarcolemma through the Na^+–K^+ pump (section 7.2 and figure 7.5). This inability leads to a decrease in the excitability of the sarcolemma and may become a cause of fatigue.

Indeed, when the frequency of nervous signals and power output are high

—such as during resistance or sprint efforts of maximal intensity lasting up to about one half minute—there is a decline in the amplitude and frequency of the muscle action potentials, which coincides with the emergence of fatigue. One can expect this decline to compromise the coupling of excitation to contraction, since it is the action potentials that trigger the liberation of Ca^{2+} from the sarcoplasmic reticulum to the cytosol.

A dietary practice that can delay perceived fatigability, in part by maintaining muscle excitation, is **caffeine** consumption. Decades of research and hundreds of publications on its effects have shown that caffeine is ergogenic in a wide variety of exercise tasks, ranging in duration from just 45 s to several hours, as reviewed by Peter Christensen and colleagues. The effective dose usually ranges from 3 to 6 mg per kilogram of body mass, taken one hour before exercise if exercise lasts up to one hour, or divided into hourly portions if exercise is longer.

Caffeine is believed to maintain muscle excitation by facilitating Ca^{2+} release from the sarcoplasmic reticulum through the ryanodine receptor (section 8.11; see also problem 7 in chapter 8). However, caffeine's ergogenic effect passes through additional modulating factors of fatigue, including psychological ones. Indeed, caffeine increases arousal and decreases the perception of effort, and a very frequent finding is lower RPE during exercise after caffeine intake. Researchers attribute these actions to caffeine's antagonizing the binding of **adenosine** to its receptors in the brain. Adenosine, a ribonucleoside introduced in section 4.9, acts in this case as a neurotransmitter and is believed to suppress arousal and promote sleep. By antagonizing adenosine, caffeine reverses these actions.

Depletion of Energy Sources

One obvious candidate for causing fatigue is lack of sources for ATP resynthesis, specifically phosphocreatine and glycogen. Phosphocreatine depletion may cause fatigue in brief maximal resistance or sprint efforts, and glycogen depletion may do so in endurance exercise tasks of moderate or high intensity lasting at least 1 h. Indeed, there is strong correlation between force reduction and phosphocreatine reduction during hard exercise. In addition, the smaller the glycogen stores in the body and, particularly, in

muscle, the earlier fatigue sets in during moderate-intensity exercise lasting over 1 h. Phosphocreatine depletion may be partly remedied by creatine supplementation, as I will discuss in the next section. Glycogen depletion may be remedied by excess carbohydrate intake, as already discussed in section 14.12.

Accumulation of Metabolic Products

One cause of fatigue (specifically, performance fatigability) during hard exercise is thought to be the accumulation of metabolic products in the sarcoplasm—products that hamper force production by the contractile machinery. These products include P_i, emanating from ATP breakdown, and H^+, emanating primarily from the anaerobic breakdown of glycogen into lactate. Edward Debold and collaborators have reviewed the evidence for a fatiguing effect of P_i and H^+ from studies with skinned single muscle fibers (that is, fibers in which the sarcolemma has been mechanically peeled or chemically permeabilized to facilitate experimentation with various solute concentrations around the myofibrils), isolated contractile proteins, and single cross-bridges.

Experiments on skinned fibers have demonstrated that high [P_i] and [H^+] can reduce maximal isometric force, shortening velocity, and power. The two ions seem to target myosin, since, in experiments with isolated proteins, they directly inhibit the ability of myosin to move relative to F-actin. Finally, experiments with single cross-bridges have revealed that, at high concentrations, P_i decreases the force-generating capacity of myosin by causing its premature detachment from F-actin after the power stroke, thus reducing the duration of myosin's strong binding to F-actin. Precisely, according to the mechanism described in section 8.7, high [P_i] hastens the transition from step *c* to step *d* in figure 8.9. This fatiguing effect of P_i is even higher at high [H^+] (pH 6.2), possibly because high [H^+] shifts reaction 9.6 to the right, thus increasing the concentration of $H_2PO_4^-$, the dihydrogen form of phosphate. The concentration of this form correlates with the decline in maximal force during in vivo fatigue more closely than the concentrations of either monohydrogen phosphate (HPO_4^{2-}) or H^+ do separately.

P_i and H^+ affect the binding of myosin to F-actin in yet another way: They

cooperate in inhibiting the binding of Ca^{2+} to troponin C, thus hampering the conformational changes in the troponin complex that allow the movement of tropomyosin away from the binding sites of myosin on F-actin (section 8.10 and figure 8.12). This detrimental effect of P_i and H^+ becomes more impactful as the rise in the cytosolic Ca^{2+} concentration upon excitation becomes smaller and smaller during fatigue. Researchers have blamed P_i for this too: They have proposed that P_i enters the sarcoplasmic reticulum, where it combines with Ca^{2+} to form calcium phosphate, an insoluble salt. The formation of salt lowers the $[Ca^{2+}]$ in the sarcoplasmic reticulum and decreases the concentration gradient across its membrane. This, in turn, decreases the rate of Ca^{2+} influx to the cytosol when the Ca^{2+} channel opens, resulting in a lower rise in the cytosolic $[Ca^{2+}]$ when the muscle is excited.

Thus, although counterarguments have been presented (for example by Håkan Westerblad), it appears that P_i and H^+ possess the ability to elicit performance fatigability and become major fatigue-inducing agents during hard exercise. Future studies are eagerly awaited to either confirm or refute this possibility in humans through in vivo fatigue paradigms. Until then, one can rely on some indirect, yet strong, evidence for a role of acidosis in the etiology of fatigue in humans during whole-body exercise.

The evidence in question comes from the ergogenic effect of sodium bicarbonate, or sodium hydrogen carbonate ($NaHCO_3$), or common baking soda. Remember that bicarbonate (HCO_3^-) accepts a proton (reverse equation 3.3) and buffers blood pH. As Christensen and colleagues conclude in a recent meta-analysis, most studies on the subject have shown that performance is improved by ingestion of 0.3 g of $NaHCO_3$ per kilogram of body mass, a practice dubbed **(sodium) bicarbonate loading**, or **soda loading**, typically one to three hours before maximal exercise tasks lasting from approximately 45 s to 8 min.

Such tasks cause massive H^+ production through the anaerobic breakdown of carbohydrates. The plasma $[HCO_3^-]$ and pH increase after ingesting $NaHCO_3$ and, although the bicarbonate anion does not seem to enter the muscle fibers, it nonetheless neutralizes the H^+ exiting the active muscles. It is believed that, by doing so, bicarbonate creates a steeper $[H^+]$ gradient across the sarcolemma, which facilitates H^+ efflux (figure 10.35) and

postpones fatigue. Bicarbonate may simultaneously increase performance *and* muscle lactate, as, for example, David Bishop and collaborators have found. This research adds to the evidence that lactate production is not a cause of fatigue, as discussed in section 10.20.

RONS accumulation during exercise has emerged as an additional cause of fatigue in recent years. Some studies have shown that oral or intravenous administration of ***N*-acetylcysteine**, an antioxidant drug, before or during exercise delays fatigue in a variety of experimental settings, including whole-body exercise in humans. The findings of these studies show that high levels of RONS in muscle decrease the force of contraction. However, Kate Rhodes and Andrea Braakhuis, in their meta-analysis, found that *N*-acetylcysteine supplementation produces a range of performance effects from beneficial to trivial to harmful and concluded that its effect remained unclear. The extent to which *N*-acetylcysteine causes side effects is also unclear. Thus, until the issue is resolved by new studies, the best protection against RONS-induced fatigue seems to be endurance training, which (among other beneficial effects) increases the muscle content of antioxidant enzymes, as discussed in the preceding section.

14.26 Recovery of the Energy State After Exercise

When exercise—whether a training session or competition—ends, it is desirable to bring the body back to the pre-exercise energy state as soon as possible in anticipation of a new exercise task. The time needed to restore the energy state is generally longer than the duration of exercise, because the replenishment of energy sources is slower than their consumption. Depending on the kind of exercise, the sources that need replenishment may include

- muscle ATP,
- muscle phosphocreatine,
- muscle glycogen,
- liver glycogen, and
- myocellular triacylglycerols.

The need to recover as soon as possible after exercise in preparation for the next exercise is aptly expressed by the quotation *"Nach dem Spiel ist vor dem Spiel" ("after the game is before the game")* by Sepp Herberger, a 20th-century German soccer player and coach.

Adipose tissue triacylglycerols are of no concern, both because they are barely affected by an exercise bout (see problems 9 and 11 in chapter 11) and because many exercisers aim at reducing rather than replenishing them.

ATP Replenishment

Muscle ATP is resynthesized in the mitochondria from ADP through oxidative phosphorylation, which is fueled primarily by the combustion of carbohydrates and lipids. The portion of ADP that may have been lost to AMP through the adenylate kinase reaction (reaction 9.3) is replenished by reversal of the reaction, because ample ATP is now available.

It is also possible to resynthesize muscle AMP from IMP (the product of AMP deamination), though not by reversal of reaction 9.7. Rather, two other reactions, linked by **adenylsuccinate** (figure 14.18) and spending one ~P, are needed for AMP resynthesis.

$$\text{IMP} + \text{asparate} + \text{GTP} \rightleftharpoons \text{adenylsuccinate} + \text{GDP} + P_i + 2\ H^+ \qquad \Delta G^{\circ\prime} = 2.7\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 14.8)}$$

$$\text{Adenylsuccinate} \rightleftharpoons \text{AMP} + \text{fumarate} \qquad \Delta G^{\circ\prime} = 2.4\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 14.9)}$$

Although reaction 14.8 has a positive $\Delta G^{\circ\prime}$, its ΔG becomes negative (and the reaction is shifted to the right) during recovery because of the ample supply of GTP, which is resynthesized from GDP with the aid of ATP by a reversal of reaction 10.11. The ample production of adenylsuccinate then makes the ΔG of reaction 14.9 negative as well and shifts it to the right. Remember that ΔG can be quite different from $\Delta G^{\circ\prime}$, depending on the actual concentrations of reactants and products (section 2.2).

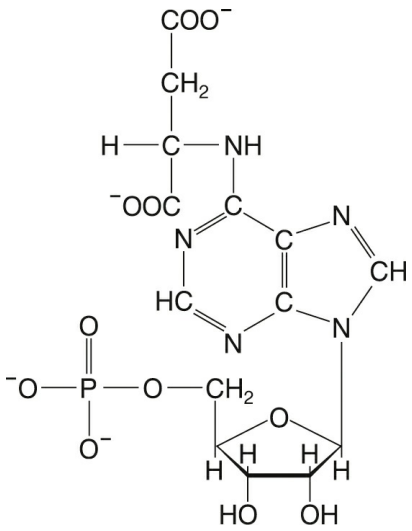

Figure 14.18 Adenylsuccinate. Adenylsuccinate is an intermediate compound in the synthesis of AMP from IMP.

Reactions 9.7, 14.8, and 14.9 are often referred to as the **purine nucleotide cycle**, because they concern the recycling of AMP and IMP, both of which are nucleotides carrying purine bases (section 4.3). However, the three reactions are accelerated at different phases, or energy states, of a muscle fiber: the first one during exercise and the latter two during recovery. In contrast, other metabolic cycles, such as the citric acid cycle, are accelerated in a concerted manner.

Phosphocreatine Replenishment

Phosphocreatine is replenished through the phosphorylation of creatine by ATP in a reversal of reaction 9.4.

$$\text{Creatine} + \text{ATP} \rightleftharpoons \text{phosphocreatine} + \text{ADP} + \text{H}^+ \qquad \Delta G^{\circ\prime} = 3\ \text{kcal} \cdot \text{mol}^{-1} \qquad \text{(equation 14.10)}$$

Again, although the reaction has a positive $\Delta G^{\circ\prime}$, its ΔG becomes negative (and the reaction is shifted to the right) during recovery because of the ample supply of ATP.

Reaction 14.10 is catalyzed by an isoform of creatine kinase that differs from the ones we met in section 9.3. The isoform in question, **mitochondrial CK**, is symbolized as **mit-CK**. Unlike CK1, CK2, and CK3, which are in the cytosol, mit-CK lies on the outer surface of the inner mitochondrial membrane. Thus, it seems to be strategically located to ensure the immediate

resynthesis of phosphocreatine by the ATP that exits the mitochondria (figure 14.19).

The time needed to resynthesize phosphocreatine depends in part on how much has been broken down. It also depends on the blood flow and oxygen delivery to the muscles that were exercised, since the ATP used in equation 14.10 derives from oxidative phosphorylation. Thus, phosphocreatine resynthesis is slow when the blood flow is constricted and fast when exercise is followed by active recovery, which maintains increased blood flow to the muscles. Evidence also suggests that the rate of phosphocreatine resynthesis relates to $\dot{V}O_2max$. This finding is in line with the dependence of the rate of phosphocreatine resynthesis on aerobic processes. If most of phosphocreatine has been depleted during exercise, the time for resynthesis is at best 3 min, and it may exceed 10 min in the worst case (figure 14.20).

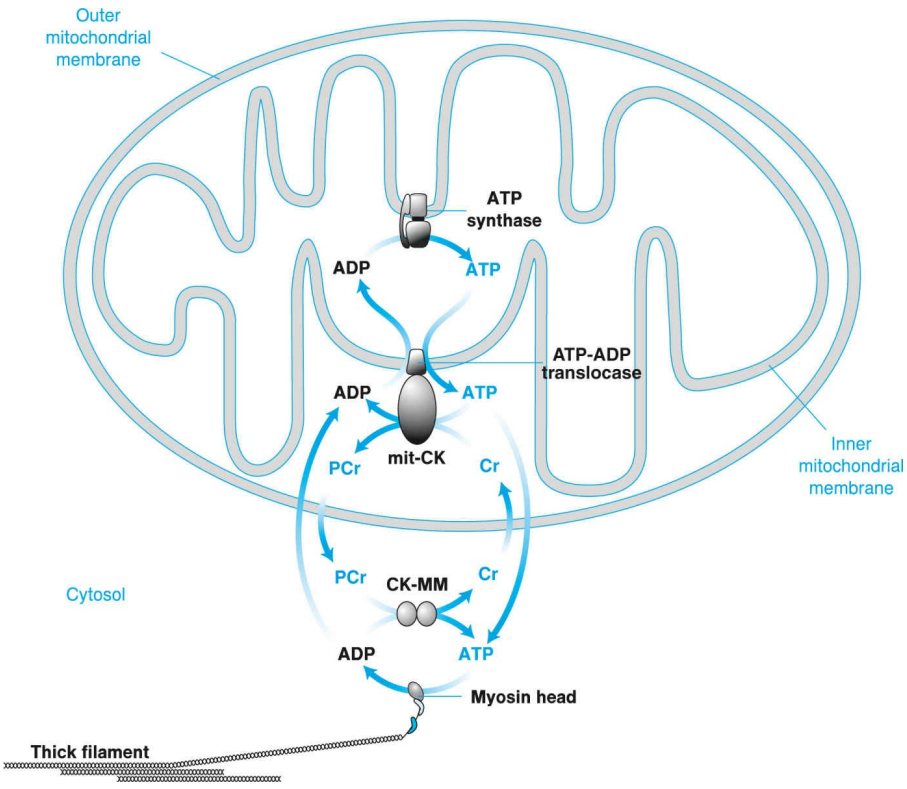

Figure 14.19 **ATP relay.** ATP synthesized in muscle mitochondria by ATP synthase exits to the intermembrane space through the ATP–ADP translocase (section 10.15 and figure 10.29) in exchange for the entry of ADP. Mitochondrial creatine kinase (mit-CK), located on the outer surface of the inner mitochondrial membrane, uses this ATP to regenerate phosphocreatine (PCr) from creatine (Cr). Phosphocreatine is then used during maximal exercise by the cytosolic creatine kinase (CK-MM, the muscle isoform) to give back ATP needed for the

movement of the myosin heads. Of course, ATP exiting the mitochondria can be used directly by myosin (long outer downward arrow); it can also be used directly or indirectly (that is, passing through phosphocreatine) by other ATPases (not shown).

Phosphocreatine resynthesis and other aspects of phosphocreatine action can benefit from creatine supplementation, as found in many original studies and several meta-analyses. In particular, two recent meta-analyses by Charlotte Lanhers and coworkers have concluded that creatine supplementation increases lower- and upper-limb strength performance in exercises lasting less than 3 min, independent of population characteristics and training protocols. In addition, a previous meta-analysis by J. David Branch found significant increases of lean body mass and performance in maximal exercise tasks lasting up to 30 s (such as laboratory strength tests, jumps, running, swimming, and cycling) with creatine supplementation. The ergogenic action of creatine weakened (though it was not abolished) in longer-lasting tasks.

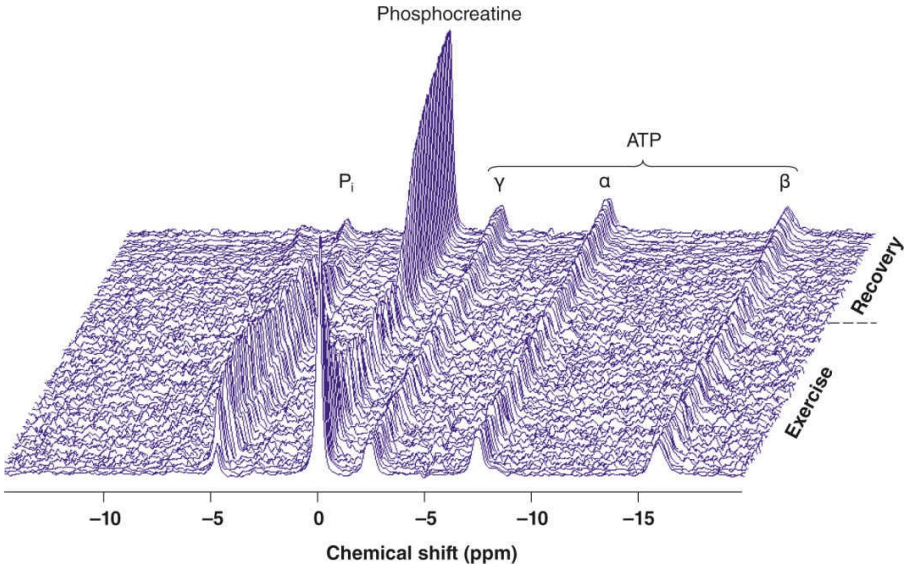

Figure 14.20 **Phosphocreatine breakdown and resynthesis.** Phosphocreatine is rapidly broken down in human tibialis anterior muscle during a series of maximal isometric contractions and rapidly resynthesized during recovery, as seen in this collection of ^{31}P NMR spectra like the one in figure 9.7. Researchers obtained one spectrum every 4 s in a so-called interleaved mode, which allows the stacking of many spectra one behind another. Notice that ATP was barely affected by the contractile activity.

Courtesy of Anne Tonson and Jane Kent.

Creatine supplementation augments the muscle creatine and phosphocreatine contents. Higher phosphocreatine may mean more ATP resynthesis during maximal exercise, and higher creatine may mean more phosphocreatine resynthesis during the rest or low-intensity intervals between repeated bouts. These two effects largely explain the ergogenic effect of creatine supplementation.

A recommended dosage of creatine is 0.3 g per kilogram of body mass per day for three to five days, followed by either repetition of this scheme every three to four weeks (with no creatine supplementation in between) or a maintenance dosage of 2 to 5 g per day. Such high amounts of creatine are rather impossible to obtain through the regular diet (which provides just about 1 g of creatine daily, as mentioned in section 9.3). Thus, one must resort to supplements, which usually take the form of creatine monohydrate (that is, crystalline creatine with water in equal molar amounts). When followed by carbohydrate intake, creatine supplementation results in even higher creatine and phosphocreatine increases in muscle.

Glycogen Replenishment

Replenishment of the muscle and liver glycogen stores after exercise requires the presence of sufficient raw material, that is, glucose. This sufficiency can be achieved only through ample carbohydrate intake. Cessation of the exercise stimulus lowers epinephrine and glucagon secretion. As the plasma concentrations of the two hormones return to baseline, their molecules dissociate from the β-adrenergic and glucagon receptors, and the receptors are deactivated. Then $G_{s\alpha}$ (figure 10.14) is deactivated, as it possesses a low intrinsic **GTPase** activity; that is, it slowly hydrolyzes GTP to GDP (figure 14.21).

As in the case of neurotransmitters (section 7.5), the binding of a hormone to its receptor is reversible.

As a result, adenylate cyclase is also deactivated, and cAMP synthesis slows down. The existing cAMP is hydrolyzed to AMP by a phosphodiesterase, like the one presented in section 11.6, and the entire

cAMP cascade is restrained. This restraint stops the activation of phosphorylase (figures 10.17 and 10.18), resulting in the slowing down of glycogenolysis. If we consume sufficient carbohydrates after exercise, insulin will be secreted and will promote glycogenesis (section 10.29 and figure 10.48), especially since exercise has already augmented muscle insulin sensitivity (see section 15.7).

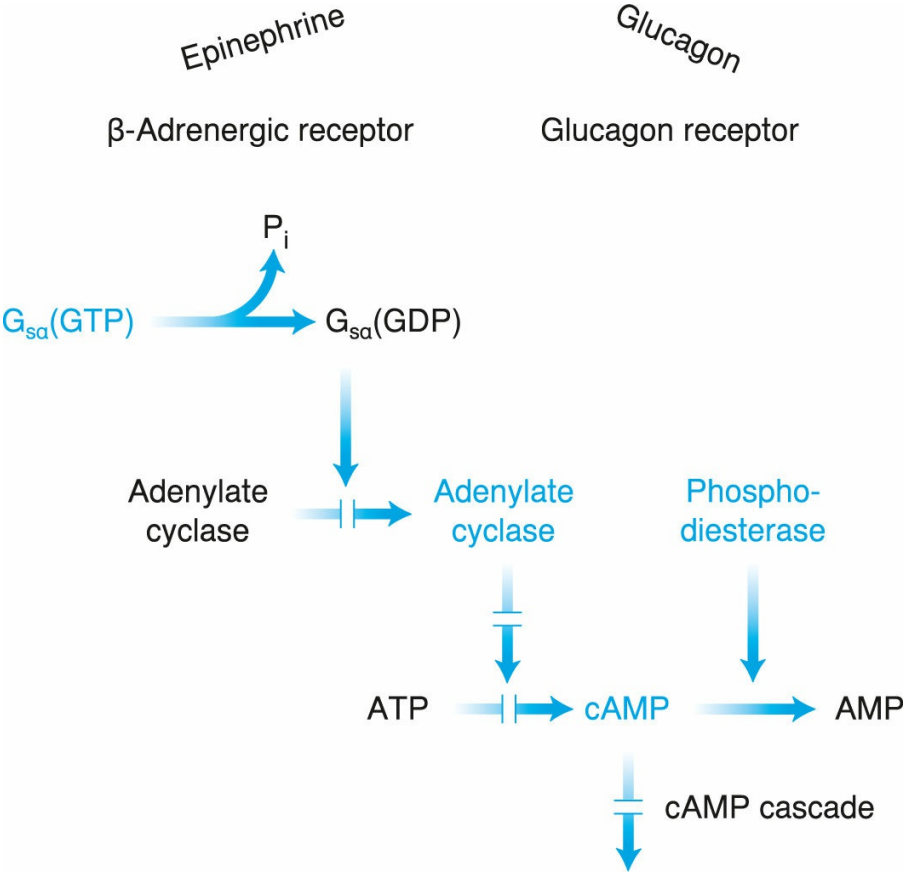

Figure 14.21 **Cyclic AMP cascade arrest.** When exercise stops, epinephrine and glucagon decrease in plasma. The two hormones detach from their receptors, and the receptors are deactivated. $G_{s\alpha}$ hydrolyzes its bound GTP to GDP, resulting in adenylate cyclase deactivation. Cyclic AMP synthesis slows down, and cAMP degradation by phosphodiesterase prevails, thus blocking the cAMP cascade.

Myocellular Triacylglycerol Replenishment

Myocellular triacylglycerols are replenished after exercise primarily from incoming fatty acids. Since there is no longer a need for increased fatty acid oxidation, most of the fatty acids entering muscle fibers are used to restore

the intracellular triacylglycerol pool. Fatty acids enter the muscle fibers from three main sources: the intestine, the liver, and adipose tissue (figure 14.22). Dietary triacylglycerols (packaged in chylomicrons) and hepatic triacylglycerols (packaged in VLDL) are hydrolyzed by muscle lipoprotein lipase (sections 11.1 and 11.19). The fatty acids produced are then taken up by the muscle fibers. Finally, the muscle fibers receive fatty acids deriving from lipolysis in the adipocytes.

EPOC

This is another example of the fifth principle of exercise metabolism presented in section III.2: Metabolism does not return to the characteristics of the resting state immediately after the end of exercise.

While recovering from exercise, we continue for some time to breathe at a rate higher than the regular resting rate. The difference between the amount of oxygen consumed during recovery and that normally consumed at rest is termed **excess postexercise oxygen consumption (EPOC)**, or **oxygen debt**. We do not know with certainty what leads to EPOC, but probable causes include the following:

- Extra oxygen may be used to reload the hemoglobin and myoglobin molecules that offered their bound oxygen for aerobic energy production during the beginning of exercise, before oxygen uptake went from baseline to the exercise level.
- Extra oxygen may oxidize fuels (mainly fatty acids) in the muscles to restore the ATP concentration and supply additional ATP for phosphocreatine replenishment. Additional ATP would also be needed in both the muscles and liver to replenish glycogen, either from ingested carbohydrates or from gluconeogenic precursors, notably lactate.
- Plasma catecholamine concentrations and body temperature rise during exercise and, naturally, do not return to resting levels immediately when exercise stops. Both have a positive effect on oxygen uptake.

14.27 Metabolic Changes in Detraining

Unlike diamonds, adaptations to training are not forever. Rather, maintaining them requires the regular application of a substantial training stimulus. If the training load drops considerably or, worse, ceases, the adaptations described in sections 14.14 through 14.17 are reversed. Athletes and exercisers often go through both voluntary and involuntary interruptions of training (for example, during the summer period, during injury or illness, and, of course, when they retire from competitive sport). It is therefore important to know how such changes affect metabolism. Unfortunately, research on changes in the body with detraining lags research on changes with training.

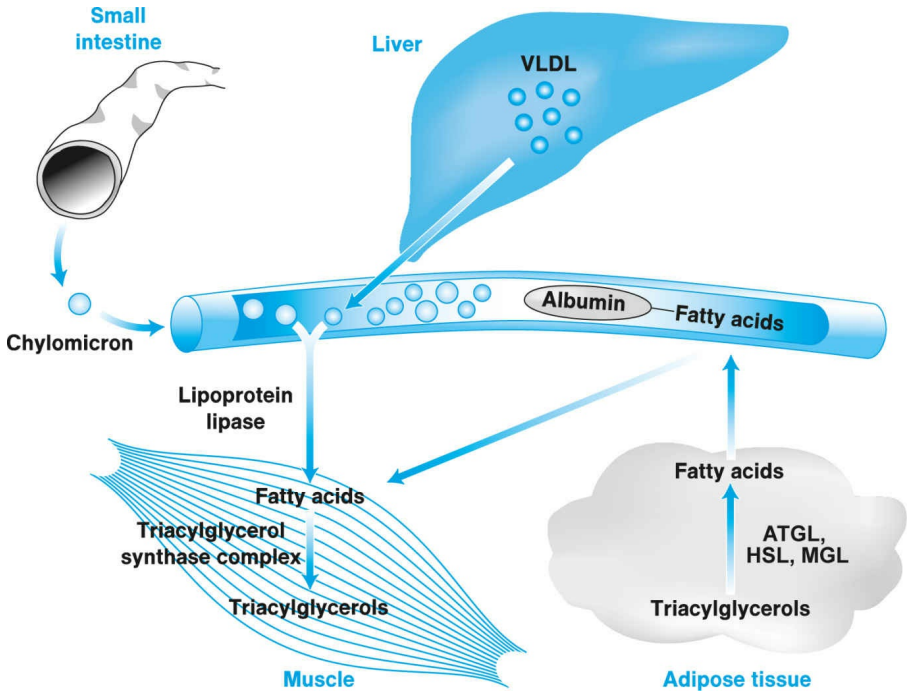

Figure 14.22 Replenishing myocellular triacylglycerols. Fatty acids from the small intestine (through hydrolysis of dietary triacylglycerols in chylomicrons by lipoprotein lipase), the liver (through hydrolysis of its triacylglycerols in VLDL by lipoprotein lipase), and adipose tissue (through hydrolysis of its triacylglycerols by ATGL, HSL, and MGL) enter muscle to restore its triacylglycerol reservoir after exercise.

The interruption of endurance training increases the proportion of carbohydrates to lipids that the body burns during exercise at a given absolute intensity. (Again, we must distinguish absolute from relative intensity,

because detraining is accompanied by a decrease in $\dot{V}O_2$max.) This increase is due to a decrease in muscle mitochondrial content, resulting in lower concentrations of the enzymes of the citric acid cycle and β oxidation. It also results from a decline in capillary density and lipoprotein lipase in the muscles. In contrast, lipoprotein lipase in adipose tissue increases, favoring fat deposition. Concomitant with these biochemical and morphological changes, there is a drop in $\dot{V}O_2$max and aerobic endurance performance. Changes are noticeable just a few weeks after training interruption.

Stopping endurance training also lowers muscle glycogen, probably due to a decline in muscle insulin sensitivity, which, in turn, probably results from a decline in the GLUT4 content of the muscles. These changes are faster than those described in the preceding paragraph: They take only a few days to appear. Finally, as we saw in section 14.19, stopping endurance training shifts the muscle fiber profile from type I to type IIX. This change is slower than those described in the previous paragraphs, taking months of inactivity to appear.

As for resistance training, the most characteristic consequence of discontinuing it is the reduction in muscle cross-sectional area and maximal strength within a few weeks. Additionally, the transition from type IIX to IIA muscle fibers that accompanies resistance training (mentioned in sections 12.12 and 14.19) is reversed with detraining. Stopping sprint training lowers enzymes of the lactate system in muscle and sprint performance. Finally, it is unclear how the discontinuation of interval training affects the adaptations it has induced, although evidence from Cesare Granata and coworkers suggests rapid reversal of muscle mitochondrial adaptations with a mere reduction in training volume.

The material covered in this section is summarized in the box titled Changes in Humans With Detraining.

Changes in Humans With Detraining

Interruption of Endurance Training

- Decrease in $\dot{V}O_2$max

- Increase in the proportion of carbohydrates to lipids used during prolonged exercise at a given absolute intensity
- Decrease in muscle mitochondrial content and, hence, mitochondrial enzymes
- Decrease in resting muscle glycogen
- Decrease in muscle capillary density
- Decrease in muscle lipoprotein lipase
- Increase in adipose tissue lipoprotein lipase
- I → IIA → IIX
- Decrease in aerobic endurance performance

Interruption of Resistance Training

- Decrease in muscle cross-sectional area
- IIA → IIX
- Decrease in maximal strength

Interruption of Sprint Training

- Decrease in muscle phosphofructokinase and lactate dehydrogenase
- Decrease in sprint performance

Several of the adaptation losses with the interruption of endurance training can be prevented or mitigated by maintaining a low training stimulus, as suggested by Iñigo Mujika and Sabino Padilla in two joint reviews. These losses include the decrease in $\dot{V}O_2max$, the increase in the carbohydrate-to-lipid ratio during exercise, the decrease in oxidative enzymes, the decrease in aerobic endurance performance, the decrease in GLUT4, and the decrease in insulin sensitivity. Similarly consoling is the situation with resistance training: Maintenance of a low training stimulus can prevent or mitigate the decline in muscle cross-sectional area and strength.

How much can one lower the training load without experiencing losses of

adaptations? According to different studies, the decrease may be as much as 60% to 90%. One needs to understand, however, that the larger the decrease in training load, the shorter the time for which the adaptations are preserved. Specifically, training frequency should not be curtailed by more than 30% for elite athletes (for example, ten training sessions per week should not be decreased to fewer than seven). Still, it is possible for athletes to maintain their competitive level under conditions not favorable for full training, provided that they have the necessary willpower.

Summary

The numerous acute and chronic effects of exercise on metabolism allow a better response of the body to the demands of increased contractile activity. The primary demand, a high energy supply, is satisfied by the cooperation of three energy systems: ATP–phosphocreatine, lactate, and oxygen. The systems rank in that order in terms of increasing energy and in the reverse order in terms of power. At least two of the energy systems participate in most exercise tasks, although one system usually stands out as dominant. The selection of energy sources during exercise is determined primarily by exercise parameters (such as intensity and duration) and characteristics of the exerciser (such as sex, age, nutritional state, training state, and the genome). Exercise intensity and duration affect the proportion of energy sources in opposite ways (figure 14.23).

As compared with men, eumenorrheic women oxidize more lipids and fewer carbohydrates and proteins during exercise at the same relative intensity thanks to their higher plasma estradiol concentration. Likewise, children use relatively more lipids than adults do in prolonged exercise at the same absolute or relative intensity because of a lower capacity to catabolize carbohydrates.

The nutritional state affects the proportion of energy sources during exercise by increasing the contribution of carbohydrates

or lipids, depending on which are consumed in high quantities. The training state affects the proportion of energy sources thanks to training-induced adaptations. More spectacular are the adaptations to endurance training, which boost the oxidative capacity of the muscles and their capacity to catabolize lipids while dampening their capacity to catabolize carbohydrates during prolonged exercise at a given absolute intensity. Interval training elicits similar adaptations to moderate-intensity continuous training with less exercise and less total time per training session. The genome affects the selection of energy sources during exercise mainly by determining the proportion of muscle fiber types. A high proportion of type I, or oxidative, fibers favors aerobic function, whereas a high proportion of type IIA, or oxidative–glycolytic, and, particularly, type IIX, or glycolytic, fibers favors anaerobic function. The proportion of muscle fiber types is primarily determined genetically, but it can change, slightly and slowly, if the contractile activity changes chronically.

Exercise metabolism is affected primarily by two hormones: epinephrine and glucagon. Other hormones play minor, if any, roles in exercise, despite playing pivotal roles in metabolism in general and despite their concentrations being affected by exercise. Exercise perturbs the redox state in favor of the oxidants, which contributes to training-induced adaptations. Fatigue, manifested as both performance fatigability and perceived fatigability, results from a variety of causes, depending on exercise parameters, characteristics of the exerciser, and environmental factors, which can be remedied in a variety of ways. After exercise, the body restores its energy sources through the uptake of excess oxygen, the aerobic resynthesis of ATP, and the consumption of food or dietary supplements. Cessation of training results in the slow loss of some adaptations and the rapid loss of others. However, several adaptations can be preserved through the maintenance of a moderate training stimulus.

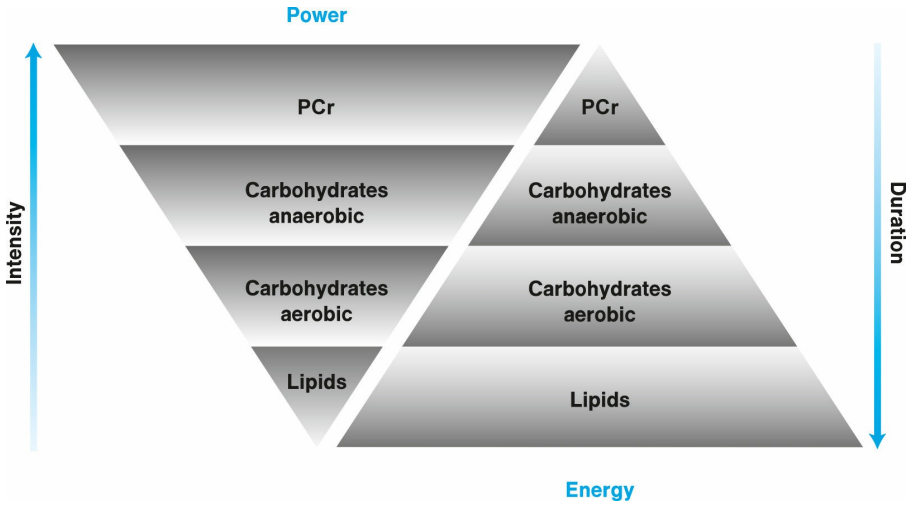

Figure 14.23 **Choice of energy sources depending on the two main exercise parameters**. As exercise intensity increases, so does the contribution of the sources producing high power (primarily phosphocreatine, PCr). As exercise duration increases, so does the contribution of the sources producing a lot of energy (primarily lipids).

Problems and Critical Thinking Questions

1. Based on figure 14.3, deduce what percentage of the energy at the 50th second of a maximal exercise bout originates from each energy system.
2. Based on figure 14.5, deduce what percentage of the total energy fueling a 50 s maximal exercise bout originates from each energy system. Compare with your answer to the previous problem.
3. Although the 100 and 200 m sprints are run at almost the same speed and, hence, the same intensity (on the basis of the information listed in table 14.3), the contribution of the ATP–phosphocreatine system to total energy differs considerably between the events. Propose an explanation for this difference.
4. Will the RER go up or down
    a. during prolonged exercise at constant intensity?
    b. as exercise intensity increases?
    c. when you exercise after carbohydrate loading, as

compared with when you eat a normal diet?

d. when you exercise after fat loading, as compared with when you eat a normal diet?

e. when you exercise after an aerobic training program, at the same absolute intensity as before training?

5. Based on the information presented in sections 14.9 and 14.10, would you expect the RER to be the same or different during prolonged exercise between

   a. a man and a eumenorrheic woman exercising at 60% of each one's $\dot{V}O_2$max (that is, at the same relative intensity)?

   b. a man and a eumenorrheic woman who have the $\dot{V}O_2$max values given in section 14.9 and who exercise at 60% of the man's $\dot{V}O_2$max (that is, at the same absolute intensity)?

   c. a boy and a girl (before the onset of puberty) exercising at the same relative intensity?

   d. a woman taking oral contraceptives and a woman not taking oral contraceptives, who exercise at the same relative intensity?

   In all cases, assume that other characteristics that could affect the RER (such as age, training state, and nutritional state) are similar in each pair.

6. Fill the following table with probable causes of fatigue during a marathon race, as well as ways to remedy them.

Cause of fatigue	Remedy

7. (Integrative problem) Suppose that, by applying a creatine supplementation protocol, an athlete managed to increase

his muscle phosphocreatine and creatine contents by 25% over those given in problem 4 in chapter 9. Suggest probable concentrations of ATP, phosphocreatine, creatine, and P_i after 3 s of maximal exercise under the same assumptions as in that problem.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

ATP–phosphocreatine system
lactate system
oxygen system, aerobic system
Fat_{max}
maximal fat oxidation, MFO
carnitine acetyltransferase
interval training
testosterone
estradiol
progesterone
steroid hormone
glucocorticoid
mineralocorticoid
androgen
estrogen
progestogen, progestagen
estrogen receptor
luteal phase
follicular phase
carbohydrate loading
fat loading

low-carbohydrate high-fat diet
ketogenic diet
medium-chain triacylglycerol, MCT
long-chain triacylglycerol, LCT
endurance training
cardiorespiratory fitness
capillary density
resistance training
sprint training
moderate-intensity continuous training, MICT
high-intensity interval training, HIIT
sprint interval training, SIT
concurrent training
interference effect, concurrent effect
type I fiber, oxidative fiber
type IIX fiber, glycolytic fiber
type IIA fiber, oxidative–glycolytic fiber
ambient temperature
hypoxia
normoxia
respiratory exchange ratio, RER
respiratory quotient, RQ
epinephrine
fight-or-flight hormone
stimulant
glucagon
insulin
growth hormone
IGF1
aldosterone

adrenal cortex
angiotensin
angiotensinogen
vasopressin, antidiuretic hormone
triiodothyronine
thyroxine
β-endorphin
redox state
redox reaction
redox pair
glutathione
reduced glutathione, GSH
reducing agent, antioxidant
oxidized glutathione, glutathione disulfide, GSSG
disulfide bond
hydrogen peroxide
reactive oxygen and nitrogen species, RONS
superoxide
superoxide dismutase, SOD
dismutation
catalase
glutathione peroxidase
glutathione reductase
NADPH oxidase
oxidative stress
hormesis
fatigue
rating of perceived exertion, RPE
hypoglycemia
dehydration

hyperthermia
motor unit firing rate
muscle afferent
caffeine
adenosine
(sodium) bicarbonate loading, soda loading
N-acetylcysteine
adenylsuccinate
purine nucleotide cycle
mitochondrial CK, mit-CK
GTPase
excess postexercise oxygen consumption, EPOC
oxygen debt

References and Suggested Readings

Alsted TJ, Nybo L, Schweiger M, Fledelius C, Jacobsen P, Zimmermann R, Zechner R, Kiens B (2009). Adipose triglyceride lipase in human skeletal muscle is upregulated by exercise training. *American Journal of Physiology Endocrinology and Metabolism* 296: E445-E453.

American College of Sports Medicine (2011). Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Medicine and Science in Sports and Exercise* 43: 1334-1359.

Armstrong N, Barker AR, McManus AM (2015). Muscle metabolism changes with age and maturation: How do they relate to youth sport performance? *British Journal of Sports Medicine* 49: 860-864.

Bishop D, Edge J, Davis C, Goodman C (2004). Induced metabolic alkalosis affects muscle metabolism and repeated-

sprint ability. *Medicine and Science in Sports and Exercise* 36: 807-813.

Branch JD (2003). Effect of creatine supplementation on body composition and performance: A meta-analysis. *International Journal of Sport Nutrition and Exercise Metabolism* 13: 198-226.

Braun B (2008). Effects of high altitude on substrate use and metabolic economy: Cause and effect? *Medicine and Science in Sports and Exercise* 40: 1495-1500.

Burke LM, Ross ML, Garvican-Lewis LA, Welvaert M, Heikura IA, Forbes SG, Mirtschin JG, Cato LE, Strobel N, Sharma AP, Hawley JA (2017). Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *Journal of Physiology* 595: 2785-2807.

Christensen PM, Shirai Y, Ritz C, Nordsborg NB (2017). Caffeine and bicarbonate for speed. A meta-analysis of legal supplements potential for improving intense endurance exercise performance. *Frontiers in Physiology* 8: 240.

Coffey VG, Hawley JA (2017). Concurrent exercise training: Do opposites distract? *Journal of Physiology* 595: 2883-2896.

Debold EP, Fitts RH, Sundberg CW, Nosek TM (2016). Muscle fatigue from the perspective of a single crossbridge. *Medicine and Science in Sports and Exercise* 48: 2270-2280.

Deschenes MR, Kraemer WJ (2002). Performance and physiologic adaptations to resistance training. *American Journal of Physical Medicine and Rehabilitation* 81: S3-S16.

Devries MC (2016). Sex-based differences in endurance exercise muscle metabolism: Impact on exercise and nutritional strategies to optimize health and performance in women. *Experimental Physiology* 101: 243-249.

Enoka RM, Duchateau J (2016). Translating fatigue to human performance. *Medicine and Science in Sports and Exercise*

48: 2228-2238.

Febbraio MA, Snow RJ, Stathis CG, Hargreaves M, Carey MF (1994). Effect of heat stress on muscle energy metabolism during exercise. *Journal of Applied Physiology* 77: 2827-2831.

Fyfe JJ, Bishop DJ, Bartlett JD, Hanson ED, Anderson MJ, Garnham AP, Stepto NK (2018). Enhanced skeletal muscle ribosome biogenesis, yet attenuated mTORC1 and ribosome biogenesis-related signalling, following short-term concurrent versus single-mode resistance training. *Scientific Reports* 8: 560.

Gagnon DD, Rintamäki H, Gagnon SS, Cheung SS, Herzig KH, Porvari K, Kyröläinen H (2013). Cold exposure enhances fat utilization but not non-esterified fatty acids, glycerol or catecholamines availability during submaximal walking and running. *Frontiers in Physiology* 4: 99.

Gastin PB (2001). Energy system interaction and relative contribution during maximal exercise. *Sports Medicine* 31: 725-741.

Gibala MJ, Hawley JA (2017). Sprinting toward fitness. *Cell Metabolism* 25: 988-990.

Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJ (2016). Liver glycogen metabolism during and after prolonged endurance-type exercise. *American Journal of Physiology Endocrinology and Metabolism* 311: E543-E553.

Granata C, Oliveira RS, Little JP, Renner K, Bishop DJ (2016). Mitochondrial adaptations to high-volume exercise training are rapidly reversed after a reduction in training volume in human skeletal muscle. *FASEB Journal* 30: 3413–3423.

Hackney AC, Lane AR (2015). Exercise and the regulation of endocrine hormones. *Progress in Molecular Biology and Translational Science* 135: 293-311.

Jeukendrup A (2014). A step towards personalized sports nutrition: Carbohydrate intake during exercise. *Sports*

Medicine 44: S25-S33.

Lanhers C, Pereira B, Naughton G, Trousselard M, Lesage FX, Dutheil F (2015). Creatine supplementation and lower limb strength performance: A systematic review and meta-analyses. *Sports Medicine* 45: 1285-1294.

Lanhers C, Pereira B, Naughton G, Trousselard M, Lesage FX, Dutheil F (2017). Creatine supplementation and upper limb strength performance: A systematic review and meta-analysis. *Sports Medicine* 47: 163-173.

Leblanc PJ, Howarth KR, Gibala MJ, Heigenhauser GJ (2004). Effects of 7 wk of endurance training on human skeletal muscle metabolism during submaximal exercise. *Journal of Applied Physiology* 97: 2148-2153.

Lundsgaard AM, Fritzen AM, Kiens B (2018). Molecular regulation of fatty acid oxidation in skeletal muscle during aerobic exercise. *Trends in Endocrinology and Metabolism* 29: 18-30.

Margaritelis NV, Theodorou AA, Paschalis V, Veskoukis AS, Dipla K, Zafeiridis A, Panayiotou G, Vrabas IS, Kyparos A, Nikolaidis MG (2018). Adaptations to endurance training depend on exercise-induced oxidative stress: Exploiting redox interindividual variability. *Acta Physiologica* 222: e12972.

Montero D, Lundby C (2017). Refuting the myth of non-response to exercise training: “Non-responders” do respond to higher dose of training. *Journal of Physiology* 595: 3377-3387.

Mujika I, Padilla S (2000). Detraining: Loss of training-induced physiological and performance adaptations. Part I: Short-term insufficient training stimulus. *Sports Medicine* 30: 79-87.

Mujika I, Padilla S (2000). Detraining: Loss of training-induced physiological and performance adaptations. Part II: Long-term insufficient training stimulus. *Sports Medicine* 30: 145-154.

Murach KA, Bagley JR (2016). Skeletal muscle hypertrophy with concurrent exercise training: Contrary evidence for an

interference effect. *Sports Medicine* 46: 1029-1039.

Murlasits Z, Kneffel Z, Thalib L (2018). The physiological effects of concurrent strength and endurance training sequence: A systematic review and meta-analysis. *Journal of Sports Sciences* 36: 1212-1219.

Nakagawa Y, Hattori M (2017). Intramyocellular lipids of muscle type in athletes of different sport disciplines. *Open Access Journal of Sports Medicine* 8:161-166.

Parkin JM, Carey MF, Zhao S, Febbraio MA (1999). Effect of ambient temperature on human skeletal muscle metabolism during fatiguing submaximal exercise. *Journal of Applied Physiology* 86: 902-908.

Powers SK, Radak Z, Ji LL (2016). Exercise-induced oxidative stress: Past, present and future. *Journal of Physiology* 594: 5081-5092.

Reid MB (2016). Reactive oxygen species as agents of fatigue. *Medicine and Science in Sports and Exercise* 48: 2239-2246.

Rhodes K, Braakhuis A (2017). Performance and side effects of supplementation with *N*-acetylcysteine: A systematic review and meta-analysis. *Sports Medicine* 47: 1619-1636.

Richter EA, Hargreaves M (2013). Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiological Reviews* 93: 993-1017.

Riddell MC (2008). The endocrine response and substrate utilization during exercise in children and adolescents. *Journal of Applied Physiology* 105: 725-733.

Ross A, Leveritt M (2001). Long-term metabolic and skeletal muscle adaptations to short-sprint training: Implications for sprint training and tapering. *Sports Medicine* 31: 1063-1082.

Taylor JL, Amann M, Duchateau J, Meeusen R, Rice CL (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Medicine and Science in Sports and*

Exercise 48: 2294-2306.

Tsiloulis T, Watt MJ (2015). Exercise and the regulation of adipose tissue metabolism. *Progress in Molecular Biology and Translational Science* 135: 175-201.

Vollaard NB, Metcalfe RS (2017). Research into the health benefits of sprint interval training should focus on protocols with fewer and shorter sprints. *Sports Medicine* 47: 2443-2451.

Westerblad H (2016). Acidosis is not a significant cause of skeletal muscle fatigue. *Medicine and Science in Sports and Exercise* 48: 2339-2342.

Zouhal H, Jacob C, Delamarche P, Gratas-Delamarche A (2008). Catecholamines and the effects of exercise, training and gender. *Sports Medicine* 38: 401-423.

CHAPTER 15

Exercise to Fight Disease

Learning Objectives

After reading this chapter, you should be able to do the following:

- Define health and provide some basic statistics about death rates from noncommunicable diseases worldwide.
- Explain how regular exercise can benefit the cardiovascular system and reduce the risk of CVD.
- Discuss the evidence for the anti-cancer effects of regular exercise and describe possible mechanisms for these effects.
- Explain how diabetes wreaks havoc in metabolism and discuss the ways by which exercise fights diabetes.
- Discuss how regular exercise helps us lose weight and describe where the weight we lose goes.
- Explain how exercise fights the health consequences of obesity.
- Describe how exercise can prevent or fight osteoporosis.
- Discuss how exercise improves brain structure and function.
- Explain how physical inactivity is detrimental to health.
- Describe how regular exercise promotes healthy aging and longevity.

In previous chapters, we have considered the numerous effects of exercise on the metabolism of mostly healthy individuals. However, not all people enjoy the benefits of healthy living. Indeed, a considerable percentage of the human population suffers from one or more chronic diseases, which hamper daily activities, harm emotional status, lower the quality of life, and cause premature death. This chapter presents information about how appropriate exercise prescriptions can mobilize biochemical mechanisms in the body to combat the detrimental (even lethal) effects of a multitude of diseases and states that predispose one to disease, including cardiovascular disease (CVD), cancer, insulin resistance, diabetes, obesity, metabolic syndrome, osteoporosis, mental disease, inactivity, and aging.

15.1 Health, Disease, and Exercise

The World Health Organization (WHO), in its constitution, defines **health** as not merely the absence of disease or infirmity but a state of complete physical, mental, and social well-being. Of the many human diseases, exercise fights primarily the noncommunicable ones, that is, those that cannot be transmitted from one person to another through contact. Noncommunicable diseases constitute a major cause of sickness and death worldwide. WHO, in its world health statistics report, estimates that 19% of the global human population (more than 7.3 billion people) will die from one of the four major noncommunicable diseases—CVD, cancer, chronic respiratory disease, and diabetes—between the ages of 30 and 70. That's about 1.4 billion people. WHO also estimates that, in 2015, 40 million deaths occurred due to noncommunicable diseases, accounting for 70% of the overall total of 56 million deaths. CVD claimed 17.7 million lives, cancer 8.8 million, chronic respiratory disease 3.9 million, and diabetes 1.6 million.

Two terms used in epidemiological studies of health and disease are **incidence** and **prevalence**. Incidence is the rate of occurrence of new cases of a disease within a certain period. Prevalence, on the other hand, is the proportion of cases of a disease in a population at a given point in time. In simpler terms, incidence shows the risk of contracting a disease, whereas prevalence shows how widespread a disease is.

On the basis of numerous observational and interventional studies, regular exercise is increasingly recognized as a powerful intervention against getting sick and dying from chronic diseases. As an example, Hannah Arem and associates pooled data from six studies, which had monitored a total of 661,137 adults of both sexes over an average of 14 years, and correlated physical activity during their leisure time with mortality. The authors concluded that even individuals performing less than the recommended minimum of physical activity presented in section 14.14—that is, 150 min of moderate-intensity exercise, or 75 min of vigorous exercise, or a combination of the two per week—had a 20% lower mortality risk than did individuals not exercising at all. The benefit was higher for those who met or exceeded the recommended minimum (31% to 39% lower mortality risk). These findings refer to mortality from all causes, but the authors found similar relationships for mortality due specifically to CVD or cancer.

The technical terms used in the scientific literature for contracting and succumbing to a disease are, respectively, **morbidity** and **mortality**.

15.2 Exercise to Fight Cardiovascular Disease

Cardiovascular disease (CVD) is a broad term encompassing various conditions—such as coronary artery disease, stroke, angina, myocardial infarction (or heart attack), heart failure, and heart arrhythmia—that involve problems of the heart and the vasculature (that is, the blood vessels). Major causes of CVD include **atherosclerosis** and **hypertension**.

Atherosclerosis consists in the presence of **atherosclerotic plaques**, that is, deposits of lipids and other components inside the walls of the blood vessels (figure 15.1). Atherosclerotic plaques narrow the vessels and diminish blood flow through them, resulting in inadequate supply of oxygen and nutrients to vital organs such as the brain and heart. A major complication of atherosclerosis is **thrombosis**, that is, the formation of a blood clot inside a blood vessel, at the site where an atherosclerotic plaque has ruptured. The clot occludes the blood flow and may cause irreparable damage in the form of

stroke or myocardial infarction. The development of atherosclerotic plaques is favored by high plasma concentrations of triacylglycerols, total cholesterol, and LDL cholesterol and, conversely, a low plasma concentration of HDL cholesterol (section 11.19). Such an abnormal lipidemic profile is often referred to as **dyslipidemia**.

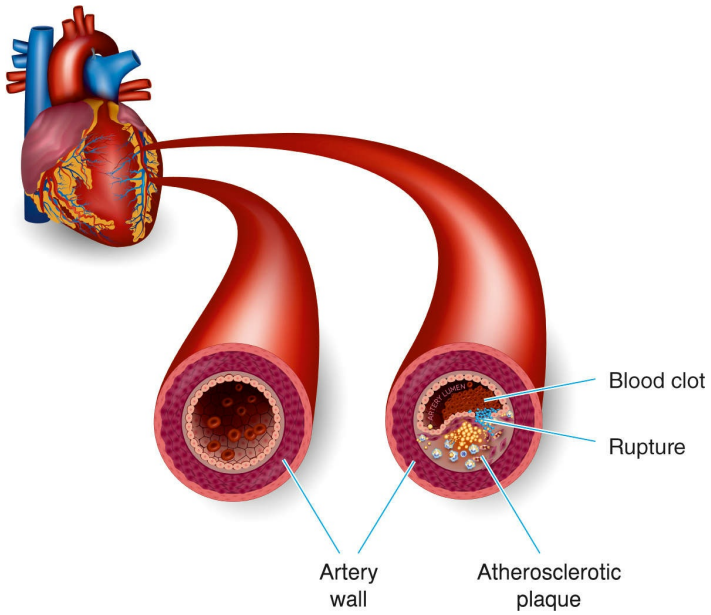

Figure 15.1 **Atherosclerosis.** Comparison of a normal artery *(left)* and an obstructed artery *(right)* in the heart reveals what atherosclerosis does: Most of the artery interior (or artery lumen) is blocked by an atherosclerotic plaque, which has ruptured. A blood clot has formed at the rupture, leading to thrombosis.

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Hypertension, on the other hand, is a persistent elevation of the blood pressure in the arteries. It is usually defined as systolic pressure above 140 mm Hg and diastolic pressure above 90 mm Hg. Hypertension can damage organs and other parts of the body, such as the heart, brain, eyes, kidneys, and legs.

As mentioned in section 15.1, CVD is the leading cause of death worldwide. Most of CVD can be prevented through healthy eating, avoidance of tobacco smoking, limitation of alcohol intake, and regular exercise. The former three lie outside the scope of this book. Let’s focus, then, on the latter.

Epidemiological studies (reviewed by Daniel Green and collaborators)

show that regular physical activity is associated with lower morbidity and mortality from CVD. In addition, in individuals with CVD, rehabilitation that includes training is associated with fewer deaths from CVD and fewer hospital admissions. These benefits equal or even exceed those provided by drugs for dyslipidemia and hypertension.

Moderate-intensity continuous training (MICT), resistance training, and interval training seem to be effective means of lowering the risk of CVD. They do so in a variety of regimens, such as those outlined in sections 14.14 for MICT, 12.12 for resistance training, and 14.17 for interval training. As reviewed in detail by Bianca Bernardo and coworkers, the benefits stem from adaptations of both constituents of the cardiovascular system—that is, the heart and the vasculature—in accordance with the third principle of exercise metabolism outlined in section III.2. Let's explore these adaptations.

15.3 Adaptations of the Heart to Training

Both endurance and resistance training result in hypertrophy of the heart, typically by 12% to 15%, as reviewed by Rick Vega and colleagues. As in the case of skeletal muscle fibers, **cardiac myocytes** (that is, cardiac muscle cells) can grow but do not proliferate. Cardiac myocytes resemble skeletal muscle fibers in that they contain myofibrils with thick and thin filaments. The sarcomeres in the myofibrils are neatly arranged, in a way that produces striations, and are surrounded by sarcoplasmic reticulum.

Cardiac myocytes contract in a manner similar to that of skeletal muscle fibers. However, unlike skeletal muscle fibers, their activity is not subject to voluntary control by the somatic nervous system; rather, it is subject to involuntary control by the autonomic nervous system, which directs them to contract and relax continuously and rhythmically. When we exercise, the autonomic nervous system is excited and makes the heart contract more frequently (thus increasing heart rate) and more forcefully (thus increasing stroke volume).

How training elicits cardiac hypertrophy is not fully understood. It is possible that exercise causes an increase in the IGF1 content of cardiac myocytes. This increase, in turn, triggers the PI3K cascade (figure 13.7),

leading to activation of PKB/Akt and subsequent cardiac myocyte growth through some unresolved steps. Evidence comes from experiments in rodents, such as mice and rats, since it is ethically unacceptable to sample the **myocardium** (that is, the heart muscle) of a living human except during heart surgery.

Training-induced cardiac hypertrophy preserves or enhances cardiac function. Thus it differs from the pathologic hypertrophy caused by prolonged hypertension, which is triggered by different signal transduction pathways and leads to a progressive decline in cardiac function. In fact, interventional studies in rodents show that training-induced hypertrophy antagonizes hypertension-induced hypertrophy of the heart, although similar evidence from observational studies in humans is rather weak, as also summarized by Vega and colleagues.

An impressive characteristic of cardiac myocytes is that they contain many more mitochondria than do skeletal muscle fibers. Actually, mitochondria may occupy as much as half of a cardiac myocyte's volume (figure 15.2). In terms of energy metabolism, a cardiac myocyte is almost exclusively aerobic, synthesizing its ATP primarily through fatty acid oxidation. Endurance training increases the ability of the heart to oxidize fatty acids, an effect probably mediated by an increase in PGC1α, the transcriptional coactivator also involved in the adaptations of skeletal muscle to endurance training (section 13.6). Again, these findings come from experiments in rodents.

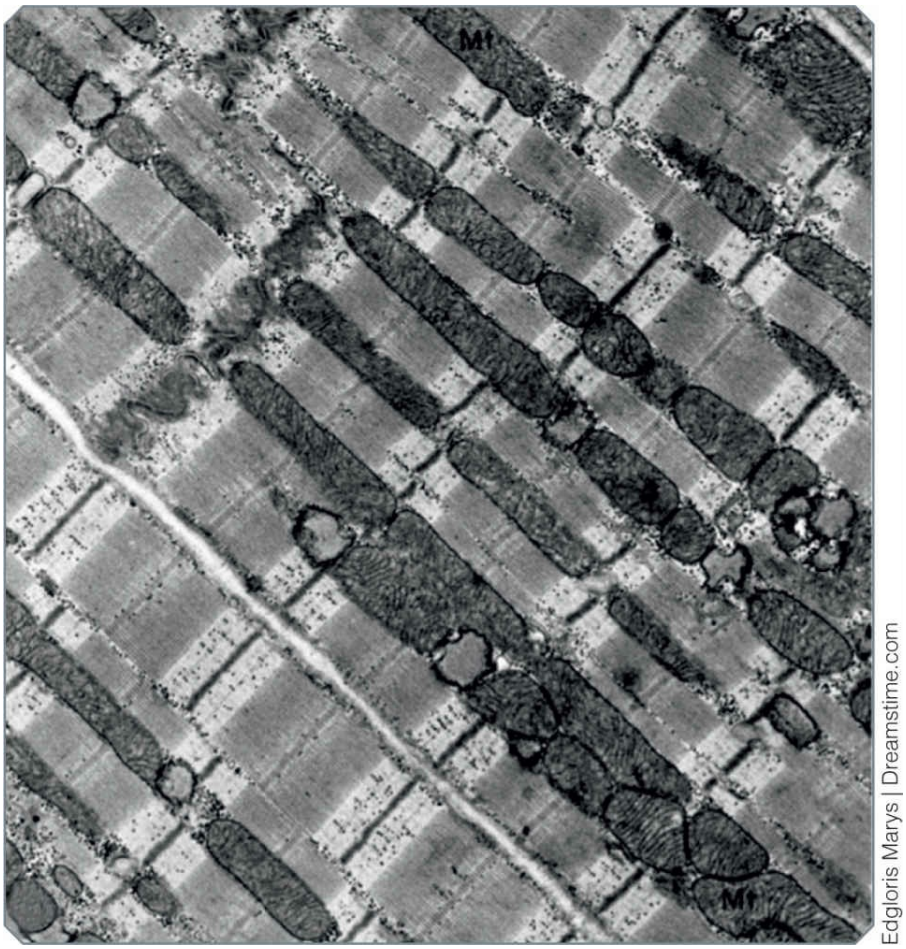


Figure 15.2 **Cardiac myocytes.** Electron micrograph of parts of cardiac muscle cells from a cat shows rows of mitochondria alternating with myofibrils. Lipid droplets are squeezed between mitochondria, as in figure 11.10.

The adaptations to training described here enhance the protection of the heart against CVD. We express this benefit by saying that *exercise is cardioprotective.* Specifically, as reviewed by Michael Wiggs and coworkers, regular exercise reduces the risk of experiencing a heart attack, protects the heart against damage resulting from a heart attack, and reduces the risk of dying during a heart attack.

In addition, training induces changes in the cardiac myocytes that protect against damage when the blood supply is restored after a myocardial infarction has deprived them of oxygen and nutrients. We refer to such damage as **ischemia-reperfusion injury**, where *ischemia* refers to deprivation of blood and *reperfusion* to restoration of blood flow. The

changes in question are not fully understood. It appears that ischemia-reperfusion results in the production of radicals that damage the cell (in particular, the mitochondria) and that training boosts the antioxidant capacity of cardiac myocytes, similar to the effect of endurance training on skeletal muscle fibers (section 14.24). Potential damage from ischemia-reperfusion is also resisted by changes in the mitochondria.

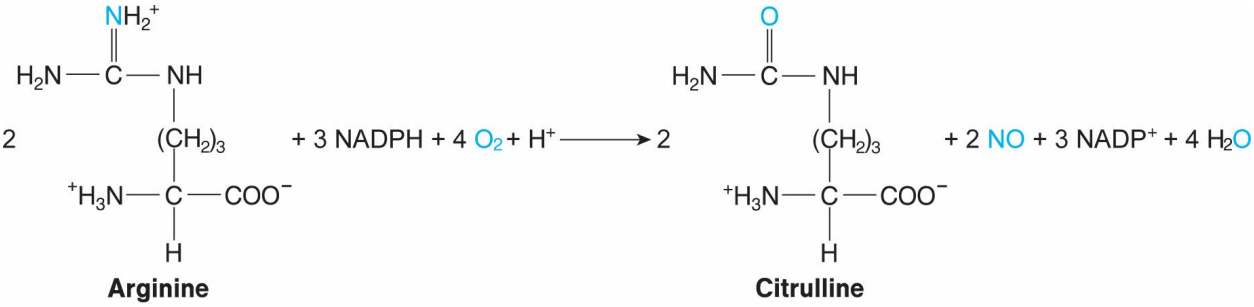
15.4 Adaptations of the Vasculature to Training

Training benefits the blood vessels as well as the heart. As discussed in sections 11.21 and 11.22, endurance and resistance training can lower the risk of atherosclerosis by decreasing plasma triacylglycerols, total cholesterol, and LDL cholesterol. On top of these effects, endurance training contributes to lowering the risk of atherosclerosis by increasing HDL cholesterol.

In addition, the increases in blood flow and pressure accompanying exercise cause structural and functional adaptations of the vascular wall that lower the risk of atherosclerosis. These beneficial effects seem to be mediated by the **endothelium**, the single layer of cells lining the interior surface of the blood vessels, in direct contact with blood on the inside and surrounded by smooth muscle cells on the outside (figure 15.3).

The endothelial cells possess a variety of proteins that convert the mechanical stimulus of the exercise-induced increase in shear stress into chemical signals. In turn, these signals activate **endothelial nitrogen oxide synthase (eNOS)**. This enzyme catalyzes the synthesis of nitric oxide (introduced in section 1.5) from arginine and oxygen in a reaction requiring NADPH as a reductant and yielding citrulline (the intermediate compound of the urea cycle introduced in section 12.9 and figure 12.10) in addition to NO.

Shear stress is the tangential force of flowing blood on the endothelium.



$\Delta G^{\circ\prime} = -301.4$ kcal · mol^{-1} (equation 15.1)

NO diffuses out of the endothelial cells and enters neighboring smooth muscle cells, where it activates **soluble guanylate cyclase**, a cytosolic enzyme that catalyzes the formation of cyclic guanylate, or cyclic GMP (cGMP), from GTP, in a manner analogous to the synthesis of cAMP (section 10.6 and reaction 10.5).

$$GTP \rightleftharpoons cGMP + PP_i \qquad \text{(equation 15.2)}$$

Cyclic GMP, in turn, activates **cGMP-dependent protein kinase**, or **PKG**, which promotes smooth muscle relaxation by activating an ion pump that removes Ca^{2+} from the cytosol, thus preventing the interaction of myosin and actin. (Ca^{2+} allows myosin binding to actin in smooth muscle cells and skeletal muscle fibers through a series of interactions differing from the one described in section 8.10.) Smooth muscle relaxation results in dilation of the blood vessels, or **vasodilation**. The capacity of the vessels to dilate in response to increased blood flow, a property termed **flow-mediated dilation**, is considered an index of cardiovascular health.

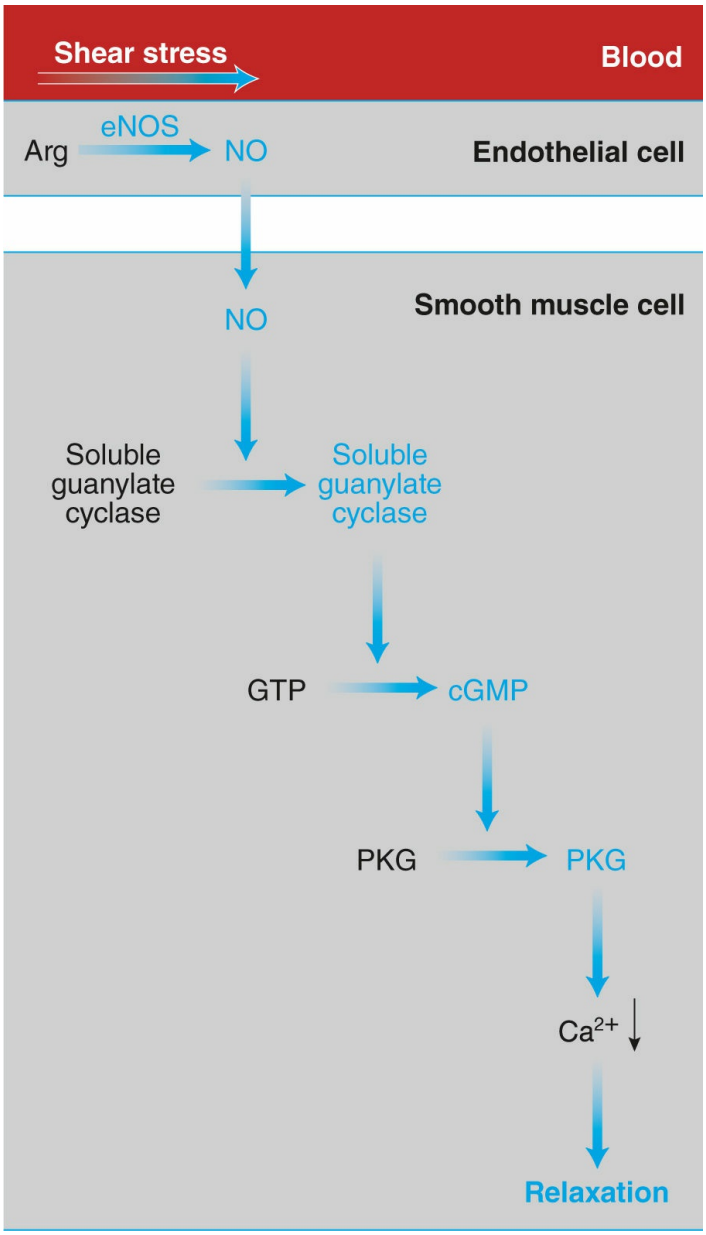


Figure 15.3 **How exercise elicits vasodilation.** The increase in the pumping activity of the heart during exercise augments the shear stress on the endothelial cells lining the blood vessels. This mechanical stimulus leads to activation of eNOS, which catalyzes the synthesis of NO from arginine. NO diffuses to the smooth muscle cells forming the walls of blood vessels and activates soluble guanylate cyclase, which catalyzes the synthesis of cyclic GMP (cGMP) from GTP. Cyclic GMP activates PKG, leading to a drop in the cytosolic Ca^{2+} concentration, relaxation of the smooth muscle cells, and vasodilation.

As Green and coworkers review, training improves flow-mediated dilation —especially in individuals with or at risk of CVD—and reduces arterial wall stiffness by enhancing the endothelium-dependent signal transduction

pathway described earlier. The enhancement includes higher eNOS content or activity. It is also possible that training influences other pathways of flow-mediated dilation. In addition, training increases the diameter of vessels—such as the coronary arteries, as well as arteries that nourish the exercising limbs—thus supplying more blood where it is needed. The stimulus, again, appears to be the increased shear stress imposed by blood on the endothelium with exercise.

Finally, training influences the smaller blood vessels: It increases the amount and diameter of the arterioles (the branches of arteries leading to capillaries) and muscle capillarization. The latter is measured as either the total number of capillaries in a muscle, or the number of capillaries per muscle fiber, or **capillary density** (defined in section 14.15 as the number of capillaries per unit of muscle cross-sectional area). Increased capillarization enhances the delivery of nutrients and O_2 to muscle, as well as the uptake of muscle CO_2 by blood.

Training-induced capillarization may be due to increased blood flow or to muscle activity itself. Both factors may promote the release of **vascular endothelial growth factor (VEGF)**, a key angiogenic (that is, vessel-generating) protein, from muscle fibers. VEGF binds to the **VEGF receptor** in the plasma membrane of endothelial cells and activates a variety of signal transduction pathways, leading to endothelial cell proliferation and, hence, formation of new capillaries.

You can see that training provides more than one way of increasing blood supply to the active muscles. It seems that these beneficial adaptations can be elicited by endurance, resistance, and interval training. Improvements in vascular function with training are evident in both healthy humans and (possibly more evident) humans with or at risk of CVD.

15.5 Exercise to Fight Cancer

As with CVD, **cancer** is a broad term that includes tens of diseases, the names of which usually refer to the affected tissue or organ—for example, lung cancer, breast cancer, and leukemia (a cancer of the bone marrow that causes high numbers of abnormal leukocytes, the cells of the immune

system). Cancer is characterized by abnormal and uncontrolled growth and proliferation of a group of cells, which form a **tumor**. Tumors interfere with normal functioning of the organs, weaken the body (because they absorb disproportionately high amounts of nutrients), and may be lethal when they spread from one organ to other places in the body. This process results from the migration of tumor cells through blood or lymph and is termed **metastasis**.

Major causes of cancer include tobacco smoking, obesity, unhealthy eating, certain infections, physical inactivity, and excessive alcohol drinking. Fewer than 10% of cancer cases are accounted for by genetic predisposition. These various causes share the fact that, one way or another, they disable the body's control of cell growth and division. This control is of paramount importance in allowing organs and systems to have proper proportions and collaborate harmoniously. Control is lost because of mutations (section 4.8) in one or several (usually the latter) genes that promote or suppress cell growth and mitosis.

Scientific evidence from epidemiological studies links physical activity with a lower risk of cancer. In one of the most comprehensive studies to date, Steven Moore and colleagues pooled data from 12 studies to assess the association of leisure-time physical activity with the risk of 26 types of cancer in 1.4 million adults of both sexes. The researchers found that high levels of physical activity were associated with lower risks of 13 of the 26 cancers, including some of the more common ones, such as lung, breast, endometrial, and colorectal cancers.

Notably, physical activity was also associated with *higher* risks for two cancers in the study by Moore and colleagues—namely, prostate cancer and malignant melanoma (a skin cancer). No biological explanation for the former association is known. However, it is possible that physically active men are more health conscious and, hence, have their prostate checked more often than do inactive men, thus increasing the possibility of diagnosing latent prostate cancer. As for malignant melanoma, its undesirable association with physical activity seems to be due to the greater sun exposure of people who exercise outdoors in light clothing. This association does not speak against regular exercise; rather, it emphasizes the need for proper sun

protection.

How exercise exerts its anti-cancer effects has not been fully established. Although researchers have proposed many mechanisms, evidence for most of them remains weak. One of the mechanisms supported by epidemiological findings in humans and experimental data in mice involves **p53**, the protein introduced in section 13.6 as a transcription factor that, when phosphorylated, induces genes encoding other transcription factors and proteins of the electron-transport chain. As we saw, all of these influences contribute to the increase in muscle mitochondrial content elicited by endurance training.

The action of p53 is much more versatile than merely promoting adaptations to endurance exercise. Most of human cancers exhibit mutations in the gene encoding p53 and a concomitant loss of p53 activity—two findings that attest to the protein's critical role in maintaining the stability of the genome and preventing tumor formation.

For this reason, p53 is characterized as a *tumor suppressor* and *guardian of the genome.* It plays its anti-cancer roles in a variety of ways, including activation of proteins that repair DNA when it is damaged, prevention of premature mitosis, and initiation of programmed cell death (termed **apoptosis**). There is evidence in mice that regular exercise lowers the risk of cancer and, at the same time, increases p53. For example, Kristin Higgins and coworkers showed that endurance training suppressed lung cancer while increasing the p53 content of the tumor (figure 15.4).

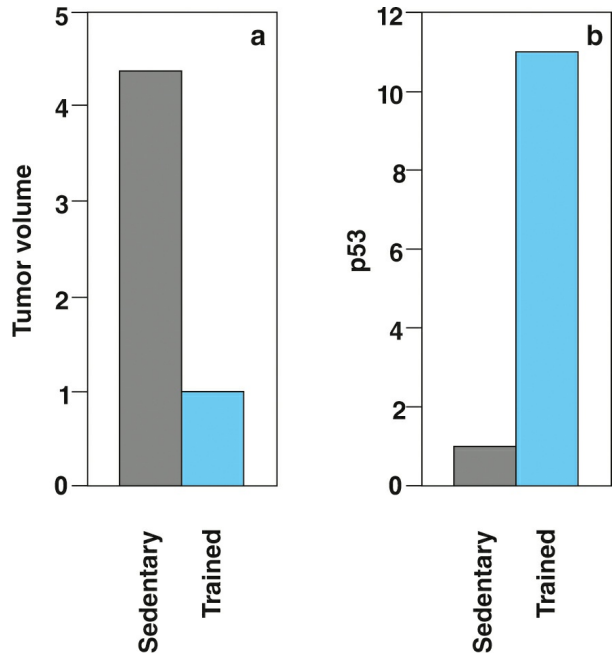


Figure 15.4 **Training may suppress tumor growth through upregulation of p53.** When lung cancer cells were injected into the circulation of mice with immune deficiency (to allow rapid tumor establishment and growth), they formed lung tumors. The mice then either remained sedentary or began exercising in running wheels. Four weeks later, the tumors were more than four times smaller in the trained mice than in the sedentary ones *(a)*. In contrast, the presence in the tumors of the tumor suppressor protein p53 was 11 times higher in the trained mice *(b)*. The graph is based on data from Higgins and coworkers. The scale on the vertical axis in each panel is relative to the lower of the two values.

One of the causes of cancer is believed to be oxidative stress (section 14.24), elicited by environmental factors such as tobacco smoking. Oxidative stress may damage DNA (figure 14.17*a*) and lead to mutations with tumor-triggering potential. Because regular exercise boosts the antioxidant defense of the body, researchers have hypothesized that it may lower the risk of cancer. However, evidence for the anti-cancer effect of exercise being due to suppression of oxidative stress is lacking.

One of the factors that help tumors grow is believed to be their ability to evade the immune system, which, normally, should detect abnormal cells and target them for destruction. Cancer cells may avoid this safeguard in part by secreting proteins that effectively blind the body's immune surveillance. There is evidence, again in mice, that regular exercise boosts this immune surveillance, thus fighting tumor growth.

As an example, Line Pedersen and collaborators found that training

decreased tumor incidence and growth through mobilization of **natural killer (NK) cells** (figure 15.5). NK cells are a class of cytotoxic (that is, cell-killing) immune cells, which are released into the circulation from the spleen, bone marrow, lymph nodes, tonsils, and thymus. Mobilization of NK cells into the tumors was due to increased epinephrine secretion during exercise and subsequent stimulation of the β-adrenergic receptor in NK cells.

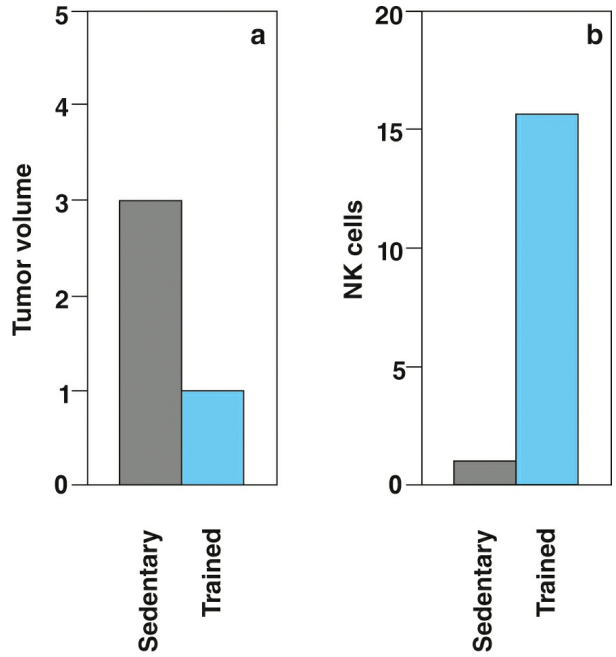


Figure 15.5 **Training may suppress tumor growth through mobilization of NK cells.** Mice that either remained sedentary or exercised in running wheels for six weeks were injected with skin cancer cells at the end of the fourth week and soon developed skin tumors. Two weeks later, the tumors were three times smaller in the trained mice than in the sedentary ones *(a)*. In contrast, the presence in the tumors of cytotoxic NK cells was more than 15 times higher in the trained mice *(b)*. The graph is based on data from Line Pedersen and collaborators. The scale on the vertical axis in each panel is relative to the lower of the two values.

In addition to its anti-cancer effects, training benefits cancer patients in a variety of other ways, as summarized by Pernille Hojman and associates. These benefits include improvements in **cardiorespiratory fitness (CRF)**, muscle strength, body composition, sense of fatigue, and mood. In addition, regular exercise improves the effectiveness of treatments, such as radiation therapy and chemotherapy, and ameliorates their side effects. Moderate-intensity to vigorous endurance training seems to be the most effective

training type, whereas resistance training may counter the significant loss of muscle mass and function seen in cancer. All in all, regular exercise secures longer lives with better quality for cancer patients.

15.6 Diabetes, a Major Metabolic Upset

Diabetes mellitus (or simply *diabetes*) is a disease caused by lack of hormone insulin or malfunctioning of the hormone's signal transduction pathway. When the former is the cause, diabetes is classified as type 1; when the latter is the cause, it is classified as type 2. Let's briefly explore the two clinical entities.

Type 1 diabetes (T1D) is primarily due to an autoimmune destruction of the β cells in the pancreas, resulting in inability of the organ to secrete insulin. It is not known what triggers such a response or how this type of diabetes can be prevented. In most cases, T1D appears early in life, and this is why it is often characterized as juvenile. It is also referred to as insulin-dependent diabetes mellitus. The disease is treated with regular insulin injections, which replenish the missing natural hormone.

> In autoimmune diseases, the body's immune system mistakes some of its own proteins or cells as foreign and raises a destructive response against them.

Type 2 diabetes (T2D) is due to a defect in the PI3K cascade (section 10.29 and figure 10.48), through which insulin exerts its multifaceted effects by initially binding to its receptor and then causing activation of PKB/Akt after several steps. Defects can appear in any of the proteins in the cascade. Thus, although the pancreas is capable of producing insulin, the hormone is incapable of acting. We express this condition with the term **insulin resistance**, reflecting that the body resists the action of insulin. For this reason, T2D is also referred to as *insulin-resistant diabetes* or *non-insulin-dependent diabetes mellitus*. It is also known as *adult-onset diabetes* because it usually appears later in life.

T2D is about 10 times as prevalent as type 1 and affects about 8% of the global adult population. Causes of T2D include obesity (section 15.8),

unhealthy eating, and physical inactivity (section 15.13). The disease is treated with drugs that lower blood glucose and, sometimes, with insulin.

If left untreated, diabetes of either type results in **hyperglycemia** (exceedingly high plasma glucose concentration) throughout the day—not just after a meal, as is normal (figure 15.6). Hyperglycemia occurs because glucose exhibits a reduced rate of disappearance from the circulation and an increased rate of appearance in it. The former is due to failure of GLUT4 to translocate to the plasma membrane from its intracellular reservoir in muscle fibers and adipocytes (figure 10.21). As a result, glucose uptake by muscle and adipose tissue (two tissues that usually add up to more than half of body mass in both sexes) drops dramatically.

The increased rate of appearance of glucose in diabetes, on the other hand, is due to excessive glucose production in the liver, since glycogenolysis and gluconeogenesis (the two glucose-producing pathways) are not restrained in the absence of insulin or in insulin resistance (section 10.29). What is more, T2D is characterized by abnormally high glucagon secretion, which further contributes to hyperglycemia through the stimulation of glycogenolysis and gluconeogenesis (section 14.22).

The abnormally high rate of gluconeogenesis in the liver of untreated diabetic patients also has another consequence: It depletes the hepatocytes of oxaloacetate, thus promoting ketogenesis (section 11.23). Ketone bodies (acetoacetate, D-3-hydroxybutyrate, and acetone) are released to the bloodstream at a rate higher than their rates of uptake by extrahepatic tissues, resulting in extraordinarily high plasma (and urine) concentrations. Being acids, acetoacetate and D-3-hydroxybutyrate lower the blood pH, causing a condition known as **ketoacidosis**, which can be life threatening. Acetone, on the other hand, being volatile, passes from blood to the exhaled air in the lungs and can be detected in the breath.

You can see that diabetes causes a major perturbation of energy metabolism, with serious repercussions for homeostasis and health. However, the negative effects of untreated diabetes are not confined to ketoacidosis. The lack of insulin action eliminates the antilipolytic action of insulin in adipose tissue (described in section 11.6 and figure 11.7). This elimination results in excessive lipolysis and abnormally high concentration of fatty acids

in plasma. Fatty acids cause insulin resistance, although there is no agreement among researchers regarding the mechanism of this effect.

Finally, diabetes affects the blood vessels, because, in addition to insulin's metabolic effects, it normally causes vasodilation. It does so by binding to its receptor in endothelial

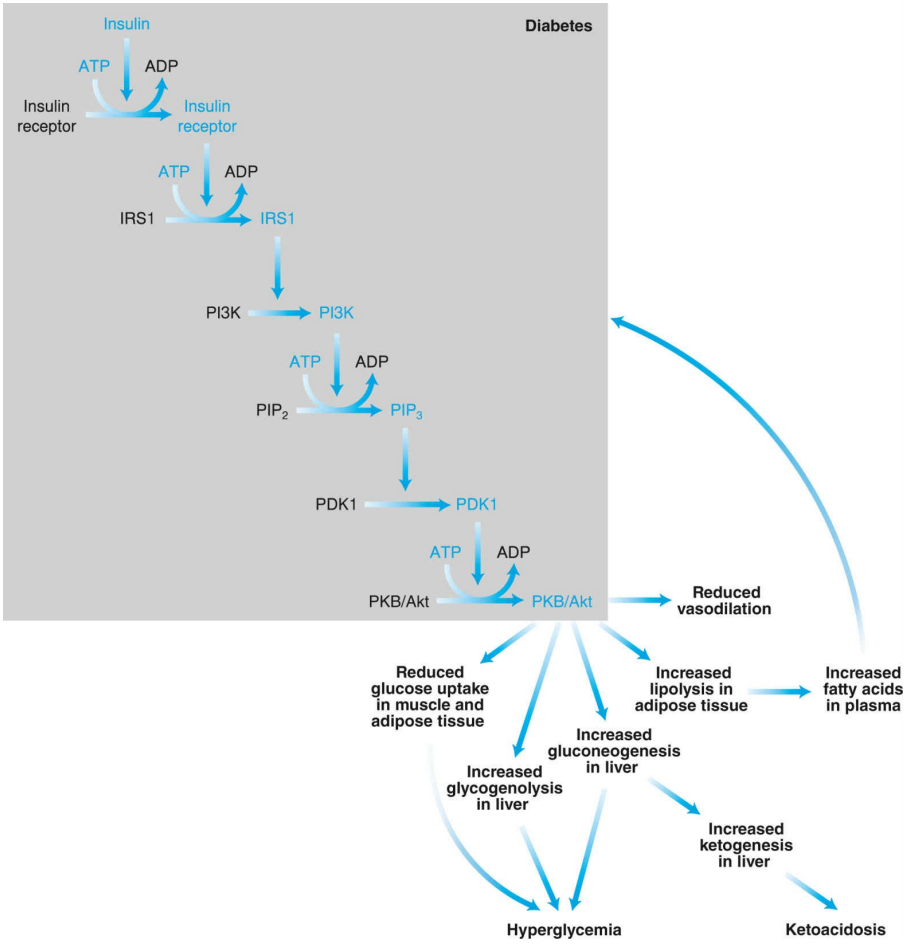


Figure 15.6 **Metabolic havoc in diabetes.** Inability of the pancreas to produce insulin (in T1D) or insulin resistance (in T2D) causes failure of one or more activation steps in the PI3K cascade (shaded area), resulting in dysfunctional PKB/Akt. This dysfunction reduces glucose uptake in muscle and adipose tissue while speeding up glycogenolysis and gluconeogenesis in the liver. These effects combine to produce persistent hyperglycemia. The use of oxaloacetate for gluconeogenesis in the liver prevents fatty acids from being oxidized and diverts them toward ketogenesis, thus causing ketoacidosis. In adipose tissue, there is no inhibitory effect of insulin on lipolysis, and the resulting high fatty acid concentration in plasma causes insulin resistance (upward curved arrow toward the shaded area). Diabetes also prevents the vasodilation normally elicited by insulin, thus reducing the delivery of glucose, fatty acids, and insulin itself to the tissues.

cells and stimulating the PI3K cascade in the usual way, leading to PKB/Akt activation. PKB/Akt phosphorylates and activates eNOS, which boosts NO synthesis (section 15.4). This insulin-induced vasodilation collaborates with insulin-induced migration of GLUT4 to the plasma membrane of muscle fibers and adipocytes, resulting in increased glucose delivery to these cells. Anton Wagenmakers and colleagues note that vasodilation also facilitates the delivery of insulin itself and the delivery of fatty

The Frequently Underestimated Value of Theory

To practice without theory is to sail an uncharted sea; theory without practice is not to set sail at all.

Mervyn Susser

Students of physical education and sport science often question the value of being taught theoretical knowledge of biological sciences such as biochemistry, as contrasted with the practical and, hence, directly applicable knowledge in other subjects. However, as I have shown in several cases throughout the book, theory provides a sound basis for practice and protects us from serious practical errors. Nevertheless, practice is the ultimate test of theory. The following is a real-life example of how theoretical knowledge and practice combined to help someone.

A senior exercise biochemist—say, Victor—once supervised an experiment involving exercising people. Every now and then, he would go back and forth between the lab and his nearby office. In one of his visits to the lab, while talking with one of his associates—say, Christopher, who was taking measurements—he detected a sweet smell in the air. He attributed it to the ethanol in the antiseptic used to disinfect the skin of the participants before blood drawing and paid no further attention.

During his next visit to the lab, half an hour later, while talking with

Christopher again, Victor detected the same smell. However, he now realized that it smelled of acetone, not alcohol. Having used both solvents for protein and lipid analyses during his many years of laboratory *practice*, he could tell the difference.

Only then did he make the connection: He knew that Christopher was suffering from T2D. He also knew that there is often acetone in the breath of a person with uncontrolled diabetes. But he knew that only in *theory*, since, not being a physician, he had had no experience of diagnosing or treating the disease. So, he asked Christopher how he was doing in terms of controlling his diabetes. Christopher replied that he had not been paying any attention to it lately.

Victor then expressed his fear that Christopher's health was in imminent danger and invited him to take a blood glucose test through a glucose meter he kept in the lab. The test produced a value of 430 mg · dL^{-1}, or 24 mmol · L^{-1}, far above the acceptable limit. Christopher said that the result might explain the mental confusion, hunger, and thirst (frequent symptoms of diabetes) he had been feeling over the past few days. He was rushed to a hospital, where his severe hyperglycemia was confirmed. He was placed under pharmacological treatment and given dietary and exercise guidance. Now his diabetes is under control and there is no smell of acetone in his breath.

acids. This delivery would prevent the accumulation of fatty acids in plasma and the resultant insulin resistance.

Conversely, insulin resistance in the endothelium prevents vasodilation and leads to diminished insulin, glucose, and fatty acid delivery to the tissues, thus exacerbating hyperglycemia and creating a vicious cycle. Moreover, hyperglycemia and insulin resistance favor the development of CVD by promoting oxidative stress and RONS generation, as explained by Francesco Paneni and coworkers.

Common effects of diabetes include damage to the eyes, kidneys, and nerves because of poor circulation in small blood vessels. Damage to the eyes (precisely, the retina) leads to gradual loss of vision and blindness. Damage

to the kidneys leads to chronic kidney disease, which may necessitate dialysis or kidney transplantation. Damage to the nerves affects the sense of touch and the feeling of pain; it may lead to foot ulcers, which often necessitate amputation.

15.7 Exercise to Fight Diabetes

Diabetes is among the chronic diseases that benefit from regular exercise. The benefit accrues in several ways. First, exercise leads to increased migration of GLUT4 from intracellular vesicles to the plasma membrane of the active muscle fibers (section 10.9 and figure 10.21). This acute effect helps to lower the plasma glucose concentration and lasts one to three days after an exercise session. Because it does not depend on insulin, it works in diabetic patients as well as in healthy individuals.

Other effects of exercise that fight diabetes are chronic rather than acute. As mentioned in section 14.15, endurance training increases the muscle GLUT4 content. This increase contributes to the increase in glucose uptake by the muscles even though a trained individual may have a lower glucose uptake as compared with an untrained one for the duration of exercise at a given absolute moderate intensity. In addition, training may increase signal biomolecules of the PI3K cascade in muscle, thus improving insulin's action. Finally, the training-induced improvement in endothelial function (described in section 15.4) also fights the detrimental effects of diabetes on the vasculature, according to what we discussed in the preceding section.

Thanks to the acute and chronic changes conferred by exercise, **insulin sensitivity** (the opposite of insulin resistance) increases, meaning that a certain plasma concentration of insulin exerts a higher effect (such as glucose uptake by muscle). This increase is seen in both type 1 and 2 diabetic patients, as well as healthy individuals, as summarized in a position statement of the American Diabetes Association, with lead author Sheri Colberg. In addition, regular exercise improves the control of blood glucose in T2D and prevents or delays the very development of diabetes.

Insulin sensitivity can be improved through moderate-intensity continuous training, resistance training, and interval training. Colberg and associates

recommend combined training for diabetic patients according to the following guidelines:

- Reduce total daily sedentary time; interrupt prolonged sitting with three to five minutes of light activity every 30 min.
- Exercise daily or at least three times per week and go no more than two consecutive days without activity (so as not to let the acute effect of exercise on insulin sensitivity wither).
- Perform both endurance and resistance training. Endurance training can be moderate-intensity continuous, interval, or both. Interval training is probably more effective than the continuous according to a meta-analysis by Charlotte Jelleyman and colleagues.
- Accumulate at least 150 min of moderate-intensity or vigorous exercise per week.
- Perform resistance exercise two or three times per week on nonconsecutive days.
- Prefer supervised training over unsupervised programs.

15.8 Obesity, a Health-Threatening Condition

Obesity is characterized by an abnormally high accumulation of body fat that may impair health. A simple measure of obesity is the BMI, although, as noted in section 11.3, it makes no distinction between lean and fat mass. Thus, persons with unusually high muscle mass and normal or low fat mass, such as strength athletes, may have BMI values that would classify them as overweight. Conversely, inactive persons with unusually low muscle mass and high fat mass may have BMI values that would classify them as normal. In such cases, assessing body fat through a variety of available techniques (such as skinfold thickness measurement, bioelectrical impedance, hydrostatic weighing, and dual-energy X-ray absorptiometry) is warranted.

These exceptions aside, BMI reflects body fat fairly well and is the predominant index of obesity, especially in epidemiological studies, in which it is not practical to subject large numbers of individuals to measurements that are more technically demanding than the measurement of body weight

and height. A BMI equal to or above 30 kg · m^{-2} classifies adults of both sexes as obese, and a BMI equal to or above 25 and lower than 30 kg · m^{-2} signifies overweight. Cut-off values are lower for children and adolescents.

Obesity is the result of long-term imbalance between energy intake and energy expenditure on the side of the former. Thus, it is caused mainly by excessive eating and inadequate physical activity, followed by genetic predisposition and other minor factors. The body stores the surplus energy primarily in the form of triacylglycerols in the adipocytes, thus increasing adipose tissue disproportionately to other tissues.

Normally, the body possesses regulatory mechanisms that balance energy intake with energy expenditure to maintain a natural and healthy weight. These mechanisms are quite complex and not fully understood. A key regulator of body weight is **leptin**, a peptide hormone secreted by adipocytes in proportion to fat mass. Leptin travels through blood to the hypothalamus in the brain, where it binds to the leptin receptor and initiates a signal transduction pathway that results in diminished appetite. Thus, leptin is part of a negative feedback loop: When fat mass increases, so does leptin secretion, and the brain directs us to eat less. This direction leads to reduced fat mass and, consequently, reduced leptin secretion, in effect releasing the brake on appetite.

Three other key regulators of body weight, all peptide hormones, are secreted by cells along the gastrointestinal tract. **Ghrelin** is secreted by cells in the stomach wall when the stomach is empty. Like leptin, it acts in the hypothalamus, albeit with an opposite effect: It stimulates appetite and hunger. When the stomach is full, ghrelin secretion drops, and the sense of hunger subsides. **Glucagon-like peptide 1 (GLP1**) and **peptide YY (PYY)** are secreted by cells in the intestinal wall in response to a meal. Acting in the hypothalamus as well, they suppress appetite, directing us to stop eating. Table 15.1 summarizes the characteristics of the four hormones regulating appetite.

Because ghrelin stimulates appetite, it is said to be **orexigenic** (meaning “generating appetite” in Greek). In contrast, leptin, GLP1, and PYY are **anorexigenic** (generating loss of appetite).

Obese people have high circulating leptin concentrations. However, leptin fails to lower their body fat because of some defect in its signal transduction pathway. This **leptin resistance** is analogous to—and frequently concomitant with—insulin resistance characterizing T2D. It is not clear whether obesity is also linked to defects in ghrelin, GLP1, or PYY signaling.

Obesity is a major risk factor for several diseases, including the three addressed in the preceding sections of this chapter: CVD, cancer (in particular, esophageal, pancreatic, liver, gallbladder, kidney, colorectal, breast, endometrial, and ovarian), and diabetes (both type 1 and type 2). Diseases linked to obesity also include musculoskeletal disorders, such as **osteoarthritis**, a disabling degeneration of the joints, first mentioned in section 6.3 under the Selenium subheading.

The prevalence of obesity has been on the rise in recent decades. The GBD (standing for Global Burden of Disease) 2015 Obesity Collaborators, a large consortium of experts, estimates that about 700 million people of all ages were obese worldwide in 2015, which constitutes about 10% of the global population. This figure represents a doubling of the prevalence since 1980. Overweight accounted for 1.6 million deaths in the same year, and obesity accounted for another 2.6 million. More than two thirds of those 4.2 million deaths resulted from CVD. The researchers concluded that the BMI associated with the lowest risk of death from all causes is 20 to 25 $kg \cdot m^{-2}$.

Table 15.1 Hormones That Regulate Appetite

Hormone	Site of synthesis	Upregulator	Effect on appetite
Leptin	Adipose tissue	Increased fat mass	Anorexigenic
Ghrelin	Stomach	Empty stomach	Orexigenic
GLP1	Intestine	Full intestine	Anorexigenic
PYY	Intestine	Full intestine	Anorexigenic

15.9 Why Obesity Is Harmful

Why does obesity raise morbidity and mortality? The connection is not known with certainty, but several possible links have emerged through research in recent decades. Two of these possibilities are discussed in this section: inflammation and reduced adiponectin.

Inflammation

Inflammation is a response by the body to a harmful stimulus—a response aimed at eliminating the insult, removing damaged cells or tissue, and initiating tissue regeneration. It involves a mobilization of immune cells, facilitated by increased blood flow, to the site of the attack. Inflammation is most easily recognized by signs such as pain, redness, swelling, heat, and loss of function following an acute attack (for example, a bee sting). However, it can also be chronic, low grade, and subtle. In addition, it can be systemic rather than localized. These are the characteristics of inflammation due to obesity.

Researchers believe that obesity triggers an immune response when enlarged adipocytes release leptin and other biomolecules, which are sensed by macrophages, a type of leukocytes that patrol tissues in search of pathogens. Macrophages are then attracted to the space between adipocytes and release inflammatory (also referred to as proinflammatory) proteins known as **cytokines** (Greek for "cell movers"). These proteins include **tumor necrosis factor α (TNFα)**, **interleukin 6 (IL6)**, and **interleukin 1β (IL1β)**. Cytokines exert autocrine, paracrine, and endocrine actions, as defined in section 13.5. They attract more leukocytes, which release more cytokines and induce inflammation.

It is also possible (as pointed out by Jason Kim) that other organs and tissues—such as muscle, heart, liver, brain, and endothelium—suffer inflammation in obesity, although the trigger for this response is not as apparent as are the enlarged adipocytes in adipose tissue. Obese individuals have high plasma concentrations of TNFα, IL6, IL1β, other cytokines, and **C-reactive protein** (which is released from the liver in response to TNFα or IL6 elevation and mediates the removal of dead cells).

Obesity-induced inflammation can cause insulin resistance through the aforementioned inflammatory cytokines. TNFα, in particular, promotes IRS1 phosphorylation at a serine residue that, contrary to tyrosine phosphorylation (section 10.29), impairs insulin signaling. This impairment may explain why obesity favors the development of T2D. There is also evidence that IL1β is involved in damage caused to pancreatic β cells in T2D that results in insulin

deficiency. It is not known how obesity relates to T1D.

An additional harmful effect of TNFα is that it promotes adhesion of monocytes (another type of leukocytes) to endothelial cells, which is considered an early event in atherosclerosis. Circulating monocytes initially stick to the luminal side of endothelial cells (that is, the side facing blood) and then pass between adjacent endothelial cells to the vessel wall (refer to figure 15.3), where they differentiate into macrophages. The latter ingest lipids and slowly turn into large cells termed *foam cells*, which form part of the atherosclerotic plaque.

Inflammatory cytokines may also be the link between obesity and cancer: TNFα, IL6, and other cytokines are implicated in cancer growth and metastasis, as reviewed by Sharon Louie and collaborators. Figure 15.7 summarizes the possible ways in which obesity may increase morbidity and mortality.

In addition to explaining the morbidity and mortality associated with obesity, inflammation may explain why abdominal, or visceral, fat (assessed through measurement of waist circumference or other techniques) is particularly unhealthy. For example, Karine Sahakyan and associates found that men and women with **central obesity**—the accumulation of excessive abdominal fat—had higher mortality risk than did persons of the same BMI without central obesity. This was so even in persons with normal BMI. Excessive visceral fat is associated with insulin resistance, dyslipidemia, and inflammation. This association may be explained by evidence that visceral fat contains more leukocytes and, hence, releases more inflammatory cytokines than does subcutaneous fat.

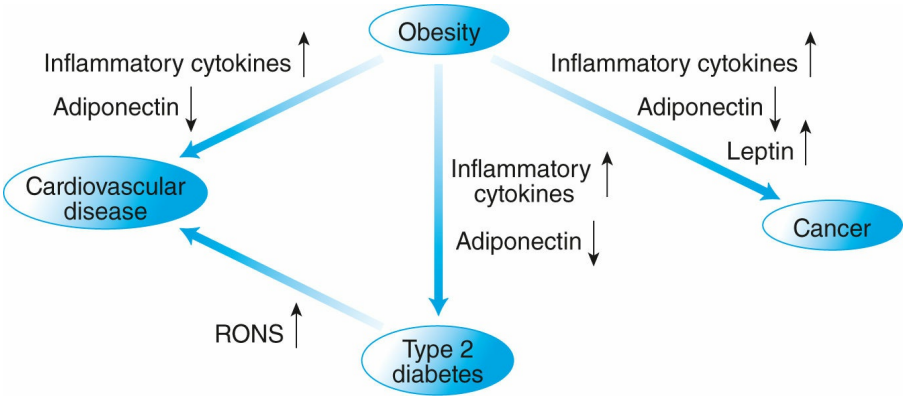


Figure 15.7 How obesity may promote disease. Inflammatory cytokines secreted by immune cells that infiltrate adipose tissue and, possibly, other tissues and organs of an obese person may promote CVD, T2D, and cancer. The decrease in adiponectin with obesity may have the same effect. The increase in leptin may favor cancer development. Diabetes causes CVD by promoting RONS generation and oxidative stress.

The negative role of central obesity in health is stressed by the fact that it is one of the criteria of the so-called **metabolic syndrome**, the other criteria being hyperglycemia, dyslipidemia (specifically, high triacylglycerols and low HDL cholesterol), and hypertension. The metabolic syndrome is the hallway, so to speak, to CVD and T2D: The risk of these diseases increases when most of the criteria for metabolic syndrome are met.

Obesity (especially central obesity), inflammation, T2D, and the metabolic syndrome are connected to **nonalcoholic fatty liver disease (NAFLD)**. NAFLD results from accumulation of excessive fat in the liver (more than 5% of liver mass) due to causes other than extreme alcohol consumption. Fat accumulation harms the liver, particularly when it evolves to non-alcoholic steatohepatitis and then to cirrhosis, which may be fatal.

Adiponectin

Adiponectin is a peptide hormone secreted by adipocytes in larger quantities than leptin. Adiponectin targets a variety of tissues, including the liver and muscle (figure 15.8). In both muscle and liver, it promotes glucose uptake and breakdown. In the liver, it slows down gluconeogenesis and fatty acid synthesis. In muscle, it increases fatty acid uptake from plasma and speeds up β oxidation. The bottom line of these actions is the stimulation of glucose and fatty acid catabolism and the inhibition of glucose and fatty acid synthesis.

Thanks to their versatile effects all over the body—and in accordance with the term *cytokine*—leptin, adiponectin, and other peptide hormones secreted by adipocytes are termed **adipocytokines** or **adipokines**.

Adiponectin exerts many of its effects on metabolism through **AMPK**, whose activation and role in the exercise-induced increase of muscle mitochondrial content we considered in section 13.6. Adiponectin binds to

adiponectin receptors in the plasma membrane of target cells, resulting in AMPK phosphorylation and activation. AMPK then phosphorylates key enzymes and other proteins, thus speeding up or slowing down the listed processes.

In addition to its metabolic actions, adiponectin exerts a variety of protective effects on the cardiovascular system, largely through AMPK activation, as discussed in detail by Chiara Caselli and coworkers. Adiponectin also restricts inflammation. Finally, it inhibits tumor growth, as reviewed by Louie and colleagues. By contrast, leptin exerts tumor-promoting effects. Characteristic of the opposite effects of the two adipokines on cancer are findings that adiponectin blocks the cancer-promoting effects of leptin in vitro, as Leo Alexandre and associates note.

The plasma adiponectin concentration decreases in obesity (figure 15.7) and increases with weight loss; it remains unclear what causes these changes. Regardless of mechanism, the drop in adiponectin concentration with obesity is thought to contribute to hyperglycemia, insulin resistance, and, eventually, T2D. In addition, the protective effects of adiponectin on the cardiovascular system dwindle, making the heart and vessels susceptible to atherosclerosis and CVD. Likewise, the anti-inflammatory action of adiponectin is lost. Finally, the drop in adiponectin and the rise in leptin may promote cancer growth in obesity.

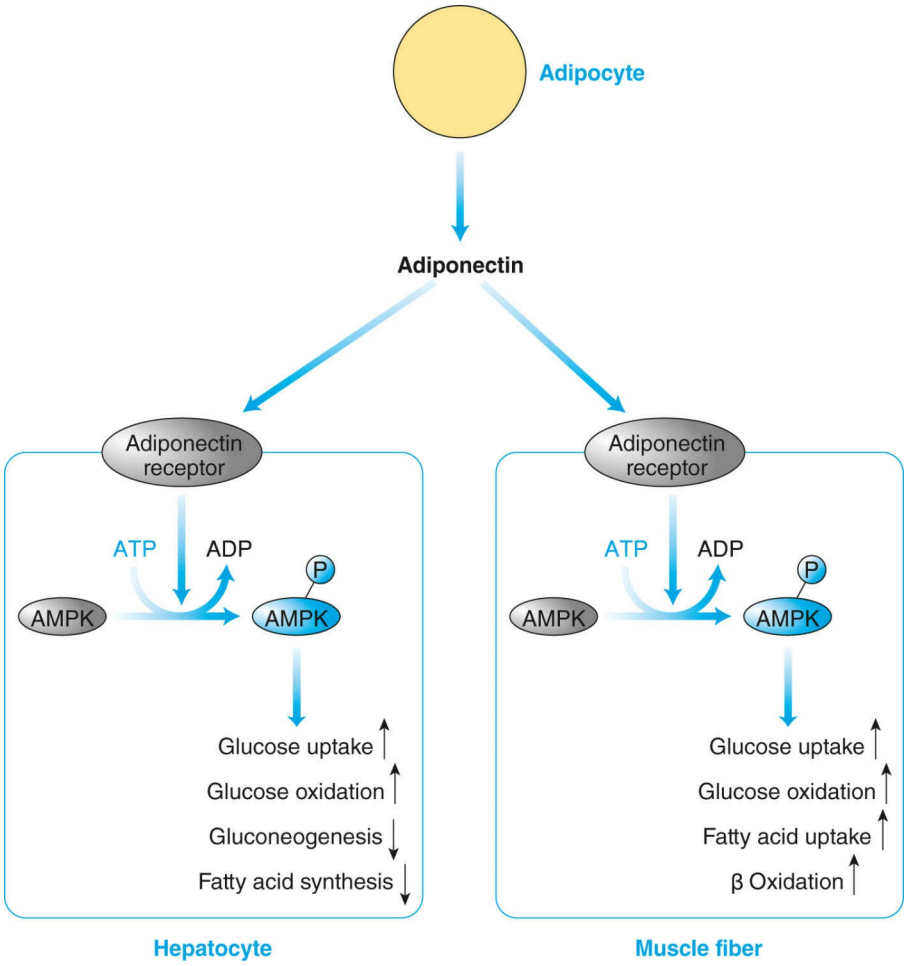


Figure 15.8 **Some metabolic effects of adiponectin.** When adiponectin is released from adipocytes, it binds to its receptors in hepatocytes and muscle fibers (as well as other cell types in the body). Adiponectin binding to a receptor results in AMPK activation through phosphorylation. In hepatocytes, AMPK promotes glucose uptake and oxidation, while inhibiting gluconeogenesis and fatty acid synthesis. In muscle fibers, AMPK promotes glucose uptake, glucose oxidation, fatty acid uptake, and β oxidation. Thus, adiponectin contributes to lowering the plasma concentrations of glucose and fatty acids.

15.10 Exercise to Fight Obesity

Regular exercise is an indispensable weapon in the fight against obesity, although it needs two "brothers in arms"—dieting and behavioral modification—which lie beyond the scope of this book. Let's focus, then, on exercise, first by considering how it promotes weight loss, second by exploring how it fights the detrimental effects of obesity on health, and third by addressing training recommendations for obese individuals.

Promoting Weight Loss

Regular exercise fights obesity primarily by increasing energy expenditure *during* exercise sessions, thus promoting weight loss. Moderate-intensity continuous training is superior to resistance training and interval training in this respect, because it causes larger energy expenditure.

An additional contribution to weight loss is made *after* an exercise session through EPOC (section 14.26), since excess oxygen consumption entails excess energy expenditure. (Remember that consumption of 1 L of O_2 is equivalent to the expenditure of about 5 kcal; figure 14.12.) It is also possible for a resistance training program to contribute somewhat to weight loss *at rest*, since it induces muscle hypertrophy, which affects resting energy expenditure in a positive manner.

Researchers have investigated whether exercise affects appetite. Increased appetite after exercise would promote eating, thus opposing the weight-reducing effect of exercise. On the other hand, decreased appetite would contribute to the weight-reducing effect of exercise. However, neither seems to be the case: A meta-analysis by Matthew Schubert and collaborators concludes that, although variable, the results of the studies on this topic suggest that people do not alter food intake in the immediate hours after exercise.

This finding is true even though exercise usually elicits favorable changes in the plasma concentrations of hormones regulating appetite, that is, decreases in the orexigenic ghrelin and increases in the anorexigenic GLP1 and PYY. On the other hand, exercise does not seem to affect the plasma concentration of the anorexigenic leptin in a consistent manner, given that researchers have reported increases, decreases, or no changes after different exercise protocols. Incidentally, this is also the case for the other adipokine, adiponectin (which does not have a clear effect on appetite).

Where Is Body Weight Lost?

Ruben Meerman and Andrew Brown asked the ostensibly simple question, “When somebody loses weight, where does it go?” They

received an astonishingly large number of wrong answers from general practitioners, dietitians, and personal trainers. Most responders (about 60%) believed that the extra weight was converted into energy or heat, which violates the principle of mass conservation (section 1.8). In other words, one cannot expect mass to just vanish as energy (unless it takes part in a nuclear reaction, which, naturally, does not take place in our bodies). Other misconceptions held that fat is excreted in the feces or converted into muscle.

To find the correct answer, we need to consider that, in order to lose weight, we have to burn fuel biomolecules. Then we need to write down the reactions of the complete oxidation of, say, glucose (reaction 10.15), palmitate (reaction 11.18), or a triacylglycerol, the latter being what is primarily burned when we lose fat. Meerman and Brown propose that the average triacylglycerol in human adipose tissue consists of a palmitoyl, an oleoyl, and a linoleoyl group (see section 5.7 and table 5.2) attached to a glycerol unit. Such a triacylglycerol has the molecular formula $C_{55}H_{100}O_6$, and the reaction of its complete oxidation is

$$C_{55}H_{100}O_6 + 77\ O_2 \rightarrow 55\ CO_2 + 50\ H_2O \qquad \text{(equation 15.3)}$$

Thus, body weight is lost in the two products of the oxidation of fuel biomolecules. One is CO_2, which we exhale. The other is H_2O, which we retain in the body as what is termed *metabolic water*; it contributes to hydration and is eventually lost through processes such as respiration, perspiration, and urination. For more detailed answers to the title question, see problems 5 and 6.

Alleviating the Detrimental Effects of Obesity

The beneficial effects of exercise on obesity may extend beyond promoting weight loss. Observational studies, summarized by Francisco Ortega and associates, provide support for the so-called **fat-but-fit paradox**: They show that the risk of all-cause and CVD mortality in obese individuals (defined by either BMI, body fat percentage, or waist circumference) who have high CRF

is similar to that in equally fit individuals with normal weight. What is more, normal-weight but unfit individuals could be at a higher risk than obese but fit individuals. It is thus possible that good CRF attenuates the consequences of obesity on health.

The reason for the protective effect of CRF against the ills of obesity is not known with certainty. Part of it may be due to heritability, although the responsible genes remain elusive. Another part must be due to regular exercise. The improvement in the lipidemic profile with training, as discussed in sections 11.21 and 11.22, seems to link CRF with the decreased risk of CVD in obese, as well as lean, people. Another candidate link between high CRF and lower metabolic complications in obesity is inflammation. As reviewed by Bente Klarlund Pedersen, exercise displays anti-inflammatory actions through a number of peptides, which are secreted by muscle and have been termed **myokines**, in accordance with cytokines and adipokines.

Of the many identified myokines, some exhibit remarkable increases in their plasma concentrations with exercise. The most renowned of these is IL6, the very protein that we met as a cytokine in the preceding section. Initially, it was thought that IL6 was produced predominantly by leukocytes in response to exercise-induced local muscle damage. However, evidence now suggests that circulating leukocytes are not the source of the increase in plasma IL6 during exercise, since exercise induces the gene encoding IL6 in muscle at both the mRNA and protein levels.

The plasma IL6 concentration rises gradually during exercise, exceeding 100 fold after prolonged exercise (more than 3 h). In addition, the plasma IL6 concentration correlates positively with exercise intensity. For the plasma IL6 concentration to increase, a large muscle mass needs to be involved in exercise (for example, in running). Training reduces IL6 production at rest and mitigates the IL6 response to acute exercise at a given relative intensity.

By now you may be confused: How can I claim that exercise displays anti-inflammatory actions when it upregulates a protein that I described as inflammatory in the preceding section? Pedersen proposes that the positive effects of IL6 are normally associated with its transient production in muscle and short-term action during and after exercise. In contrast, its negative effects are associated with a persistently elevated systemic concentration,

characteristic of chronic inflammatory states such as obesity.

Once released in the circulation, IL6 causes leukocytes to produce two cytokines, **interleukin 1 receptor antagonist (IL1ra)** and **interleukin 10 (IL10)**. These cytokines exert anti-inflammatory actions by blocking the signal transduction pathway of the inflammatory and pancreas-damaging IL1β and by inhibiting the synthesis of the also-inflammatory TNFα, respectively. Inhibition of TNFα is beneficial for insulin sensitivity, because TNFα causes insulin resistance, as described in the preceding section.

In addition, acting in a paracrine or autocrine fashion, IL6 may increase basal and insulin-stimulated glucose uptake by muscle fibers, thus again contributing to insulin sensitivity. Enhanced IL6 release during exercise may also signal the liver to deliver more glucose to muscle and may increase lipolysis and fat oxidation in muscle. Finally, by suppressing TNFα, IL6 released during exercise may fight CVD and cancer, according to the discussion in the previous section.

Another myokine, which has attracted much attention since its recent discovery by Pontus Boström and coworkers, is **irisin**. This myokine is released not only from muscle fibers but also from adipocytes (which qualifies it as an adipokine too). It has been linked to a variety of biological effects, the most interesting of which is the browning of white fat. Specifically, irisin induces a change in gene expression in white adipocytes of mice that makes them assume a brown adipocyte phenotype, including the synthesis of UCP1 (section 11.18).

Because brown fat spends more energy than does white fat, researchers are considering irisin as a means of fighting obesity. However, there is no evidence that irisin can turn white fat into brown fat in humans. The plasma irisin concentration exhibits a modest increase after exercise in humans, and the increase is higher in fit exercisers than in unfit ones, as concluded in a meta-analysis by Jill Fox and collaborators.

Exercise seems to alleviate yet another disease linked to obesity, as described in the previous section: NAFLD. A meta-analysis by Christina Katsagoni and colleagues concluded that regular exercise lowers the serum concentrations of alanine aminotransferase (section 12.5) and aspartate aminotransferase (section 12.9), which serve as indices of liver damage, as

discussed in section 18.9. In addition, regular exercise lowers the fat content of the liver, independent of change in body weight, in patients with NAFLD.

Training Recommendations for Obese Individuals

Recommendations for training intended to prevent body weight gain, reduce body weight, and prevent body weight regain are similar to those presented in sections 14.14 and 12.12 for the general population, as well as section 15.7 for diabetic patients. The American College of Sports Medicine, in a position stand with lead author Joseph Donnelly, provides the following guidelines for adults.

- To prevent weight gain (specifically, greater than 3%), accumulate 150 to 250 min of moderate-intensity or vigorous exercise per week, with an energy expenditure of 1,200 to 2,000 kcal.
- To achieve a clinically significant weight loss of at least 5%, accumulate at least 225 min of exercise per week. Weight loss hinges on exercise time: Roughly speaking, every 50 min of exercise per week results in loss of 1 kg in six months. Thus, a person exercising for 250 min per week will lose 5 kg in six months (solely from exercise).
- To maintain weight after weight loss, accumulate 200 to 300 min of exercise per week. Regular exercise appears to be indispensable for the maintenance of weight loss.
- Physical activity increases weight loss if combined with moderate dieting but not with severe dieting, that is, energy intake below the resting energy expenditure. The reason probably relates to survival-related metabolic adaptations, which cancel the effect of exercise on weight loss.

Resistance training does not appear to be an effective means of weight loss. Thus, the recommendation regarding resistance training for obese and overweight individuals is the same as for lean ones: Exercise all major muscle groups two or three times per week to maintain or increase muscle strength.

15.11 Exercise to Fight Osteoporosis

Osteoporosis is a disease characterized by abnormally low **bone mineral density**, or **BMD** (that is, mineral mass per bone volume), and, hence, bone mass. As such, osteoporosis reduces bone strength and increases the risk of fractures, even with minor stress. BMD increases throughout childhood and adolescence, culminating at the age of 18 to 20 and remaining maximal for about a decade. Thereafter, it gradually declines. The risk of osteoporosis can be reduced through adequate calcium intake and adequate vitamin D biosynthesis (through sunlight exposure) or intake to facilitate dietary calcium absorption (sections 6.2 and 6.3).

Like many other tissues, bone goes through a continuous process of forming new tissue and breaking down old tissue; the latter is termed **resorption**. Bone formation and resorption are handled by specialized cells, called **osteoblasts** and **osteoclasts**, respectively. Cells of a third type, **osteocytes** (the most common in mature bone tissue), maintain the balance between bone formation and removal. Osteocytes convert mechanical signals imposed on a bone into chemical ones, which, in turn, modulate bone mass.

Osteoporosis is more prevalent in women than in men because women lose BMD more rapidly after the age of 30. It is particularly prevalent after menopause, due primarily to the decline in estrogen levels. Although both bone resorption and formation increase after menopause, the increase in the former is larger than in the latter, resulting in a negative bone mass balance.

BMD increase is promoted by regular exercise that includes resistance training or activities bearing the weight of the body (such as walking, running, and jumping, as opposed to cycling and swimming). It is possible that osteocytes sense the increased load from such exercise and signal increased bone formation over resorption. Researchers believe that accruing maximal BMD by early adulthood is crucial for preventing osteoporosis in later life and that this accrual may be achieved by regular exercise during childhood and adolescence. It is remarkable that physically active children and adolescents have 10% to 20% higher BMD than their inactive counterparts, as reviewed by Frank Booth and colleagues.

In addition, evidence exists (such as that from Tracey Howe and

collaborators) that regular exercise elicits a slight increase in BMD. What is more, regular exercise reduces the incidence of bone fractures in patients with osteoporosis. An additional training type (apart from endurance and resistance) that contributes to the latter is balance and flexibility training (such as tai chi), which helps prevent falls, especially in elderly persons.

15.12 Exercise to Fight Mental Dysfunction

Exercise has the potential to modify brain structure and function. Many studies in humans and experimental animals have shown that exercise changes the structure of circuits of neurons by stimulating the formation of new dendrites and synapses (section 7.1 and figure 7.2) in existing neurons. Exercise even stimulates the formation of new neurons, a process termed **neurogenesis**. This process takes place through complex signal transduction pathways, which involve changes in metabolism and gene expression and about which we have limited knowledge.

Good evidence also suggests that exercise improves cognition and mood, as summarized in a meta-analysis by Kristin Szuhany and colleagues. A protein that appears to play a prominent role in training-induced adaptations of the brain and to mediate training-induced improvements in mental function is **brain-derived neurotrophic factor**, or **BDNF**. This protein is synthesized in the brain, where it supports the growth and survival of existing neurons. In addition, it promotes the differentiation of neural stem cells into new neurons. BDNF is also synthesized in other tissues, including muscle.

BDNF is upregulated by exercise. Studies in mice have shown that physical activity increases BDNF in the hippocampus and that this effect is crucial for the formation, survival, and function of new neurons. The hippocampus is a part of the brain, present in each of its two hemispheres, that is important for learning and memory. In humans, the meta-analysis conducted by Szuhany and colleagues concluded that acute exercise increases the plasma BDNF concentration and that this effect is intensified by training. In addition, Kirk Erickson and coworkers found that endurance training for one year increased the size of the hippocampus in older humans and that this increase was associated with improved memory and higher circulating BDNF

concentration. The brain is the main source of circulating BDNF in response to exercise; muscle BDNF does not seem to enter the circulation.

The effect of acute and chronic exercise on BDNF seems to be smaller in women than in men. Remarkably, regular exercise may be more effective in raising the BDNF concentration in psychiatric patients than in healthy individuals, as suggested in the meta-analysis by Szuhany and colleagues. The ability of regular exercise to raise the low resting BDNF concentration in depressed patients seems to be similar to that of antidepressant drugs. This increase may be the mechanism of the modest beneficial effect of regular exercise on depression according to evidence summarized by Bente Klarlund Pedersen and Bengt Saltin. Exercise and training of either the endurance or the resistance type can upregulate BDNF, although more studies support the effects of endurance than of resistance training.

Pedersen and Saltin also summarize evidence for a moderate beneficial effect of regular exercise on anxiety, stress, and schizophrenia. Exercise seems to achieve this effect through the distraction that diverts the patients' attention away from these diseases or their symptoms.

15.13 Detriments of Physical Inactivity

Like obesity, **physical inactivity** has been on the rise in recent decades. WHO estimates that 31% of the global population aged 15 and over were insufficiently active in 2008. The limit between sufficiently and insufficiently active was set at 150 min of moderate-intensity activity (or less in the case of vigorous activity) per week, accumulated across work, home, transport, and leisure.

The high prevalence of inactivity can be attributed to several factors related to technological advances. For one, people spend more time sitting during occupational and domestic activities because of the mechanization and automation of many work-related and household activities. In addition, increased urbanization has created an environment that discourages physical activity. People prefer to use passive, rather than active, means of transportation (for example, cars rather than bicycles). Finally, people have decreased participation in physical activity during leisure time, showing a

preference for television viewing or computer-based sitting activities over outdoor activities.

Ample and convincing epidemiological evidence indicates that physical inactivity increases morbidity and mortality. Specifically, inactive individuals (also referred to as *sedentary* in the literature) have higher prevalence of CVD, T2D, breast cancer, colorectal cancer, dementia, depression, and anxiety. WHO estimates that some 3.2 million deaths are attributable to inactivity worldwide each year. Unfortunately, as Booth and associates note, the detrimental effects of chronic inactivity on health remain silent for years before they reach overt clinical symptoms. Thus, it is imperative not to wait for disease to settle in but to prevent it through lifelong exercise.

Unsurprisingly, physical inactivity exerts a detrimental effect on CRF: Comparisons between inactive and active individuals have yielded large differences in $\dot{V}O_2max$ (lower in the former by about one third) over a wide age span (20 to 80). Low CRF is associated with increased prevalence of hypertension, dyslipidemia, inflammation, insulin resistance, central obesity, metabolic syndrome, and T2D, as well as increased mortality, as reviewed by Booth and associates.

Equally unsurprising, physical inactivity exerts detrimental effects on muscle mass and strength. Studies in both experimental animals and humans show a rapid decline in the rate of protein synthesis when muscles remain inactive (figure 15.9). In humans, the rate of muscle mass loss is about 0.5% per day, much higher than the rate of muscle mass gain with resistance training (0.1% to 0.2%; section 12.12). The term **muscle atrophy** is used as the antipode of *muscle hypertrophy*.

The mechanisms explaining how physical inactivity exerts its detrimental effects on health have been studied less than the mechanisms explaining how physical activity exerts its beneficial effects. One could argue that the former are the reverse of the latter, and this is certainly the case in many instances. However, there may be more to inactivity. Let’s consider two examples.

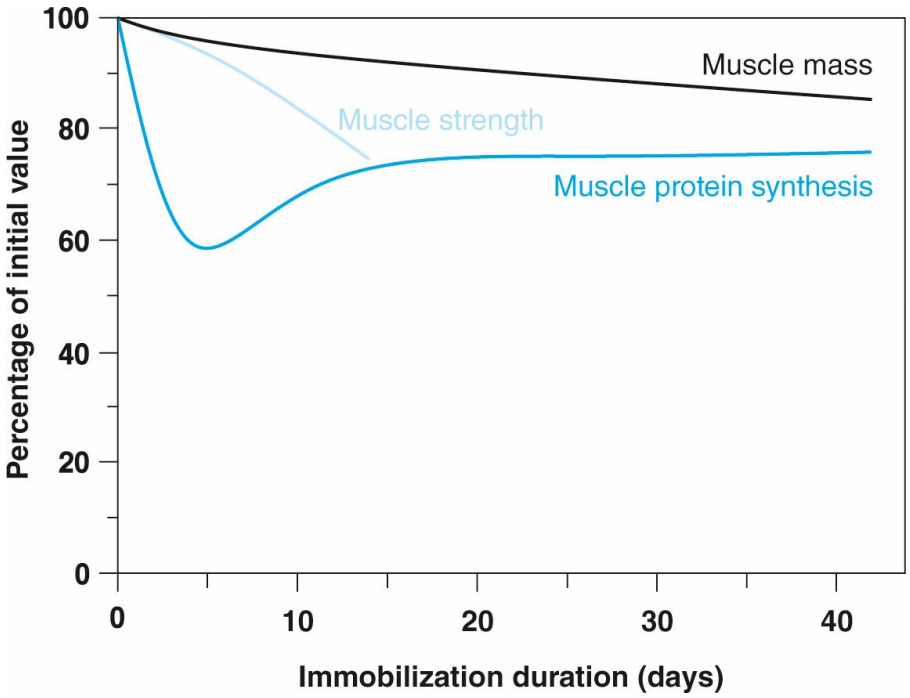


Figure 15.9 **Physical inactivity compromises muscle size and function.** Muscle protein synthesis, muscle mass, and muscle strength show rapid declines in humans after immobilization of a leg. The graph has been created on the basis of studies reviewed by Booth and associates and by Dirks and associates. Direct data are lacking about the effect of inactivity on muscle proteolysis.

The first example relates to CVD. One would expect that, since physical activity lowers CVD risk by promoting, among other things, vasodilation (section 15.4), physical inactivity would raise the risk simply by not promoting vasodilation. However, Dick Thijssen and colleagues have reviewed evidence that, in addition, inactivity promotes the opposite effect, **vasoconstriction**.

The second example relates to osteoporosis. Again, one would expect that, since physical activity lowers osteoporosis risk by promoting bone formation (section 15.11), physical inactivity would raise the risk simply by not promoting bone formation. However, it seems that osteocytes sense the reduced load placed on the bones of inactive persons and signal increased bone resorption.

Therefore, inactivity may augment morbidity and mortality not only passively but also actively. By doing so, it speeds up biological aging, which brings us to the next section.

15.14 Exercise for Healthy Aging and Longevity

Aging is a natural, inevitable process for humans and other species. It is not a disease, but it constitutes a risk factor for many diseases because the function of practically all systems in the body gradually declines after the second or third decade of life, for largely unknown reasons. This decline exerts a negative effect on physical, mental, and social functions. Age-related changes include the following:

- Decline in CRF ($\dot{V}O_2max$)
- Decline in HRmax
- Deterioration of the lipidemic profile (see sections 17.6 and 17.7)
- Reduction in insulin sensitivity
- Increased atherosclerosis and hypertension
- Decline in muscle mass and strength, referred to as **sarcopenia** ("lack of flesh" in Greek)
- Decline in muscle mitochondrial function
- Attenuation of the anabolic responses to protein intake and resistance exercise in muscle
- Reduction in BMD, leading to osteoporosis and loss of stature
- Emergence of osteoarthritis
- Decline in cognition, which may lead to **dementia**, the most common type of which is **Alzheimer's disease**, involving the degeneration of neurons in the brain
- Emergence of **Parkinson's disease**, another neurodegenerative disease, characterized by trembling, rigidity, and difficulty in walking, mainly due to death of cells in the brain's basal ganglia (figure 7.1)
- Reduced visual and auditory acuity
- Decline in female fertility, culminating at menopause
- Loss of skin elasticity and appearance of wrinkles
- Increased hair loss and discoloration

Several of these changes elicit reduced mobility and increase one's dependence on assistance in performing activities of daily living. This situation is encapsulated in the term **frailty**.

The number of aged individuals as a percentage of the global population has been on the rise throughout the second half of the 20th century and during the current century. Between 1950 and 2010, the proportion of people aged 60 and over increased from 8% to 11% (according to Gene Shackman and colleagues) thanks to increased **life expectancy**. The estimated average life expectancy at birth worldwide has risen from 65.3 years in 1990 to 71.5 years in 2013, according to GBD 2013 DALYs and HALE Collaborators.

The increase in life expectancy is primarily due to public health measures, followed by improved environment and nutrition. During the same period, the so-called **healthy life expectancy** (defined as the average number of years a person can expect to live in full health, that is, excluding years lived in disease, injury, or both) rose from 56.9 to 62.3 years. Thus, healthy life expectancy (also referred to by the shorter term **healthspan**) amounts to 87% of life expectancy (or **lifespan**), leaving out eight to nine years in poor health.

Naturally, these figures are averages that do not distinguish physically active from inactive individuals. The question then arises, "Does lifespan or healthspan differ between the two categories?" The answer seems to be yes: Studies, summarized by Duck-chul Lee and collaborators, confirm that regular exercise promotes longevity. Specifically, researchers estimate that active individuals live about three years longer than sedentary individuals. It is unclear how many years one must exercise in order to reap this benefit.

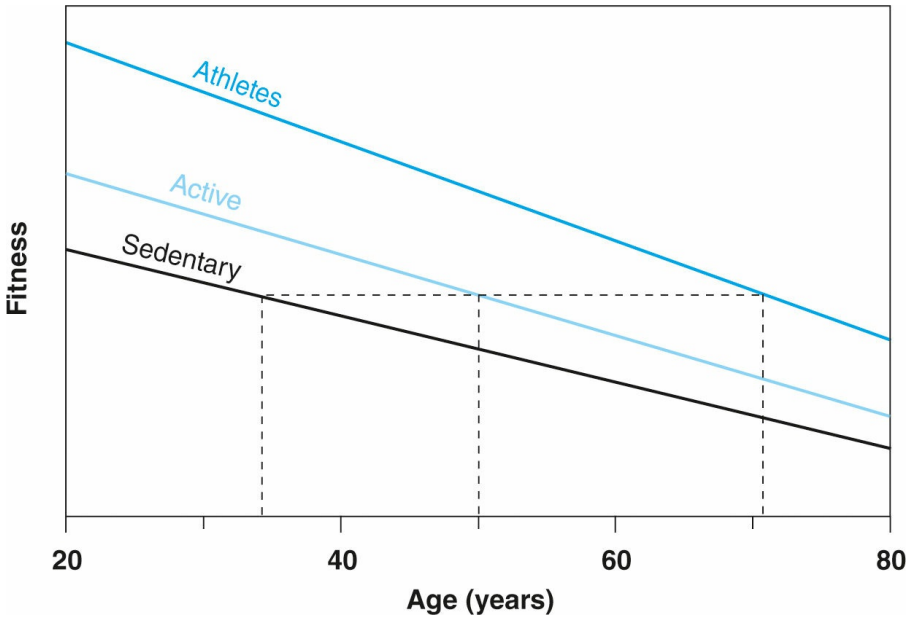


Figure 15.10 **Lifelong exercise delays the decline in fitness.** As we age, physical fitness (expressed in terms of factors such as $\dot{V}O_2$max, muscle strength, and speed) declines in a roughly linear fashion. However, the line corresponding to physically active individuals lies above the line corresponding to sedentary ones, and the line corresponding to lifelong athletes lies even higher. As a result, at any age, athletes have higher fitness than do other active individuals, and the latter have higher fitness than do sedentary individuals. What is more, a given fitness level (horizontal broken line) is seen in young sedentary individuals, middle-aged active individuals, and old athletes—and the difference between the former and the latter spans three to four decades. In effect, active people and athletes look biologically younger than do inactive people; conversely, inactive people look biologically older than do active people and athletes.

Another issue that remains unclear is the effect of physical activity on healthspan, since specific data are lacking. Nevertheless, it is reasonable to assume that regular exercise increases healthspan as well as lifespan by fighting chronic disease in the ways discussed in previous sections. In addition, regular exercise delays the usual declines in CRF, muscle mass, and muscle strength as we age. As a result, at any age, exercisers have higher fitness values than do sedentary individuals. In other words, the fitness level of a young sedentary individual is also the fitness level of an older exerciser (figure 15.10). This difference is particularly evident in lifelong athletes, the so-called **master athletes**. Regular exercise does not, however, affect the decline in HRmax, which, therefore, can be roughly calculated as 220 – age regardless of training state.

Physical activity promotes healthy aging also by slowing the progression of neurodegenerative diseases such as dementia and Parkinson's disease. A multitude of studies, summarized by Pedersen and Saltin, have shown that regular exercise prevents Alzheimer's disease and, in general, dementia. However, evidence is limited for an effect on cognitive function or on the ability to perform activities of daily living in people who have already developed the disease. Physical activity may protect against dementia by increasing BDNF, as described in section 15.12. There is also evidence for a positive effect of training on motor performance in Parkinson's patients, although the mechanism is unknown.

15.15 Benefits From Regular Exercise in Regard to Other Diseases

I have discussed the chronic noncommunicable diseases that are accompanied by the strongest evidence for a therapeutic effect of regular exercise. People who suffer from other diseases may also benefit from physical activity, albeit in a nonspecific manner. That is, physical activity may alleviate suffering not by targeting the specific biochemical mechanisms responsible for the disease but by improving CRF and muscle strength, just as it does in healthy individuals. These diseases include the following:

- **Chronic obstructive pulmonary disease**, a common chronic respiratory disease characterized by breathlessness due to gradual and irreversible decrease in lung function
- **Bronchial asthma** (or plain **asthma**), another common chronic respiratory disease characterized by occasional constriction of the bronchi due to inflammation
- Osteoarthritis, the degenerative disease of the joints mentioned in section 15.8 as a consequence of obesity
- **Rheumatoid arthritis**, an autoimmune inflammatory disease of the joints that results in severe disability and pain, while often extending its devastating effects to the heart, vessels, lungs, and skin
- **Multiple sclerosis**, the debilitating neurological disease described in

section 7.4

Exercise may also exert an analgesic (that is, pain-relieving) effect on diseases such as osteoarthritis and rheumatoid arthritis. This effect may occur either through improvement in joint mobility and muscle function or as a direct effect on the sensation of pain, although the mechanism of such an effect is not known. You may find a detailed discussion of the role of training in the diseases listed here in the paper by Pedersen and Saltin.

Because these diseases usually compromise the patient's physical abilities, regular exercise is a valuable addition to the interventions (pharmacological, surgical, dietary, and behavioral) aimed at alleviating the symptoms. Thus, *exercise is medicine* in numerous diseases, including those for which it can lessen the burden on the body and mind, as well as those for which it directly affects the etiology.

15.16 A Final Word on the Value of Exercise

Pamela Das and Richard Horton make the insightful remark that we should not view physical activity only in the context of promoting health and fighting disease, because the benefits of physical activity are even more far-reaching: "Being physically active is a major contributor to one's overall physical and mental well-being. Positive outcomes include a sense of purpose and value, a better quality of life, improved sleep, and reduced stress, as well as stronger relationships and social connectedness. Additionally, promoting active modes of travel, such as walking and cycling, are good for the environment, which, in turn, also has a positive impact on health."

All in all, exercise is an excellent weapon in our hands (and legs). It effectively safeguards health against disease and improves health if disease has settled in. It does so at no cost or, at any rate, at a cost that we can control, in the sense that we can choose how much to spend on sporting gear, facilities, and services. This control is not usually the case with medical costs, wherein others determine how much we need to spend in order to get proper treatment. Exercise is also safe as long as we perform it under proper supervision or after having received expert instruction and as long as we avoid excesses that may lead to injury or overtraining, a topic that I will

address at the end of this book.

Summary

Regular exercise is a powerful weapon against morbidity and mortality from chronic diseases, including CVD, cancer, diabetes, obesity, osteoporosis, and mental disease. Regular exercise fights CVD by protecting both the heart and blood vessels. The heart becomes stronger by hypertrophy, possibly through the stimulation of the PI3K cascade by IGF1, and resists heart attacks. The vessels experience less atherosclerosis thanks to an improvement in the lipidemic profile and enjoy better dilation thanks to increased production of NO. Training also promotes muscle capillarization through upregulation of VEGF. Physical activity lowers the risk of many types of cancer, in part by increasing p53, a tumor suppressor and guardian of the integrity of the genome, and in part by boosting immune surveillance by NK cells.

Diabetes involves a major metabolic upset of the body, with life-threatening consequences. Fortunately, acute exercise increases the migration of GLUT4 to the plasma membrane of muscle fibers, thus lowering plasma glucose without the need for insulin, an effect that lasts one to three days. Chronic exercise augments the muscle GLUT4 content, thus aiding in the control of hyperglycemia. In addition, training may enhance the PI3K cascade, thus stimulating the action of insulin. Also, by improving blood flow, training increases the delivery of insulin, glucose, and fatty acids to tissues.

Obesity raises morbidity and mortality in several ways, including inflammation by cytokines, resulting in insulin resistance, atherosclerosis, and cancer. Visceral obesity is particularly harmful and is linked to the metabolic syndrome and NAFLD. Adiponectin, a highly beneficial hormone, is reduced in obesity, which contributes to morbidity. Regular exercise fights

obesity, primarily through the increase in energy expenditure during each exercise session. In addition, regular exercise may alleviate the detrimental effects of obesity by inducing the secretion of anti-inflammatory myokines from muscle.

Regular exercise promotes bone health by increasing BMD, thus helping to prevent osteoporosis. Physical activity fights mental dysfunction by upregulating several beneficial biomolecules. One of them, BDNF, induces the formation of new neuronal circuits and neurons, resulting in improved cognition and mood, with potentially beneficial effects on depression, anxiety, stress, and schizophrenia. Physical inactivity is a risk factor for CVD, T2D, cancer, and mental disease. In addition, it reduces fitness and accelerates biological aging. Indeed, sedentary individuals, as compared with active individuals and athletes, have lower fitness levels at all ages, suffer from more chronic diseases, and live shorter lives. Exercise is an effective, low-cost, and safe medicine.

Problems and Critical Thinking Questions

1. List beneficial effects of regular exercise on the cardiovascular system and ways in which regular exercise reduces the risk of CVD.
2. To substantiate the role of epinephrine in the exercise-induced mobilization of NK cells against tumors, Pedersen and coworkers repeated the experiment described in figure 15.5, with the following modification: They administered propranolol, a drug that blocks the β-adrenergic receptor, daily to both the sedentary and exercising mice. What do you think was the outcome that led the researchers to confirm the role of epinephrine?
3. Summarize the ways in which exercise fights diabetes.
4. How does regular exercise help us lose weight, and how does it fight the health consequences of obesity?

5. According to the information presented in the highlight box (titled Where Is Body Weight Lost?), is most of our fat lost as CO_2 or H_2O?
6. During their long trips in the desert, camels rely on fat stored in their humps as a source of energy. Is it just energy that they get from fat? Explain your answer.
7. How can exercise prevent or fight osteoporosis? What types of training would you recommend for this purpose?
8. How does exercise improve brain structure and function?
9. How is physical inactivity detrimental to health?
10. Lee and coworkers address the argument that active people live longer by the same amount of time for which they have exercised throughout their lives and that exercise may not be worthwhile because the longevity bonus is negated by an equal amount of time spent exercising. Setting aside the insinuation that exercise is an unpleasant thing (one that you do by compulsion), calculate how much time you would need to exercise from the age of 6 until the age of 72 (the current average lifespan) if you spend 150 min per week on moderate-intensity activities. How does that compare with the three years of life that you would gain?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

health
incidence
prevalence
morbidity
mortality
cardiovascular disease, CVD

atherosclerosis
hypertension
atherosclerotic plaque
thrombosis
dyslipidemia
cardiac myocyte
myocardium
ischemia-reperfusion injury
endothelium
endothelial nitrogen oxide synthase, eNOS
soluble guanylate cyclase
cGMP-dependent protein kinase, PKG
vasodilation
flow-mediated dilation
capillary density
vascular endothelial growth factor, VEGF
VEGF receptor
cancer
tumor
metastasis
p53
apoptosis
natural killer cell, NK cell
cardiorespiratory fitness, CRF
diabetes mellitus
type 1 diabetes, T1D
type 2 diabetes, T2D
insulin resistance
hyperglycemia
ketoacidosis

insulin sensitivity
obesity
leptin
ghrelin
glucagon-like peptide 1, GLP1
peptide YY, PYY
orexigenic
anorexigenic
leptin resistance
osteoarthritis
inflammation
cytokine
tumor necrosis factor α, TNFα
interleukin 6, IL6
interleukin 1β, IL1β
C-reactive protein
central obesity
metabolic syndrome
nonalcoholic fatty liver disease, NAFLD
adiponectin
adipocytokine, adipokine
AMPK
adiponectin receptor
fat-but-fit paradox
myokine
interleukin 1 receptor antagonist, IL1ra
interleukin 10, IL10
irisin
osteoporosis
bone mineral density, BMD

resorption
osteoblast
osteoclast
osteocyte
neurogenesis
brain-derived neurotrophic factor, BDNF
physical inactivity
muscle atrophy
vasoconstriction
sarcopenia
dementia
Alzheimer's disease
Parkinson's disease
frailty
life expectancy, lifespan
healthy life expectancy, healthspan
master athlete
chronic obstructive pulmonary disease
bronchial asthma, asthma
rheumatoid arthritis
multiple sclerosis

References and Suggested Readings

Alexandre L, Long E, Beales IL (2014). Pathophysiological mechanisms linking obesity and esophageal adenocarcinoma. *World Journal of Gastrointestinal Pathophysiology* 5: 534-549.

Arem H, Moore SC, Patel A, Hartge P, Berrington de Gonzalez A, Visvanathan K, Campbell PT, Freedman M, Weiderpass E, Adami HO, Linet MS, Lee IM, Matthews CE (2015). Leisure time physical activity and mortality: A detailed pooled analysis

of the dose-response relationship. *JAMA Internal Medicine* 175: 959-967.

Bernardo BC, Ooi JYY, Weeks KL, Patterson NL, McMullen JR (2018). Understanding key mechanisms of exercise-induced cardiac protection to mitigate disease: Current knowledge and emerging concepts. *Physiological Reviews* 98: 419-475.

Booth FW, Roberts CK, Thyfault JP, Ruegsegger GN, Toedebusch RG (2017). Role of inactivity in chronic diseases: Evolutionary insight and pathophysiological mechanisms. *Physiological Reviews* 97: 1351-1402.

Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Boström EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Højlund K, Gygi SP, Spiegelman BM (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481: 463-468.

Caselli C, D'Amico A, Cabiati M, Prescimone T, Del Ry S, Giannessi D (2014). Back to the heart: The protective role of adiponectin. *Pharmacological Research* 82: 9-20.

Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, Horton ES, Castorino K, Tate DF (2016). Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. *Diabetes Care* 39: 2065-2079.

Das P, Horton R (2012). Rethinking our approach to physical activity. *Lancet* 380: 189-190.

Dirks ML, Wall BT, van Loon LJC (2017). Interventional strategies to combat muscle disuse atrophy in humans: Focus on neuromuscular electrical stimulation and dietary protein. *Journal of Applied Physiology* 125: 850-861.

Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK (2009). Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for

adults. *Medicine and Science in Sports and Exercise* 41: 459-471.

Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM, Wojcicki TR, Mailey E, Vieira VJ, Martin SA, Pence BD, Woods JA, McAuley E, Kramer AF (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America* 108: 3017-3022.

Fox J, Rioux BV, Goulet EDB, Johanssen NM, Swift DL, Bouchard DR, Loewen H, Sénéchal M (2017). Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: A meta-analysis. *Scandinavian Journal of Medicine and Science in Sports* 28: 16-28.

GBD 2013 DALYs and HALE Collaborators (2015). Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013: Quantifying the epidemiological transition. *Lancet* 386: 2145-2191.

GBD 2015 Obesity Collaborators (2017). Health effects of overweight and obesity in 195 countries over 25 years. *New England Journal of Medicine* 377: 13-27.

Green DJ, Hopman MTE, Padilla J, Laughlin MH, Thijssen DHJ (2017). Vascular adaptation to exercise in humans: Role of hemodynamic stimuli. *Physiological Reviews* 97: 495-528.

Higgins KA, Park D, Lee GY, Curran WJ, Deng X (2014). Exercise-induced lung cancer regression: Mechanistic findings from a mouse model. *Cancer* 120: 3302-3310.

Hojman P, Gehl J, Christensen JF, Pedersen BK (2018). Molecular mechanisms linking exercise to cancer prevention and treatment. *Cell Metabolism* 27: 10-21.

Howe TE, Shea B, Dawson LJ, Downie F, Murray A, Ross C, Harbour RT, Caldwell LM, Creed G (2011). Exercise for

preventing and treating osteoporosis in postmenopausal women. *Cochrane Database of Systematic Reviews* 4: CD000333.

Jelleyman C, Yates T, O'Donovan G, Gray LJ, King JA, Khunti K, Davies MJ (2015). The effects of high-intensity interval training on glucose regulation and insulin resistance: a meta-analysis. *Obesity Reviews* 16: 942-961.

Katsagoni CN, Georgoulis M, Papatheodoridis GV, Panagiotakos DB, Kontogianni MD (2017). Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism* 68: 119-132.

Kim JK (2012). Endothelial nuclear factor κB in obesity and aging: Is endothelial nuclear factor κB a master regulator of inflammation and insulin resistance? *Circulation* 125: 1081-1083.

Lee DC, Brellenthin AG, Thompson PD, Sui X, Lee IM, Lavie CJ (2017). Running as a key lifestyle medicine for longevity. *Progress in Cardiovascular Diseases* 60: 45-55.

Louie SM, Roberts LS, Nomura DK (2013). Mechanisms linking obesity and cancer. *Biochimica et Biophysica Acta* 1831: 1499-1508.

Meerman R, Brown AJ (2014). When somebody loses weight, where does the fat go? *British Medical Journal* 349: g7257.

Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, Keadle SK, Arem H, Berrington de Gonzalez A, Hartge P, Adami HO, Blair CK, Borch KB, Boyd E, Check DP, Fournier A, Freedman ND, Gunter M, Johannson M, Khaw KT, Linet MS, Orsini N, Park Y, Riboli E, Robien K, Schairer C, Sesso H, Spriggs M, Van Dusen R, Wolk A, Matthews CE, Patel AV (2016). Association of leisure-time physical activity with risk of 26 types of cancer in 1.44 million adults. *Journal of the American Medical Association Internal Medicine* 176: 816-825.

Ortega FB, Ruiz JR, Labayen I, Lavie CJ, Blair SN (2017). The Fat but Fit paradox: What we know and don't know about it. *British Journal of Sports Medicine* 52: 151-153.

Paneni F, Beckman JA, Creager MA, Cosentino F (2013). Diabetes and vascular disease: Pathophysiology, clinical consequences, and medical therapy: Part I. *European Heart Journal* 34: 2436-2443.

Pedersen BK (2017). Anti-inflammatory effects of exercise: Role in diabetes and cardiovascular disease. *European Journal of Clinical Investigation* 47: 600-611.

Pedersen BK, Saltin B (2015). Exercise as medicine—evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scandinavian Journal of Medicine and Science in Sports* 25 Supplement 3: 1-72.

Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, Johannesen HH, Becker JC, Pedersen KS, Dethlefsen C, Nielsen J, Gehl J, Pedersen BK, Thor Straten P, Hojman P (2016). Voluntary running suppresses tumor growth through epinephrine- and IL-6-dependent NK cell mobilization and redistribution. *Cell Metabolism* 23: 554-562.

Sahakyan KR, Somers VK, Rodriguez-Escudero JP, Hodge DO, Carter RE, Sochor O, Coutinho T, Jensen MD, Roger VL, Singh P, Lopez-Jimenez F (2015). Normal-weight central obesity: Implications for total and cardiovascular mortality. *Annals of Internal Medicine* 163: 827-835.

Schubert MM, Desbrow B, Sabapathy S, Leveritt M (2013). Acute exercise and subsequent energy intake. A meta-analysis. *Appetite* 63: 92-104.

Shackman G, Wang X, Liu YL (2012). Brief review of world demographic trends—trends in age distributions. Global Social Change Research Project. http://gsociology.icaap.org/report/demsumAging.html.

Szuhany KL, Bugatti M, Otto MW (2015). A meta-analytic review

of the effects of exercise on brain-derived neurotrophic factor. *Journal of Psychiatric Research* 60: 56-64.

Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, Green DJ (2010). Impact of inactivity and exercise on the vasculature in humans. *European Journal of Applied Physiology* 108: 845-875.

Vega RB, Konhilas JP, Kelly DP, Leinwand LA (2017). Molecular mechanisms underlying cardiac adaptation to exercise. *Cell Metabolism* 25: 1012-1026.

Wagenmakers AJ, Strauss JA, Shepherd SO, Keske MA, Cocks M (2016). Increased muscle blood supply and transendothelial nutrient and insulin transport induced by food intake and exercise: Effect of obesity and ageing. *Journal of Physiology* 594: 2207-2222.

Wiggs MP, Duarte AF, Powers SK (2015). Exercise can protect against a broken heart. *Current Sports Medicine Reports* 14: 6-8.

World Health Organization (n.d.). Constitution of WHO. www.who.int/about/mission.

World Health Organization (n.d.). World Health Statistics 2017. http://who.int/gho/publications/world_health_statistics/2017.

PART IV

Biochemical Assessment of Exercisers

“Read and strive and fight,” he said. “Each to his own weapons,” he said.

Odysseus Elytis (Nobel Prize in Literature, 1979), *Worthy It Is*

Based on the vast array of exercise-induced biochemical changes (both acute and chronic) that we examined in part III, we can collect precious information about an exerciser by measuring a variety of biomolecules in his or her body. I will use the term *biochemical assessment of exercisers* to describe measurements performed not (or mainly not) for research purposes but, rather, for evaluating the condition of the exercising individual. Such assessment interests primarily athletes and the professionals who support them: coaches, trainers, physicians, physical therapists, and dietitians. Nevertheless, people who exercise for recreational or therapeutic purposes can also benefit from biochemical tests.

Blood is the most suitable biological material for the biochemical assessment of exercisers, since it is relatively easy to sample and provides a satisfactory amount of information. Naturally, muscle tissue can provide more information, because muscle is the primary setting of energy metabolism. In addition, one can measure in muscle a number of substances

that do not appear in blood (for example, phosphocreatine and glycogen). However, muscle biopsy is painful and requires highly specialized personnel, as well as advanced analytical techniques for the measurement of analytes in small samples. Urine and saliva are sometimes used to measure substances that exhibit concentration changes with exercise. However, their utility in assessing exercisers has yet to be established.

Of the hundreds of biochemical parameters that can be measured in blood and are treated by **clinical chemistry**, I present those that I consider the most useful in assessing an exerciser. Measurement of these parameters is relatively easy, because there are commercially available kits and automated instruments (analyzers) that produce fast, accurate, and reliable results at a low to moderate cost. In addition, there are portable analyzers for parameters, such as lactate and glucose, that enable measurements during exercise in almost any setting. For each parameter, we will discuss the information it provides, its usual values, the factors influencing its values, and the importance of measuring it in exercisers.

IV.1 Blood

Before I begin presenting the parameters that we recruit for the biochemical assessment of exercisers, I need to say a few words about the biological specimen we use. Blood is not a homogeneous fluid. Hence, if we obtain a small amount of it by **venipuncture** (that is, piercing of a vein with a needle) and centrifuge it, blood will separate into roughly two parts of different densities: a dark-red sediment and a yellowish supernatant (figure IV.1). The sediment contains mainly erythrocytes. The supernatant is usually clear and occupies a larger volume than the sediment. The supernatant is called **serum** or **plasma**, depending on how the blood was collected.

When blood is collected after a fatty meal, the supernatant is not clear; rather, it is turbid, due to the presence of chylomicrons (section 11.1). Because of their large size, chylomicrons scatter light and give the supernatant a cloudy appearance.

What is serum, and what is plasma? Blood is usually drawn with a syringe and decanted into a test tube, or it is drawn directly into an evacuated test

tube. As soon as blood contacts surfaces other than its natural surroundings (the vessel walls), it begins to coagulate, forming a clot. Coagulation is usually complete within 30 min at room temperature. If we subsequently centrifuge the blood sample, the clot is squeezed to the bottom of the test tube. The supernatant in this case is what we call *serum*.

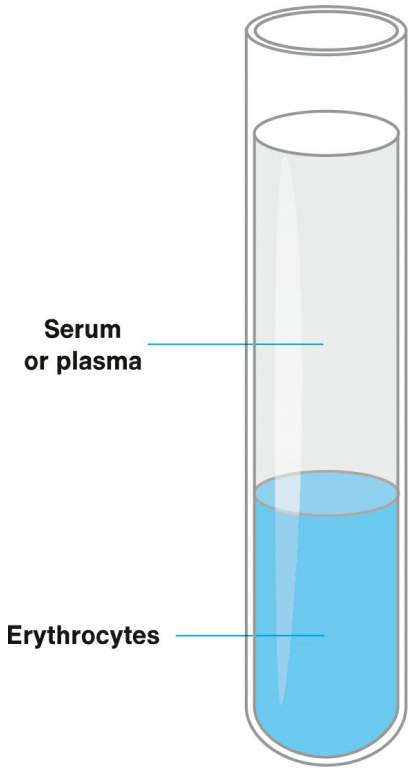


Figure IV.1 Separation of blood components. A test tube filled with blood presents two distinct layers after centrifugation. Erythrocytes form most of the sediment, whereas serum (if blood has clotted) or plasma (if clotting has been prevented) forms the supernatant.

Alternatively, we can prevent coagulation by collecting blood in a test tube containing an **anticoagulant**. Anticoagulants are substances that inhibit one or more steps in the complex coagulation process; the most frequently used anticoagulants are **heparin** and **ethylenedinitrilotetraacetate**, or **EDTA**. Blood containing an anticoagulant will not clot, and we can centrifuge it immediately. In this case, we call the supernatant *plasma*, the same as the natural fluid that runs in our vessels.

Serum and plasma differ with respect to certain components, but most substances have the same concentration in the two fluids and can be

measured equally well in both. In some cases, we prefer plasma, and in some others we use whole blood. I will point out these cases as we go.

IV.2 Aims and Scope of the Biochemical Assessment

The biochemical assessment of exercisers has two main aims:

- Protecting or improving health
- Increasing performance

The former goal refers to all exercisers, whereas the latter applies primarily to athletes. Good health is the sound foundation on which sport performance is built. Thus, it must be the primary concern of a biochemist—and any professional, for that matter—who monitors athletes.

As for the scope of the assessment, one can measure biochemical parameters in samples collected at different time points relative to exercise. We may discern three types of cases.

- Samples collected at rest. In this case, we examine the basal state of the body or the adaptations brought about in the basal state by previous exercise.
- Samples collected during exercise. Such samples inform us about acute exercise-induced changes when compared with samples collected at rest.
- Samples collected after exercise. “After” can range from a few seconds to hours or even days from the end of exercise. Depending on when exactly a sample is taken, it can show acute effects of exercise, duration of changes elicited by exercise, or changes with delayed onset. Of course, the farther we get from the end of exercise, the more we approach the first case.

IV.3 The Reference Interval

Suppose that the measurement of glucose in the serum of an athlete produced

a value of 90 mg · dL^{-1}. To decide whether this value is normal or abnormal, we usually ask, "How much should it be?" or "What are the normal values?" If we are told that normal values range from 74 to 106 mg · dL^{-1}, then we decide that 90 mg · dL^{-1} is a normal value. The range of 74 to 106 mg · dL^{-1} is the **reference interval** of glucose. We can therefore define the reference interval roughly as *the range of values that a parameter usually gets.* The lower and upper ends of the reference interval are the lower and upper **reference limits**.

How are reference intervals determined? Investigators do this by analyzing biological samples taken from an adequate and representative part of the population (typically, several hundred people). As you might expect, the value of a parameter differs from one individual to another. However, there is usually an accumulation of values around a central one, and the farther we get from it, the fewer values we encounter (figure IV.2). On such a **frequency plot**, we can locate the lower 2.5% and the upper 2.5% of the values and reject them as being extreme. The range that includes the remaining 95% of the values is the reference interval. Thus, the exact definition of the reference interval is *the range of values of a parameter in the vast majority (usually the central 95%) of a population.*

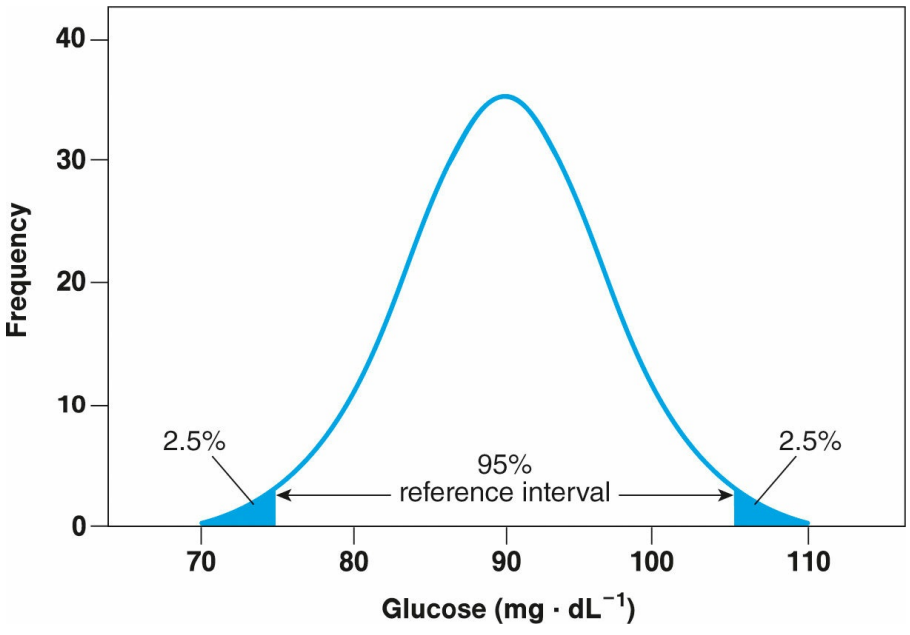


Figure IV.2 **The reference interval.** After measuring a biochemical parameter (such as the serum glucose concentration) in many individuals, we can construct a frequency plot showing

the number of individuals that have each value. By rejecting the lower and upper 2.5% of the values, we find the reference interval. The curve in this example depicts an ideal distribution called the *normal distribution,* which is not the case for all biochemical parameters.

You may have noticed that the reference interval is determined through a mathematical approach with no involvement of biochemistry (except, of course, for the measurement of the parameter). This is why I prefer the rather neutral and objective term *reference interval* instead of the more frequently used terms *normal values* and *normal range*. What I mean is that we are not certain whether the values exhibited by 95% of the population are normal or, conversely, whether the values exhibited by the remaining 5% are abnormal. Instead, the former are simply values that we refer to in order to make decisions. One case of reference intervals that are not normal (in the sense that they include values associated with increased health risks) is that of reference intervals of serum lipids (sections 17.6 and 17.7).

The reference interval of a parameter depends on two main factors: the method of measurement and the reference population.

- **Method of measurement.** Most parameters can be measured by use of more than one method, and different methods often produce different results. A frequent source of disagreement between methods is the occurrence of positive or negative errors because of substances coexisting with the substance of interest in the sample. Nevertheless, differences are generally small between acceptable methods of measuring a parameter. Although I provide reference intervals for most parameters discussed in part IV based on the scientific literature, you should rely on the reference intervals provided by the laboratory that has performed a test to evaluate yourself or an exerciser.
- **Reference population.** The values of many parameters depend on factors such as sex and age and thus have different reference intervals for different population groups. When this is the case, I cite reference intervals for both sexes and for the entire age range of exercisers (from children to older people). A parameter may also be influenced by an individuals' way of life, including diet and physical activity. This is particularly true of athletes, as I will note on several occasions.

I close this section by clarifying two more issues regarding reference intervals. First, in clinical chemistry, the concentration of a substance is usually expressed as mass per volume (for example, mg · dL^{-1}), although an effort is being made to establish units of chemical quantity per volume (for example, mmol · L^{-1}). Where the mass per volume unit is more customary, I cite reference intervals in both kinds of units, along with information about how to convert mass per volume into chemical quantity per volume.

Table IV.1 Parameters Useful in Biochemical Assessment of Exercisers

Class	Parameters
Iron status	Hemoglobin
	Iron
	Total iron-binding capacity
	Transferrin saturation
	Soluble transferrin receptor
	Ferritin
Metabolites	Lactate
	Glucose
	Triacylglycerols (triglycerides)
	Total cholesterol
	HDL cholesterol
	LDL cholesterol
	Glycerol
	Urea
	Ammonia
	Creatinine
	Uric acid
	Glutathione
Enzymes	Creatine kinase
	γ-Glutamyltransferase
	Antioxidant enzymes
Hormones	Cortisol
	Testosterone

Second, because the values of many biochemical parameters are influenced by food intake, reference intervals refer to the fasted state (approximately 12 h after the last meal), the one that I named *postabsorptive*

state in section 10.29.

IV.4 Classes of Biochemical Parameters

To facilitate examination of the parameters that are useful in biochemical assessment of exercisers, I have divided the parameters into four classes:

- Parameters of the iron status
- Metabolites
- Enzymes
- Hormones

Table IV.1 summarizes the parameters that we will examine within the framework of these classes.

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

clinical chemistry
venipuncture
serum
plasma
anticoagulant
heparin
ethylenedinitrilotetraacetate, EDTA
reference interval
reference limit
frequency plot

CHAPTER 16

Iron Status

Learning Objectives

After reading this chapter, you should be able to do the following:

- List parameters of iron status that are useful in biochemical assessment of exercisers.
- Define each of these parameters.
- Describe what each parameter shows.
- Discuss the factors that influence each parameter.
- Explain the importance of measuring these parameters in exercisers.
- Define anemia, iron deficiency, and iron deficiency anemia.
- Discuss whether athletes have higher prevalence of these disorders.

Iron holds a central place in oxygen transport, storage, and utilization for aerobic ATP resynthesis. This importance derives from the fact that iron is part of heme, the prosthetic group of myoglobin (section 3.10), hemoglobin (section 3.11), and proteins of the electron-transport chain (section 10.14). In

fact, iron is precisely the element in heme that binds O_2, and the iron content of tissues is one of the factors that determines the aerobic capacity of an individual. As a result, given the great importance of aerobic metabolism during exercise—even hard or maximal exercise—the adequacy of iron in the body is closely linked to health, fitness, sport performance, and recovery from exercise, as highlighted throughout part III. Thus, the interest of athletes in iron adequacy is fully justifiable.

Several parameters are used to assess **iron status**, that is, the concentration of iron in the various compartments of the body. We will examine six of these parameters:

- Hemoglobin
- Iron
- Total iron-binding capacity
- Transferrin saturation
- Soluble transferrin receptor
- Ferritin

Why do we need this many parameters to assess the status of a single chemical element? The reason is that each parameter sheds light on a different facet of iron status.

- Hemoglobin concentration shows the capacity of blood to absorb atmospheric oxygen in the lungs and carry it to the tissues.
- Iron concentration (in serum) informs us about the amount of iron available for uptake by the tissues.
- Total iron-binding capacity is the amount of iron that plasma can carry.
- Transferrin saturation and soluble transferrin receptor concentration are indices of the adequacy of iron in the body.
- Ferritin concentration reflects the amount of iron stored in the tissues.

Let’s examine these parameters one by one.

16.1 Hemoglobin

Hemoglobin is the oxygen-carrying protein in blood. It is found in the erythrocytes and is the most abundant component of blood (accounting for about 14% of its mass) next to water. A minimal amount of hemoglobin can be found in plasma as a result of the normal wear and tear of erythrocytes. Plasma hemoglobin does not take part in oxygen transport and is removed from the circulation by the liver.

We measure hemoglobin in whole blood after we have blocked coagulation by adding an anticoagulant. The reference interval depends on age and sex (table 16.1). Both the lower and upper reference limits rise as boys enter adolescence due to the anabolic action of testosterone, the secretion of which by the testes increases at that time (see section 18.7). Because females have little testosterone in their bodies throughout life, their blood hemoglobin concentration remains similar to that of boys—and lower than that of men.

Table 16.1 Reference Intervals of the Hemoglobin Concentration in Human Blood

Age (years)	Reference interval ($g \cdot dL^{-1}$)		Reference interval ($mmol\ globin \cdot L^{-1}$)[a]	
	Male	Female	Male	Female
5-8	11.5-14.5	11.5-14.5	7.2-9.1	7.2-9.1
9-11	12.0-15.0	12.0-15.0	7.5-9.4	7.5-9.4
12-14	12.0-16.0	11.5-15.0	7.5-10.0	7.2-9.4
15-17	12.3-16.6	11.7-15.3	7.7-10.4	7.3-9.6
18-44	13.2-17.3	11.7-15.5	8.3-10.8	7.3-9.7
45-64	13.1-17.2	11.7-16.0	8.2-10.8	7.3-10.0
65-74	12.6-17.4	11.7-16.1	7.9-10.9	7.3-10.1

[a]To convert $g \cdot dL^{-1}$ into $mmol \cdot L^{-1}$, divide by 1.6.

Data from Yip, Johnson, and Dallman (1984); Wu (2006).

Blood hemoglobin content increases if we ascend from sea level or low altitude to high altitude, where the oxygen concentration in the air is lower. As the $[O_2]$ in the air drops, so does the $[O_2]$ in blood, which promotes **erythropoietin** production in the kidneys. Erythropoietin is a protein hormone causing erythrocyte formation from progenitor cells in the bone marrow, followed by erythrocyte release to blood. As a result, hemoglobin mass and concentration increase. These changes are reversed upon returning to sea level or low altitude. The time frame of the positive and negative changes in hemoglobin varies widely across individuals, ranging generally from a few days to a few weeks.

As discussed in section 14.15, endurance training increases hemoglobin mass in the body. However, this increase is usually not reflected in the blood hemoglobin concentration, because of a concomitant increase in plasma volume due to increases in the size and number of blood vessels, as described in section 15.4.

Blood hemoglobin concentration (but not hemoglobin mass) increases during exercise, as body water is lost through sweating and plasma water is forced out of the vessels by the increased blood pressure. This effect is called **hemoconcentration**, although **hemocondensation** would be a more appropriate term. It lasts maximally 1 h after the end of exercise and makes the measurement of hemoglobin useful in calculating blood volume changes. For example, if the hemoglobin concentration is 14.6 $g \cdot dL^{-1}$ at rest and 15.2 $g \cdot dL^{-1}$ after exercise, then, since concentration is inversely proportional to volume, the blood volume after exercise, relative to rest, will be 0.96 (14.6/15.2), or 96% of the blood volume at rest. We then declare that there is a hemoconcentration of 4% (100 – 96).

Sometimes, mainly for research purposes, we are interested not in the *blood* volume change with exercise but in the *plasma* volume change. To calculate plasma volume changes, we need to consider changes in the volume of the blood cells. For this reason, and for the sake of the ensuing discussion on anemia, I will make a short detour to the hematologic parameters.

Hematologic Parameters

Hematologic parameters relate to the blood cells. They are measured in whole blood (in which we have blocked coagulation), and the most common ones are **hematocrit**, **erythrocyte count**, **leukocyte count**, and **platelet count**.

Hematocrit (meaning “judge of blood” in Greek), also referred to as **packed cell volume**, is the volume of the erythrocytes as a percentage of blood volume. The reference intervals are presented in table 16.2. As expected, hematocrit relates closely to blood hemoglobin concentration and displays the same variation with age and sex, being highest in adult males. Like hemoglobin, it shows the oxygen-carrying capacity of blood. Finally, exercise and high altitude have the same effects on hematocrit as they have on hemoglobin concentration.

Table 16.2 Reference Intervals of Hematocrit and Erythrocyte Count in Human Blood

	Hematocrit		Erythrocyte count ($M \cdot \mu L^{-1}$)[a]	
Age (years)	Male	Female	Male	Female
6-8	33-41	33-41	3.8-4.9	3.8-4.9
9-11	34-43	34-43	3.9-5.1	3.9-5.1
12-14	35-45	34-44	4.1-5.2	3.8-5.0
15-17	37-48	34-44	4.2-5.6	3.9-5.1
18-44	39-49	35-45	4.3-5.7	3.8-5.1
45-64	39-50	35-47	4.2-5.6	3.8-5.3
65-74	37-51	35-47	3.8-5.8	3.8-5.2

[a] $M \cdot \mu L^{-1}$: millions per microliter.
Data from Yip, Johnson, and Dallman (1984); Wu (2006).

The erythrocyte, leukocyte, and platelet counts express the number of the corresponding cells in 1 µL of blood. Erythrocytes are by far the most abundant blood cells and measure millions per microliter ($M \cdot \mu L^{-1}$). In contrast, leukocytes and platelets measure thousands per microliter ($k \cdot \mu L^{-1}$). Erythrocyte count goes hand in hand with hemoglobin concentration and hematocrit, being of the same utility. The reference intervals are shown in table 16.2.

Erythrocyte count, along with hemoglobin concentration and hematocrit, increases with **blood doping**, which involves transfusion of a large volume of blood into an athlete before an event. The same effect can be achieved through injection of erythropoietin or synthetic analogs of it. Both practices increase aerobic endurance performance and are prohibited in sport. The WADA Prohibited List includes blood doping as a prohibited method under the "manipulation of blood and blood components" category and erythropoietin as a prohibited substance under the "peptide hormones, growth factors, related substances, and mimetics" category.

Leukocyte count is indicative of the state of the immune system, of which leukocytes are part. The reference intervals are as follows:

- 4.5 to 13 $k \cdot \mu L^{-1}$ in children and adolescents
- 4.5 to 11 $k \cdot \mu L^{-1}$ in adults

Leukocyte count increases in cases of infection as part of the immune system's response to the threat posed by the pathogens; this condition is called **leukocytosis**. In contrast, leukocyte count decreases when the immune system is suppressed, and this condition is termed **leukopenia** ("lack of white cells" in Greek). The leukocyte count also increases after hard or prolonged exercise and stays high for a few hours.

Finally, platelet count is indicative of the state of the blood clotting system, of which platelets are part. The reference interval is 150 to 400 $k \cdot \mu L^{-1}$. This parameter too may increase transiently after exercise.

Let's return now to the discussion in the previous section regarding change in plasma volume with exercise. As mentioned, in order to calculate it, we need to consider not only the change in blood volume but also the change in volume of the blood cells. Taking the change in erythrocyte volume (hence, in hematocrit) as representative of the change in volume of all blood cells, we can arrive through mathematical calculations at the following formula:

$$\frac{\text{Plasma volume post}}{\text{Plasma volume pre}} = \frac{\text{hemoglobin pre}}{\text{hemoglobin post}} \cdot \frac{(100 - \text{hematocrit post})}{(100 - \text{hematocrit pre})} \qquad \text{(equation 16.1)}$$

The formula connects the postexercise to the pre-exercise plasma volume through the corresponding hemoglobin and hematocrit values. Because hemoconcentration causes the postexercise value of each of the two parameters to be higher than its pre-exercise value, both fractions on the right side of equation 16.1 are less than one. Hence, the fraction on the left side is also less than one, meaning that the plasma volume decreases after exercise. For an example, see problem 1.

Sports Anemia Does Not Exist

The condition in which an individual's hematocrit and blood hemoglobin are below the corresponding lower reference limits is termed **anemia**. This condition can be inherited, as, for example, in **sickle cell anemia** and **thalassemia**. In the absence of a genetic background, the main cause of anemia is nutritional—namely, inadequate intake of iron (see section 16.7), proteins (needed for hemoglobin synthesis), or certain vitamins (vitamin B_6,

folate, and vitamin E), as discussed in sections 6.1 and 6.2. Women should be particularly watchful about anemia if they lose a lot of blood during menstruation.

Are athletes at higher risk of anemia than members of the general population? The scientific literature of previous decades has provided an affirmative answer, leading to the introduction of the term **sports anemia**. Admittedly, reasons exist for reduction in an athlete's hemoglobin and hematocrit. One is erythrocyte destruction in the blood vessels of the feet when they hit the ground, if an athlete is involved in activities such as running and jumping. Another is erythrocyte loss in the stool as capillaries break in the digestive tract during exercise, particularly prolonged endurance exercise.

Athletes may also display **hemodilution**, a transient increase in blood volume above normal as water returns to the vessels following exercise-induced hemoconcentration. An example is shown in figure 16.1.

If we exercise regularly, hemodilution settles as an adaptation to training, fading away three to five days after training interruption. Although it entails a drop in hematocrit and hemoglobin concentration, hemodilution does not compromise the oxygen-carrying capacity of blood, because it does not affect the total erythrocytes or hemoglobin mass. Nevertheless, it may push a low hematocrit and a low hemoglobin value below their lower reference limits, thus raising a red flag for anemia. Some authors have proposed the term **pseudoanemia** ("false anemia" in Greek) to describe the "anemia" that is due to hemodilution.

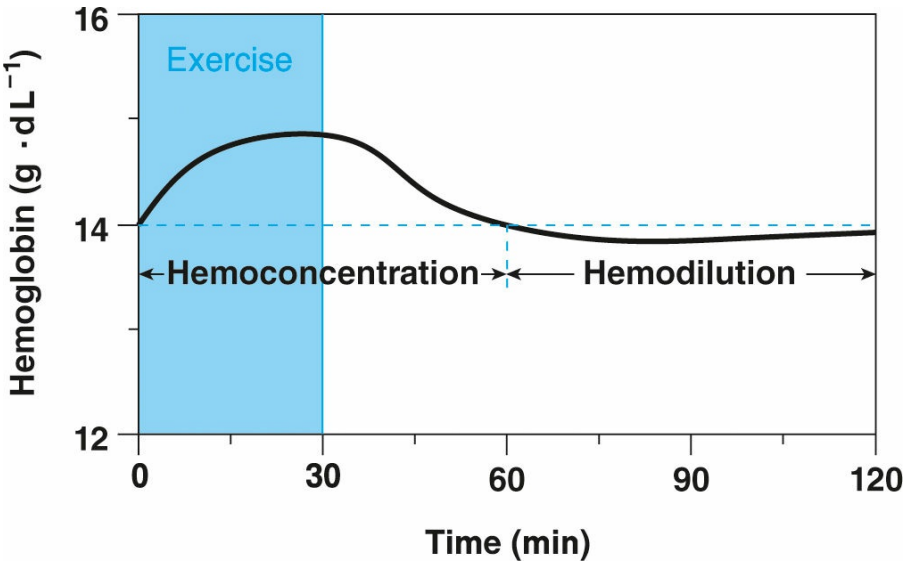


Figure 16.1 Exercise-induced hemoconcentration and hemodilution. If we perform prolonged moderate-intensity or hard exercise, blood hemoglobin concentration increases due to hemoconcentration. During recovery, water returns to the blood vessels, bringing hemoglobin concentration back to baseline. If the returning water exceeds the blood water lost during exercise, it causes hemodilution.

Despite all of the possible effects of exercise on hematocrit and hemoglobin concentration, most studies that have compared athletes and properly matched nonathletes have shown similar prevalence of anemia and similar hematocrit and hemoglobin values in the two groups. Thus, it seems that there is no considerable effect of exercise training on these parameters and no sports anemia in reality.

16.2 Iron

Iron commutes among tissues as Fe^{3+} bound mainly to **transferrin**, or **siderophilin**, its transport protein in plasma. The reference intervals of the serum iron concentration are as follows:

- 50 to 120 µg · dL^{-1}, or 9.0 to 21.5 µmol · L^{-1}, in children and adolescents
- 65 to 175 µg · dL^{-1}, or 11.6 to 31.3 µmol · L^{-1}, in men
- 50 to 170 µg · dL^{-1}, or 9.0 to 30.4 µmol · L^{-1}, in women

To convert μg · dL^{-1} into μmol · L^{-1}, divide by 5.585.

The higher the circulating iron concentration is, the higher the amount of iron that the tissues can absorb and incorporate in proteins such as hemoglobin, myoglobin, and components of the electron-transport chain. Thus, a high iron concentration (within its reference interval) is desirable. Most people can achieve this by eating foods rich in iron, as listed in section 6.3; indeed, serum iron concentration relates positively to iron intake. The literature reports similar serum iron concentrations in athletes and nonathletes.

Athletes sometimes follow dietary regimens, such as weight loss diets, that provide insufficient amounts of iron. Thus, measuring serum iron is important for assessing adequate intake. On the other hand, iron supplementation is customary in sport, even when there is no apparent reason for it. Measuring iron is important in this case too, in order to prevent unnecessary and potentially harmful overdosing if the upper reference limit is exceeded.

Serum iron shows short-term iron intake and changes easily when iron intake is modified. Therefore, measurement of iron should be accompanied by measurement of the indices discussed in the next sections.

16.3 Total Iron-Binding Capacity

As mentioned in the previous section, iron is carried in plasma primarily by transferrin. However, transferrin is only partly saturated with iron (by about one quarter to one third). Serum iron concentration in the hypothetical case in which the iron-binding proteins in plasma are fully saturated is the **total iron-binding capacity**, or **TIBC**. The reference interval is 250 to 425 μg · dL^{-1}, or 44.8 to 76.1 μmol · L^{-1}.

Interest in TIBC stems from the fact that the body reacts to a shortage of iron by increasing the plasma transferrin concentration. This response is believed to result in maximizing the capture of the little available iron, and it requires prolonged lack of iron before manifesting itself. Thus, TIBC values above the upper reference limit are indicative of prolonged iron depletion.

16.4 Transferrin Saturation

Transferrin saturation, or **iron saturation**, is serum iron concentration as a percentage of TIBC.

$$\text{Transferrin saturation} = \frac{[Fe^{3+}]}{\text{TIBC}} \cdot 100 \qquad \text{(equation 16.2)}$$

For example, if $[Fe^{3+}]$ is 84 μg · dL^{-1} and TIBC is 300 μg · dL^{-1}, then the transferrin saturation is 28%. The reference intervals of transferrin saturation are as follows:

- 20% to 50% in males
- 15% to 50% in females

Transferrin saturation relates positively to the adequacy of iron in the body, since an adequacy of iron causes a high numerator and low denominator in equation 16.2. In fact, because it combines two parameters, transferrin saturation changes relatively more than does $[Fe^{3+}]$ or TIBC separately. As a result, transferrin saturation is considered a more sensitive index of iron status (see problem 5) and is used as a criterion of iron deficiency (see section 16.7).

16.5 Soluble Transferrin Receptor

The **transferrin receptor** is an integral protein of the plasma membrane of almost all cells (erythrocytes being a notable exception), consisting of two identical 95 kDa polypeptide chains. The receptor binds iron-laden transferrin and mediates iron uptake through endocytosis of the receptor-transferrin complex. Once in the cytoplasm, iron is released and used for cellular needs, whereas transferrin and the receptor are recycled to the plasma membrane.

Being a membrane protein, the transferrin receptor is poorly soluble in water. Nevertheless, part of it is detected in plasma as a water-soluble form produced by proteolytic removal of the extracellular segments from the original polypeptide chains. This **soluble transferrin receptor (sTfR)** circulates in plasma bound to transferrin and is what we measure in serum.

The sTfR concentration relates inversely to iron adequacy because, as with

transferrin, the body reacts to iron depletion by increasing the amount of the transferrin receptor in the plasma membrane of the cells. By extension, the serum sTfR concentration also increases. On the basis of this association, the sTfR concentration is used as a criterion of iron deficiency, although it is not used as widely as other parameters of iron status. The reason is that its measurement is expensive and not well standardized, as discussed by Christine Pfeiffer and Anne Looker. The reference interval is 2.9 to 3.8 mg · L^{-1}.

16.6 Ferritin

Ferritin is our iron-storing protein. It consists of 24 subunits having a total molecular mass of 480 kDa and forming a huge (in molecular terms) shell, which encloses as many as 4,500 Fe^{3+} ions in an internal cavity measuring 12 nm in diameter, as reviewed by Elizabeth Theil and coworkers. Ferritin is found mainly in hepatocytes and macrophages of the liver, spleen, and bone marrow. Its amount in the body relates positively to that of iron.

A minuscule portion of intracellular ferritin, roughly proportional to its quantity, leaks into plasma because of the natural wear of cells. Thus, the serum ferritin concentration serves as an index of the iron stores in the body and is used as a criterion of iron deficiency. The reference intervals of serum ferritin concentration are as follows:

- 7 to 140 μg · L^{-1} in children and adolescents
- 20 to 250 μg · L^{-1} in men
- 10 to 120 μg · L^{-1} in women

16.7 Iron Deficiency

Iron deficiency is an alarming condition, because it may lead to reduced synthesis of hemoglobin, myoglobin, and proteins of the electron-transport chain. Usually, it can be rectified by eating iron-rich foods and taking iron supplements in moderate dosages.

Prolonged iron deficiency exhausts the iron stores in the body, leading to

iron deficiency anemia of the microcytic hypochromic type (section 6.3). This condition (like anemia from other causes) is characterized by paleness, weakness, low aerobic capacity, and reduced ability to keep warm in a cold environment. To reverse iron deficiency anemia, one needs to take iron supplements in high dosages.

To declare a person iron deficient, it is preferable to examine two parameters of iron status so as to reduce the chance of a wrong diagnosis due to an accidental change in one parameter only. As mentioned before, two such parameters are transferrin saturation and ferritin concentration. Figure 16.2 clarifies the diagnostic criteria of the conditions discussed in the present chapter.

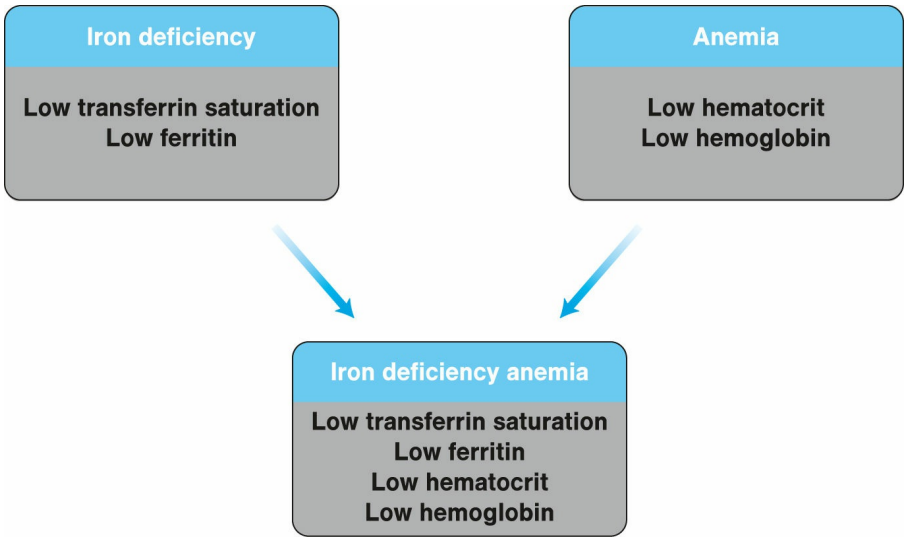


Figure 16.2 Iron deficiency and anemia. These two different conditions have different indices. Nevertheless, iron deficiency can cause anemia, in which case we have iron deficiency anemia and a combination of indices. There are also other biochemical indices of iron deficiency.

By analogy to the question posed in section 16.1 about anemia, we now ask, “Are athletes at higher risk of iron deficiency than the general population?” The answer, again, seems to be negative. Although there is no agreement in the literature, most studies that used an appropriate control group showed no significant differences between athletes and nonathletes regarding the indices of iron status or the prevalence of iron deficiency. The reason may be that exercise does not increase iron loss to any great extent.

The only documented increased loss is through the stool, and the frequently mentioned loss through sweat is actually negligible. It is also possible that most athletes replenish any possible increased iron losses by eating more food to meet their increased energy demands.

Summary

Iron status is intimately linked to health and aerobic capacity. Of the parameters describing iron status, blood hemoglobin shows the capacity to carry oxygen; serum iron shows the circulating amount of iron; total iron-binding capacity shows the maximal capacity of plasma to carry iron; transferrin saturation and the soluble transferrin receptor show the adequacy of iron in the body; and ferritin shows the amount of stored iron. The picture is completed by hematologic parameters, such as hematocrit and erythrocyte count, and leukocyte and platelet counts inform us about the state of the immune and blood-clotting systems, respectively.

Blood hemoglobin concentration rises during exercise due to hemoconcentration and drops during recovery because of hemodilution. Low hemoglobin and hematocrit signify anemia, whereas low transferrin saturation and ferritin signify iron deficiency. Iron deficiency may lead to anemia unless diagnosed early enough and reversed by increased iron intake. Athletes do not seem to be at higher risk of iron deficiency or anemia than the general population.

Problems and Critical Thinking Questions

1. A runner had a blood hemoglobin concentration of 15.4 g · dL^{-1} and a hematocrit of 44.2 at rest. Immediately after exercise, these values changed to 15.8 g · dL^{-1} and 44.9. Calculate the change in plasma volume.
2. Although equation 16.1 was developed for cases of

hemoconcentration, it can be applied equally well to cases of hemodilution. As a follow-up to the previous problem, suppose that, one hour after exercise, the hemoglobin and hematocrit values were 15.2 g · dL^{-1} and 43.8. Calculate the change in plasma volume from the resting state.

3. Which doping substance and which doping method affect the blood hemoglobin concentration, hematocrit, and erythrocyte count?
4. How does iron deficiency affect the serum TIBC, transferrin saturation, sTfR concentration, and ferritin concentration?
5. The serum iron concentration and TIBC of a person were 90 and 300 µg · dL^{-1}, respectively, six months ago. Because of poor nutrition, iron has now decreased by 10% and the TIBC has increased by 10%. Calculate the change in transferrin saturation.
6. An athlete has a high serum ferritin concentration but low transferrin saturation. What do you conclude, and what would you advise him or her to do?
7. In contrast, another athlete has low ferritin and high transferrin saturation. What would you conclude?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

iron status
hemoglobin
erythropoietin
hemoconcentration, hemocondensation
hematocrit, packed cell volume
erythrocyte count
leukocyte count

platelet count
blood doping
leukocytosis
leukopenia
anemia
sickle cell anemia
thalassemia
sports anemia
hemodilution
pseudoanemia
iron
transferrin, siderophilin
total iron-binding capacity, TIBC
transferrin saturation, iron saturation
transferrin receptor
soluble transferrin receptor, sTfR
ferritin
iron deficiency anemia

References and Suggested Readings

Buratti P, Gammella E, Rybinska I, Cairo G, Recalcati S (2015). Recent advances in iron metabolism: Relevance for health, exercise, and performance. *Medicine and Science in Sports and Exercise* 47: 1596-1604.

Pfeiffer CM, Looker AC (2017). Laboratory methodologies for indicators of iron status: Strengths, limitations, and analytical challenges. *American Journal of Clinical Nutrition* 106: 1606S-1614S.

Theil EC, Tosha T, Behera RK (2016). Solving biology's iron chemistry problem with ferritin protein nanocages. *Accounts of Chemical Research* 49: 784-791.

Wu A (2006). *Tietz Clinical Guide to Laboratory Tests* (Saunders, Philadelphia).

Yip R, Johnson C, Dallman PR (1984). Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. *American Journal of Clinical Nutrition* 39: 427-436.

CHAPTER 17

Metabolites

Learning Objectives

After reading this chapter, you should be able to do the following:

- List metabolites that are useful in biochemical assessment of exercisers and assign them to classes of energy sources in exercise whose metabolism they reflect.
- Discuss the usefulness of measuring blood lactate in exercise.
- Design exercise protocols to achieve maximal lactate production and assess anaerobic capacity.
- Design exercise protocols to construct a lactate–intensity curve and assess aerobic endurance.
- Describe how you would test an improvement in aerobic endurance through blood lactate measurements.
- Discuss the usefulness of measuring serum or plasma glucose and ammonia in exercise.
- Describe a healthy lipidemic profile in quantitative terms and discuss the value of monitoring it in exercisers.
- Explain why glycerol is the best index of lipolysis in the circulation.
- Discuss the utility of measuring serum urea and creatinine in

exercisers.

- Explain the information gained from measuring uric acid and glutathione in exercisers.

This chapter examines the utility of measuring biochemical parameters related to the four classes of energy sources that we explored in part III, albeit in a slightly different order: carbohydrates, lipids, proteins, and compounds of high phosphoryl-transfer potential. In addition, we will discuss the value of monitoring two indices of the redox state. These various parameters are itemized in table 17.1.

17.1 Lactate

L-Lactate is the end product of anaerobic carbohydrate breakdown. It is also the metabolite displaying the most spectacular concentration changes in muscle and blood with exercise. As a result, its measurement offers a wealth of information regarding the effects of exercise on metabolism. We usually determine lactate in whole blood rather than in plasma or serum. Besides measuring lactate in laboratory spectrophotometers, one can use practical and dependable portable devices that produce a lactate reading within seconds of applying a few microliters of capillary blood from a fingertip or earlobe.

Table 17.1 Metabolites Useful in Biochemical Assessment of Exercisers

Parameter	Relevance
Lactate	Carbohydrate metabolism
Glucose	
Triacylglycerols	Lipid metabolism
Total cholesterol	
HDL cholesterol	
LDL cholesterol	
Glycerol	
Urea	Amino acid metabolism

Ammonia	
Ammonia	Metabolism of compounds with high phosphoryl-transfer potential
Creatinine	
Uric acid	
Uric acid	Redox state
Glutathione	

The blood lactate concentration can go from about 1 mmol · L^{-1} at rest to more than 20 mmol · L^{-1} after maximal exercise lasting at least one half minute. Efforts of lower intensity result in lower lactate concentrations, as carbohydrates are broken down primarily aerobically. The lactate concentration is also lower than 20 mmol · L^{-1} after maximal efforts lasting less than one half minute, since the lactate produced in the exercising muscles is not sufficient to elicit such a high concentration in blood.

When measuring lactate after hard or maximal exercise of short duration, remember that it takes some minutes for lactate concentration to peak in blood (figure 10.50). Thus, a blood sample taken right after the end of exercise will not produce the peak value. To find it, we need to perform repeated samplings spaced maximally 2 min apart. If we measure lactate on the spot with a portable device, we can stop when we get a value that is lower than the previous one and declare the previous one to be the peak value.

If, on the other hand, we collect blood samples with the intent to analyze them later in the lab, I recommend obtaining a sample at 2, 4, 6, 8, and 10 min after the end of exercise. This practice rarely misses the peak value. If you measure the same person after the same test again in the future, you may reduce the number of samples depending on the outcome of the initial test, hoping that the blood lactate kinetics will be the same, although there is no guarantee of that. Finally, if all you can afford is a single measurement, the preferred sampling time would be 3 to 5 min after the end of exercise.

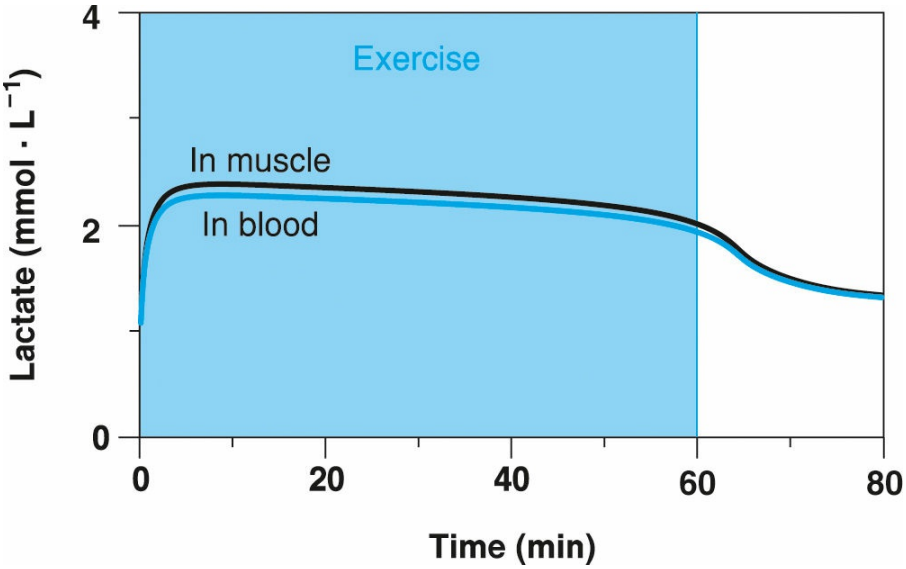


Figure 17.1 Lactate concentration in moderate-intensity exercise. Muscle and blood lactate concentrations peak a few minutes after the start of exercise at constant moderate intensity and drop slightly as exercise continues. After the end of exercise, both return gradually to baseline.

During prolonged exercise at constant moderate intensity, blood lactate declines gradually (figure 17.1), paralleling the decline in energy contribution from carbohydrates (figure 14.4). If intensity fluctuates, as in ball games and interval training, then lactate concentration lies between the values corresponding to the highest and lowest intensities.

Apart from the exercise parameters (that is, intensity, duration, and program) already discussed, blood lactate response depends on the characteristics of the exerciser and on the environmental factors listed in section 14.4 as affecting the choice of energy sources during exercise. Here's how.

- **Sex.** Males have higher blood lactate concentrations than females do during exercise at the same relative intensity (from low all the way to maximal). This difference seems to result from males' using a higher proportion of carbohydrates to lipids during exercise (section 14.9) and having a higher muscle content of phosphorylase, phosphofructokinase, and lactate dehydrogenase (enzymes that are critical for lactate production, according to the discussion in chapter 10), as reviewed by Anne-Marie Lundsgaard and Bente Kiens.

- **Age.** As discussed in section 14.11, children have lower blood lactate concentrations than adults do during exercise at a given relative intensity (again, from low all the way to maximal), apparently because they have lower muscle glycogen and lactate dehydrogenase. These differences fade as children reach adolescence and enter adulthood.
- **Nutritional state.** A carbohydrate-rich diet before exercise, carbohydrate intake during exercise, or a combination of the two augments blood lactate concentration during exercise at a given absolute intensity, because relatively more carbohydrates are used (section 14.12). As a result, you need to ensure the same diet for about two days before an exercise test and the same (or no) carbohydrate intake during the test if you wish to compare exercisers in terms of blood lactate. You should take the same precautions when testing an individual at different times during a training period to evaluate adaptations to training (see section 17.4).
- **Training state.** Endurance training lowers blood lactate concentration during exercise at a given absolute moderate intensity, as discussed in section 10.15. If exercise is performed at the same relative intensity before and after endurance training, then blood lactate is similar.
- **Genome.** The higher the percentage of type II fibers is in the exercising muscles, the higher the blood lactate concentration during exercise at a given absolute intensity. The reason is that type II fibers have higher contents of glycolytic enzymes and lactate dehydrogenase than type I fibers do, as discussed in section 10.19.
- **Ambient temperature.** There is no agreement among researchers as to the effect of ambient temperature on blood lactate concentration during exercise, just as there is no agreement regarding the effect of ambient temperature on the proportion of energy sources (section 14.21). It seems that the outcomes of studies on this topic are influenced by factors such as exercise type and training state of the participants.
- **Hypoxia.** Blood lactate does not differ between exercises at sea level and high altitude (or, more generally, between exercises in normoxia and hypoxia), provided they are performed at the same relative

intensity, which means lower absolute intensity in hypoxia (section 14.21). If exercise is performed at the same absolute intensity (which translates into lower relative intensity in normoxia), then blood lactate is higher in hypoxia (figure 17.2). Thus, relative, rather than absolute, exercise intensity is what determines blood lactate during exercise in normoxia and hypoxia.

When performing blood lactate measurements that will be used in comparisons (either on the same exerciser or between exercisers), it is important to sample the same site and use the same kind of blood or component of blood. As summarized by Oliver Faude and coworkers, plasma (from venous blood) displays the highest values, followed, in sequence, by capillary blood from a fingertip, capillary blood from an earlobe, and whole (venous) blood. In addition, one should use the same portable analyzer or laboratory method to ensure valid comparisons.

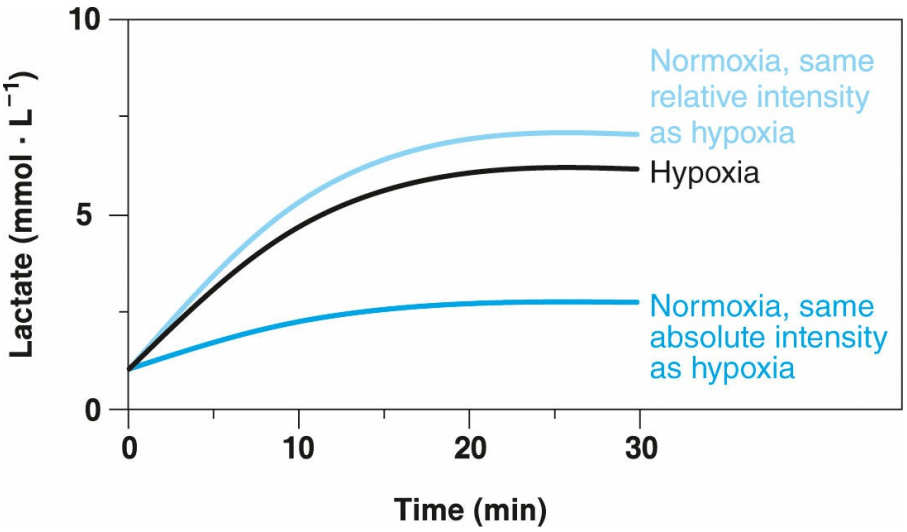


Figure 17.2 **How hypoxia influences blood lactate concentration in exercise.** When we perform moderate-intensity or hard exercise at high altitude (or hypoxia, in general) blood lactate is similar to that when we perform exercise at the same relative intensity at sea level (or normoxia, in general)—say, 70% of our $\dot{V}O_2max$ in each case. However, because our $\dot{V}O_2max$ is lower in hypoxia, absolute intensity is lower in hypoxia. If we exercise at that absolute intensity in normoxia (corresponding to about 50% of normoxic $\dot{V}O_2max$), then blood lactate is lower. The graph has been constructed on the basis of similar findings by Barry Braun and coworkers (in women) and Glenn Wadley and coworkers (in males).

The wide range of values of blood lactate concentration, and the plethora of factors that influence it, render it a sensitive and highly useful marker of the effects of exercise on metabolism. Let's be more specific. We can locate the utility of measuring lactate in three areas:

1. Estimating the anaerobic lactic capacity
2. Programming training
3. Estimating aerobic endurance

17.2 Estimating Anaerobic Lactic Capacity

High anaerobic lactic capacity (that is, capacity of the lactate system to regenerate ATP) interests athletes in events relying primarily or largely on the lactate system, such as sprint and middle-distance running and swimming. Because sprint training increases the muscle content of the enzymes of the lactate system (section 14.16), it possesses the potential to increase the power of the system and, hence, the maximal rate of lactate production. However, this potential may not materialize if it stumbles upon inhibition of muscle function by a decrease in cytosolic pH. Thus, if a sprint training program manages to increase blood lactate after maximal exercise (notably, after an event-specific exercise), the increase should be attributed to at least one of the following factors:

- Increased ability of the muscles to produce lactate thanks to upregulation of the enzymes of the lactate system
- Increased buffer capacity (consult section 1.12) of the muscles, blood, or both, which mitigates the drop in muscle pH, delays the development of fatigue, and lets the muscles work at high power output, thus producing more lactate at the same time
- Faster exit of lactate, along with H^+ (section 10.23 and figure 10.35), from the contracting muscle fibers, which, again, mitigates the drop in muscle pH

Any of these adaptations may increase performance. Thus, high blood lactate concentration after maximal exercise is desirable. In fact, several

researchers have found a positive relationship between postexercise blood lactate concentration and performance in events such as the 400 and 800 m runs.

17.3 Programming Training

Success in sport usually depends on a combination of capacities, which athletes develop through different types of training. Often, the main factor determining the type of training is exercise intensity. In such cases, measuring blood lactate helps define the desired intensity.

Programming training on the basis of exercise intensities dictated by blood lactate concentrations is considered superior to programming on the basis of heart rates because blood lactate relates more closely to muscle metabolism and muscle adaptations to training. Of course, this approach is less practical and carries a certain cost, since it requires blood sampling, consumables, and technical equipment for measuring lactate. Thus, one could determine training intensities based on lactate at the beginning of a training program, monitor training through daily heart rates, and resort to lactate periodically (every few weeks) to fine-tune intensities.

How do we establish a relationship between training intensity and blood lactate concentration for an athlete? We need to construct a lactate–intensity plot like the one shown in figure 10.52. How do we do so? There are several tests, which can be divided into two categories: continuous and intermittent. Let's explore them.

Continuous tests are mostly suitable for laboratory settings, in which there is a stationary ergometer such as a treadmill or bicycle. The athlete (or exerciser, in general) starts at a low speed or power and goes through five to nine steps of gradually increasing intensity until exhaustion. This process is often referred to as a *maximal graded exercise test*. The duration of each step should be 3 to 5 min to let lactate reach a steady state, and blood sampling should be performed within the last half minute of each step. Step duration and increment size (in speed or power) affect the lactate values and, hence, the shape of the lactate–intensity plot. In addition, because each step carries the echo, so to speak, of the previous one (that is, the starting point of the

lactate value is the value of the previous step, not the resting value), the lactate measured in it may not be the same as the lactate measured when the same step is carried out alone.

If a plot is to be constructed in the field, for example at a track or swimming pool, then continuous tests are impractical, because the athlete is not staying at a fixed place. In such cases, intermittent tests are preferable, in which the athlete performs rather brief (1 to 4 min) exercise bouts of gradually increasing intensity (for example, from 60% to 100% of maximal speed in increments of 5% to 10%) and stops after each bout to provide blood and recover. It is preferable to take more than one blood sample after each bout in order not to miss the peak lactate concentration (section 17.1).

Each step in an intermittent test must be performed at a steady speed, so that the lactate measured in the end reflects this speed. Trained athletes are capable of this with some guidance from their coach or with the aid of devices that give the tempo through auditory or visual signals during each bout. Ideally, the interval between steps should be sufficient for complete lactate removal from blood and recovery of the athlete's strength before the next step. However, this period may exceed one half hour and therefore result in an unacceptably prolonged test. Thus, a compromise may be needed. In my experience, the interval may be shortened to between 10 min (after the first bout) and 15 min (before the last bout) without seriously affecting the results of the test.

Many exercise biochemists and physiologists prefer to use a lactate–intensity plot as is (that is, without any further processing) to decipher and prescribe desired exercise intensities (figure 17.3), usually in terms of speed (as in running, cycling, or swimming). Here are three rules of thumb:

- Intensities eliciting lactate values up to about 2 mmol · L^{-1} characterize light to moderate-intensity exercise and are suitable for active recovery (section 10.32) and enhancing aerobic endurance, especially in persons with low fitness levels, such as nonathletes and patients.
- Intensities corresponding to lactate values between about 2 and 4 mmol · L^{-1} serve the development of aerobic endurance through moderate-intensity to hard exercise.

- Intensities pushing the blood lactate concentration above 4 mmol · L^{-1} typify hard to maximal exercise serving a variety of purposes, from developing aerobic endurance (through interval training) to developing maximal speed (through sprint training).

Other exercise scientists prefer not to use fixed lactate values to prescribe training intensities but to determine several reference points, such as those listed in section 10.33, through graphical or mathematical processing of the lactate–intensity plot. Faude and colleagues summarize how such reference points are determined. These points may be useful in planning training as long as no inferences of biochemical or physiological relevance are made. This is particularly true of the "anaerobic threshold," which, as discussed in sections 10.33 and 14.7, marks no passage from mostly aerobic to mostly anaerobic metabolism. In addition, there is no evidence that training at a "threshold" is more effective than training within an intensity range such as the ones just outlined, as Ralph Beneke and colleagues point out.

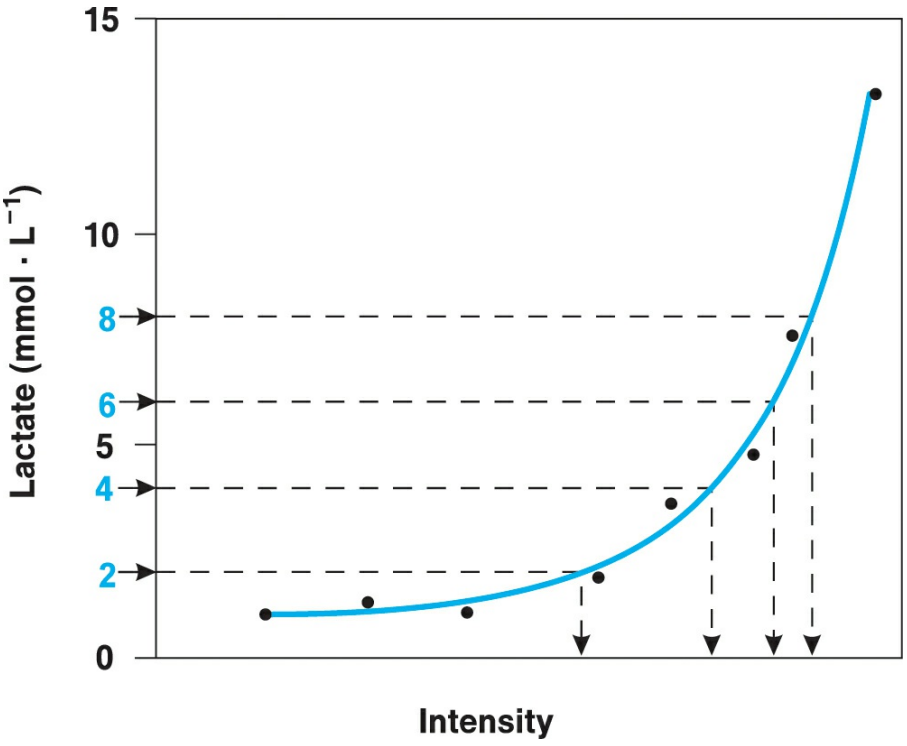


Figure 17.3 Programming training on the basis of a lactate–intensity plot. After performing an exercise test involving steps or bouts of gradually increasing intensity and blood lactate measurement, we can place the pairs of intensity and lactate as dots on a graph.

We can then draw a line through the dots and use it to prescribe exercise intensities such as those corresponding to 2, 4, 6, and 8 mmol · L^{-1}.

Similarly, there is no evidence that setting training intensities not based on fixed lactate values is better than setting intensities based on fixed values. In fact, the review of 32 studies by Faude and coworkers shows similar correlation coefficients of aerobic endurance performance with exercise intensities established through the two approaches.

A different test used to establish a relationship between exercise intensity and blood lactate concentration is the one aimed at determining **maximal lactate steady state**, or **MLSS** (figure 17.4). In this test, the athlete is asked to perform rather long (at least 30 min) bouts on separate days, each at a fixed intensity but gradually increasing from one day to another. The MLSS is defined as the highest intensity that does not elicit a continuous rise in the blood lactate concentration during a bout (precisely, no more than 1 mmol · L^{-1} between 10 and 30 min). The obvious disadvantage of this test is that it cannot be completed in a single day, which makes it difficult to accommodate with an athlete's training routine.

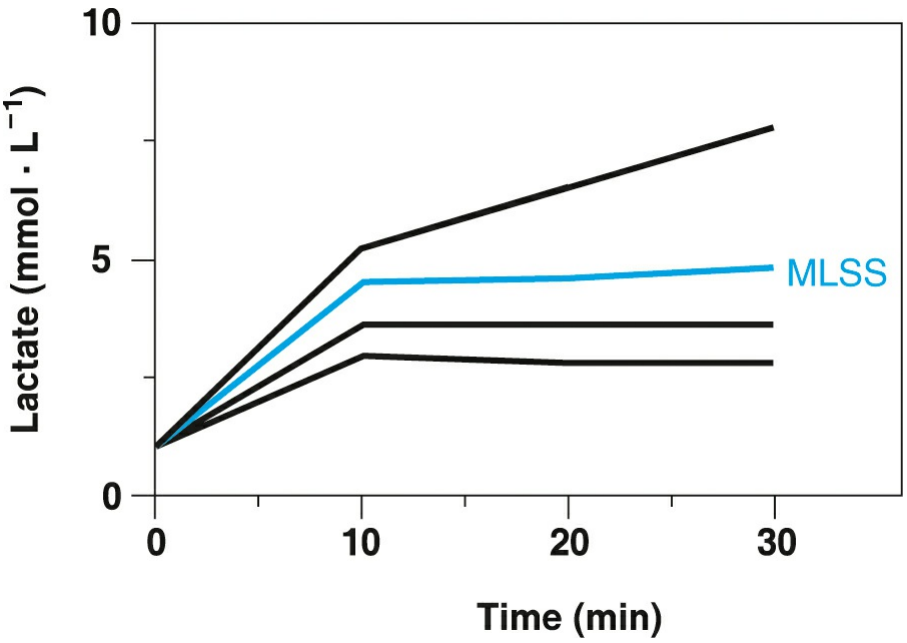


Figure 17.4 **Determining maximal lactate steady state.** An exerciser performs a number of bouts on separate days, each at a constant intensity for at least 30 min. Intensity is gradually increased from day to day until blood lactate concentration cannot be held constant. The

intensity of the previous bout (that is, the highest intensity that did not elicit a continuous rise in lactate) is declared the MLSS.

For maximal economy of time and money, tests of just two bouts have been developed. Obviously, such a test does not enable one to construct an entire lactate–intensity plot. Nevertheless, one can determine a couple of useful intensities, such as the one corresponding to a concentration of 4 mmol · L^{-1}, which is considered efficient in developing aerobic capacity. A two-bout test requires the expertise of the coach in selecting the two intensities so that the lower one elicits a lactate concentration slightly below the target concentration and the higher one a lactate concentration slightly above the target concentration.

17.4 Estimating Aerobic Endurance

It may seem odd at first that a product of anaerobic metabolism, such as lactate, can be useful in estimating aerobic endurance. But if you think about how interlaced the energy systems are (as analyzed in chapter 14), you will realize that there is no oddity here. However, the connection between lactate and aerobic endurance is the opposite of that between lactate and anaerobic capacity: The higher the aerobic endurance, the lower the lactate concentration.

This argument has been confirmed by many studies (reviewed by Faude and collaborators), which show a strong positive relationship between performance in endurance events and reference exercise intensities (such as speed corresponding to a blood lactate concentration of 4 mmol · L^{-1}) determined though a lactate–intensity plot, as described in the previous section. In simple terms, this means that *a high endurance performance goes with being fast while keeping lactate low.*

This connection is explained by the adaptations that accompany endurance training (sections 14.14 and 14.15), in particular the increase in the proportion of lipids to carbohydrates broken down during exercise at a given absolute moderate intensity. Because of the decreased carbohydrate breakdown, less lactate is produced in the muscles and less lactate appears in blood. It is also possible that endurance training increases the rate of

disappearance of lactate from blood. Possible mechanisms include an increase in MCT1 in the plasma membrane of type I muscle fibers (section 10.23) and an increase in the rate of gluconeogenesis in the liver and kidneys. (Remember that lactate is a raw material for gluconeogenesis, as discussed in sections 10.25 through 10.27.)

A lower lactate concentration during exercise at a given absolute intensity means a higher intensity for a given lactate concentration. It is exactly this higher intensity that characterizes adaptations to endurance training and the increase in aerobic endurance. In fact, a compilation of studies by Faude and colleagues shows that the reference intensities mentioned earlier correlate with aerobic endurance performance better than $\dot{V}O_2max$ does. This correlation may exist because performance in an endurance event depends less on $\dot{V}O_2max$ and more on how high an intensity the athlete can maintain for a long time.

Another advantage of estimating aerobic endurance based on lactate values is that it requires submaximal efforts, which are feasible for frail or diseased individuals. In contrast, measuring $\dot{V}O_2max$ requires a maximal effort, which may be difficult, impossible, or dangerous for such individuals.

The increase in the exercise intensity corresponding to a given blood lactate concentration can be used to monitor adaptations to endurance training. Indeed, if we construct a lactate–intensity plot before and several weeks after the beginning of a training program, the latter plot will be to the right of the former (figure 17.5). For the plots to be comparable, all factors that may affect the lactate values, as discussed in section 17.2, should be controlled. These factors include nutritional state, ambient temperature, test site, blood sampling site, specimen (whole blood or plasma), method of measurement, and test protocol.

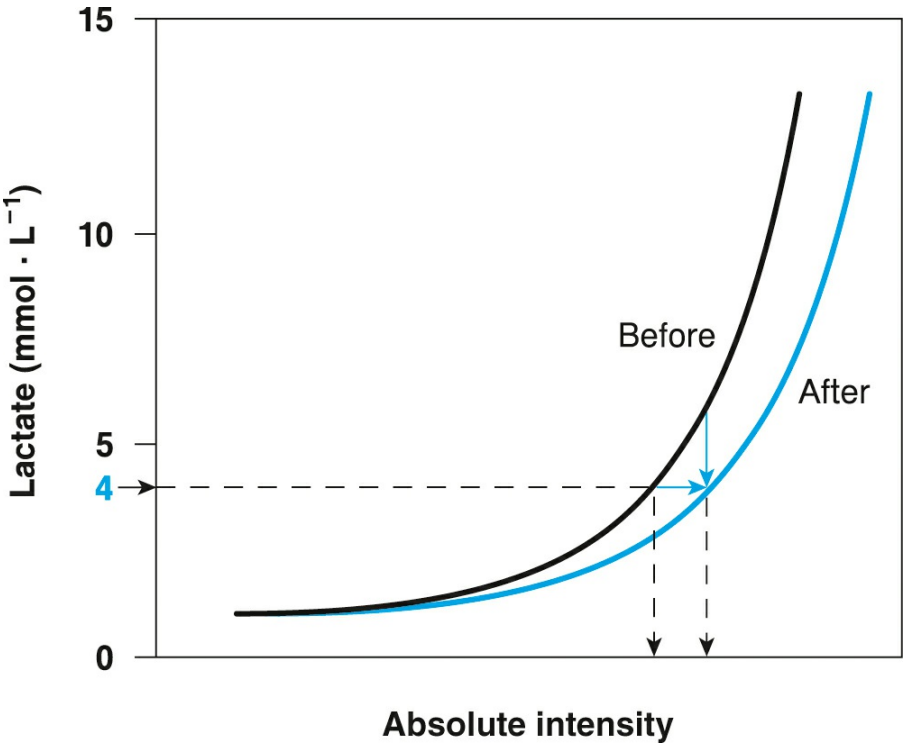


Figure 17.5 Effect of endurance training on the lactate–intensity plot. Endurance training shifts the plot to the right. After training, a given blood lactate concentration is achieved at a higher absolute exercise intensity (horizontal colored arrow), and a given intensity corresponds to a lower blood lactate concentration (vertical colored arrow).

A point of consideration regarding intermittent tests (described in the previous section) is whether the duration of the intervals between successive steps should be identical in the pre- and post-training tests or adjusted to training-induced adaptations. In other words, it might be preferable to change interval duration, roughly proportionally to the lactate value achieved after a step, so that the exerciser can start the next step with a more-or-less fixed blood lactate concentration. This calibration becomes particularly important during the latter stages of a test, which are characterized by high intensities and high lactate values.

This suggestion does not violate the requirement to implement the same parameters for the pre- and post-training tests in order to make them comparable. Rather, it focuses on controlling not the rest duration alone but the proportion of lactate concentration to duration of rest. Since these tests commonly use portable lactate analyzers that produce immediate readings, one can adjust interval duration based on a lactate reading and a

predetermined algorithm with relative ease.

Two final precautions are worth taking to ensure comparable tests. First, they should be performed at roughly the same hour of the day to dispel the possibility of diurnal variation in the postexercise lactate values (although there seems to be no agreement among researchers on whether such variation really exists, as reviewed by Stefanos Nikolaidis and coworkers). Second, it is advisable for the exerciser to avoid hard exercise during the day preceding the test in order to ensure normal glycogen stores and optimal performance.

Diurnal variation is the fluctuation of a biological parameter over the 24 hours of the day.

The value of monitoring adaptations to endurance training is twofold: It lets us find out whether a training program works, and it lets us upgrade the training intensities to push the adaptations forward. Otherwise, there will be no further adaptations. This is the **progression principle** in training.

17.5 Glucose

Glucose (often termed *sugar* in biochemistry lab reports) is kept within a narrow concentration range in plasma thanks to the actions of glucagon, epinephrine (possibly), insulin, and glucose itself (the latter through feedback inhibition). As analyzed in chapter 10, the former two tend to increase plasma glucose, whereas the latter two tend to decrease it. The importance of measuring glucose lies in the fact that it shows the balance between the biochemical mechanisms that raise and lower it.

All reference intervals in this chapter are from Alan Wu unless otherwise stated.

The reference interval for plasma or serum glucose concentration is as follows:

- 60 to 100 mg · dL^{-1}, or 3.3 to 5.6 mmol · L^{-1}, in children and adolescents
- 74 to 106 mg · dL^{-1}, or 4.1 to 5.9 mmol · L^{-1}, in ages 18 to 60

- 82 to 115 mg · dL^{-1}, or 4.6 to 6.4 mmol · L^{-1}, in ages 61 to 90

> To convert mg · dL^{-1} into mmol · L^{-1}, divide by 18.

It is desirable to have a serum glucose concentration below 100 mg · dL^{-1} (5.6 mmol · L^{-1}). Values above 125 mg · dL^{-1} (6.9 mmol · L^{-1}) are characteristic of **diabetes mellitus**, whereas values in between are ascribed to **prediabetes**. Values below the lower reference limits are indicative of **hypoglycemia**, which may result from inadequate carbohydrate intake; thus, the advice given to exercisers with fasting hypoglycemia is to eat more carbohydrates.

Exercise may upset glucose homeostasis in plasma if an imbalance develops between glucose uptake by the exercising muscles and glucose supply by the liver. In section 10.30, we discussed the effects of different exercises and of carbohydrate intake before and during exercise on plasma glucose concentration. Of all the possible scenarios, the one that calls for the most attention is the risk of hypoglycemia during very prolonged exercise, particularly if carbohydrate intake is inadequate. Monitoring blood glucose during such exercise lets us spot this danger and deter it by modifying exercise parameters, nutrition, or both. Luckily, portable glucose analyzers produce readings from a few microliters of capillary blood within seconds. The glucose concentration in whole blood is about 90% of the corresponding serum concentration.

17.6 Triacylglycerols

Triacylglycerols (usually referred to as **triglycerides** in biochemistry lab reports) constitute one of the parameters of the **lipidemic profile**, a term introduced in section 11.22. They are present in plasma as part of **lipoproteins** (section 11.19). **VLDL** are the major carriers of triacylglycerols in the postabsorptive (fasting) state. Serum triacylglycerol concentration is of interest mainly because of its relationship with the risk of atherosclerosis (section 11.21). The Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (hereafter, Expert Panel) has introduced the

following limits (in milligrams per deciliter) for the adult population:

To convert mg · dL^{-1} into mmol · L^{-1}, divide by 88.5.

- < 150, normal
- 150 to 199, borderline high
- 200 to 499, high
- ≥ 500, very high

The reference interval of serum triacylglycerol concentration depends strongly on age, sex, and race. Hence, the scientific literature contains numerous reference intervals, which are impossible to list here. As an example, Wu lists reference intervals for black and white Americans of both sexes and various ages. In general, the reference limits, especially the upper ones, tend to increase with age and are higher in males than in females. They are also higher in white people than in black people.

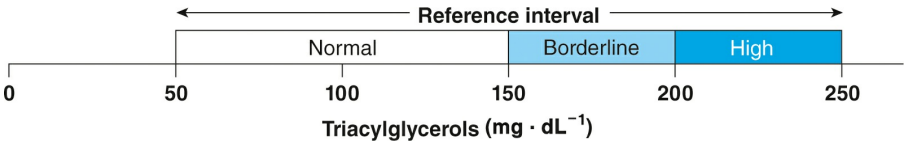


Figure 17.6 Usual may not be normal. Reference intervals of serum triacylglycerol concentration for almost all ages, sexes, and races extend from the normal all the way to the high values, as in this example of a typical reference interval of 50 to 250 mg · dL^{-1}. Thus, large numbers of people have values that are undesirable (full color) or somewhat undesirable (faint color).

All upper reference limits of triacylglycerols fall within the borderline-high or high areas (figure 17.6), which means that a considerable portion of the population has values that are not desirable. Thus, serum triacylglycerol concentration is a case in which the usual values differ from the normal ones. This is an example of why using the term *normal values* to describe the reference interval may be misleading, as pointed out in section IV.3.

Measuring triacylglycerols in an exerciser's serum is interesting after acute and, particularly, chronic exercise. As mentioned in section 11.21, both types of exercise can decrease serum triacylglycerol concentration under

certain conditions. Thus, measuring that concentration from time to time contributes to monitoring the health of exercisers and helps assess a training program's effectiveness in improving the lipidemic profile.

17.7 Cholesterol

Like triacylglycerols, **cholesterol** is present in plasma as part of lipoproteins. The main carriers of cholesterol are **LDL** and **HDL**, and VLDL also contain a small amount of it. Three cholesterol-related serum parameters of the lipidemic profile are of interest for health: total, HDL, and LDL cholesterol. As in the case of triacylglycerols, numerous reference intervals of the cholesterol parameters can be found in the literature, depending on age, sex, and race (see, for example, Wu). Let's examine each parameter.

Total Cholesterol

Serum total cholesterol concentration relates strongly to the risk of atherosclerosis. The Expert Panel has set the following limits (in $mg \cdot dL^{-1}$):

> To convert $mg \cdot dL^{-1}$ into $mmol \cdot L^{-1}$, divide by 38.6 (for all cholesterol parameters).

- < 200, desirable
- 200 to 239, borderline high
- ≥ 240, high

The reference limits of serum cholesterol concentration, especially the upper one, tend to increase with age, but differences between sexes and races are not as distinct as in triacylglycerols. Again, all upper reference limits lie within the borderline-high or high zones (figure 17.7*a*), meaning that many people are at risk of atherosclerosis.

HDL Cholesterol

As discussed in section 11.22, HDL hinder the development of atherosclerosis; hence, HDL cholesterol is nicknamed **good cholesterol**.

Because of this, it is desirable to have high HDL cholesterol, unlike triacylglycerols, total cholesterol, and LDL cholesterol. The Expert Panel considers HDL cholesterol concentrations under 40 mg · dL^{-1} to be low and concentrations at or over 60 mg · dL^{-1} to be high.

The reference interval of HDL cholesterol depends primarily on sex and race. Women have higher upper and lower reference limits than men do, and black people have higher and lower limits as compared with white people. Nearly all lower reference limits are below 40 mg · dL^{-1} (figure 17.7*b*), which means that a large part of the population has undesirably low HDL cholesterol values.

LDL Cholesterol

The measurement of LDL cholesterol requires equipment that is not available in most laboratories performing routine tests. Thus, we usually calculate this parameter by subtracting HDL cholesterol and VLDL cholesterol from total cholesterol. VLDL cholesterol, in turn, is calculated by dividing the triacylglycerol concentration by 5, if both are measured in mg · dL^{-1}, or 2.18, if both are measured in mmol · L^{-1}. For this approximation to be acceptable, the triacylglycerol concentration must not exceed 400 mg · dL^{-1}, or 4.52 mmol · L^{-1}. Otherwise, LDL cholesterol needs to be measured directly.

As discussed in section 11.22, LDL are atherogenic lipoproteins; hence, LDL cholesterol is the **bad cholesterol**. As a result, it is desirable to have low LDL cholesterol. The Expert Panel has determined the following limits (in mg · dL^{-1}):

- < 100, optimal
- 100 to 129, near optimal
- 130 to 159, borderline high
- 160 to 189, high
- ≥ 190, very high

The upper and lower reference limits of LDL cholesterol tend to increase with age. All upper reference limits lie within the borderline-high, high, or very high zones (figure 17.7*c*). Again, this shows that a substantial number of

people have undesirably high LDL cholesterol values.

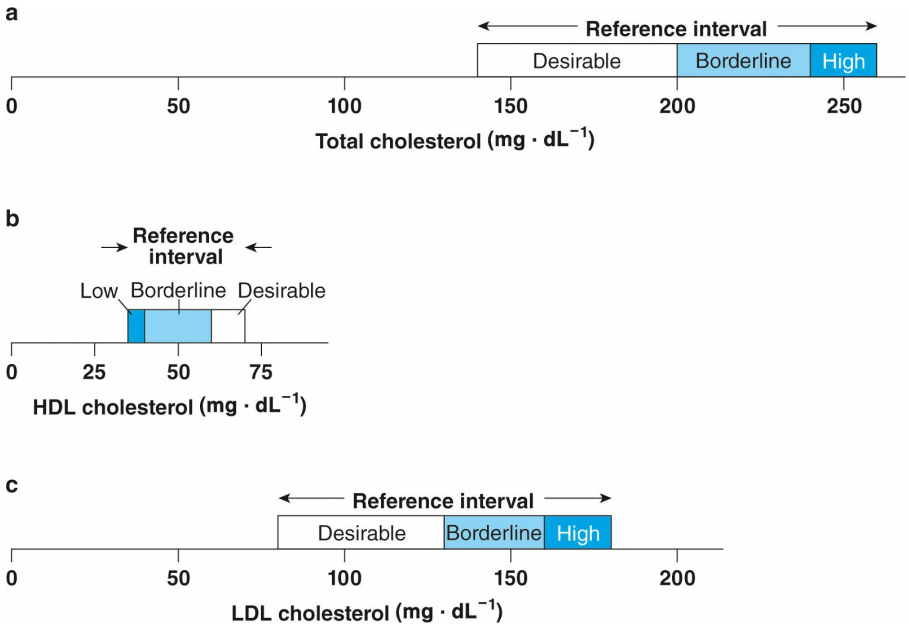


Figure 17.7 The burden of serum cholesterol. Reference intervals of serum total cholesterol *(a),* HDL cholesterol *(b),* and LDL cholesterol *(c)* concentrations for almost all ages, sexes, and races extend from normal to undesirable values, as in these typical examples. Thus, substantial parts of the human population are at high (full color) or moderately high risk of atherosclerosis and CVD (faint colors). This fact stresses the importance of exercise (along with diet) in improving and protecting the health of these people.

17.8 Recapping the Lipidemic Profile

The purpose of determining the lipidemic profile in exercisers is the same as it is for everyone, that is, to assess their risk of atherosclerosis and CVD in order to take corrective measures, if necessary. An additional purpose specific to exercisers is to evaluate the effectiveness of acute exercise or of training programs in improving the lipidemic profile. Some, but not all, types of exercise and training possess the potential to increase HDL cholesterol and decrease total cholesterol, LDL cholesterol, and triacylglycerols (sections 11.21 and 11.22).

A final purpose of determining the lipidemic profile concerns exercisers who use **anabolic androgenic steroids**, a regrettably frequent practice discussed in section 18.7. This practice lowers HDL cholesterol, thus raising

the risk of atherosclerosis and CVD. This risk, in turn, necessitates careful monitoring if we cannot talk the user out of using steroids.

All of these purposes justify our strong interest in the lipidemic profile and make it worthwhile to assess on a regular basis in exercisers. Table 17.2 summarizes the information presented in the previous two sections about the normal, borderline, and abnormal values of the lipidemic profile.

Table 17.2 Desirable, Borderline, and Undesirable Serum Lipid Concentrations in Adults

	Triacylglycerols	Total cholesterol	HDL cholesterol	LDL cholesterol
Desirable	< 150	< 200	≥ 60	< 130
Borderline	150-199	200-239	40-59	130-159
Undesirable	≥ 200	≥ 240	< 40	≥ 160

Values are in mg · dL^{-1}.

Apart from concentration values, the ratio of total cholesterol to HDL cholesterol and the ratio of LDL cholesterol to HDL cholesterol are often used to estimate the risk of atherosclerosis. The two ratios are termed **atherogenic indices**, and we want them to be as low as possible. The desirable values are as follows:

$$\frac{\text{Total cholesterol}}{\text{HDL cholesterol}} < 5$$

$$\frac{\text{LDL cholesterol}}{\text{HDL cholesterol}} < 4$$

17.9 Glycerol

Plasma **glycerol** originates primarily from the complete hydrolysis of triacylglycerols in cells. The reference intervals for plasma glycerol concentration are 0.06 to 0.23 mmol · L^{-1} in ages 3 to 10 and 0.03 to 0.19 mmol · L^{-1} in ages 11 to 80. Glycerol is not measured in serum. The acceleration of lipolysis during exercise raises both the rate of appearance of glycerol in plasma and the plasma glycerol concentration, which can reach 0.6 mmol · L^{-1}.

Although produced along with fatty acids upon triacylglycerol hydrolysis,

glycerol exhibits simpler kinetics in plasma during exercise—that is, a relatively steady increase for as long as exercise lasts. As a result, it is considered a better index of the lipolytic rate than are fatty acids, which do not respond in a steady manner (figure 11.20). Thus one can estimate how strong a lipolytic stimulus is by measuring plasma glycerol before, during, and after exercise.

17.10 Urea

Urea is the main product of nitrogen disposal in humans and derives primarily from amino acid degradation (section 12.9). The reference intervals for serum urea concentration are as follows:

- 11 to 39 mg · dL^{-1}, or 1.8 to 6.5 mmol · L^{-1}, in children and adolescents
- 13 to 43 mg · dL^{-1}, or 2.2 to 7.2 mmol · L^{-1}, in ages 18 to 60
- 17 to 49 mg · dL^{-1}, or 2.8 to 8.2 mmol · L^{-1}, in ages 61 to 90

Certain laboratories report **urea nitrogen** instead of urea. Urea nitrogen has different reference intervals only in terms of mg · dL^{-1}. These are as follows:

- 5 to 18 mg · dL^{-1} in children and adolescents
- 6 to 20 mg · dL^{-1} in ages 18 to 60
- 8 to 23 mg · dL^{-1} in ages 61 to 90

To convert mg · dL^{-1} into mmol · L^{-1}, divide by 6.

Serum urea concentration is affected by the amount of protein consumed. If protein intake is low, then urea concentration is also low. If protein intake is high, through ingestion of either normal food or supplements, as is the practice of many exercisers, then urea concentration rises, because a large part of the protein consumed is degraded. Thus, the measurement of serum urea serves to protect athletes against nutritional excesses. Unfortunately, the inconsistent changes in serum urea concentration during exercise (as described in section 12.10) limit its usefulness as an index of amino acid metabolism in exercise.

Serum urea concentration also rises when the kidneys do not work properly, which hinders the removal of urea from blood. Hence, serum urea concentration serves as an index of kidney function.

17.11 Ammonia

Ammonia (NH_3) appears in place of **ammonium** (NH_4^+, the predominant form at physiological pH) in biochemistry lab reports. Its major sources in the human body are AMP deamination (section 9.5) and amino acid deamination (section 12.5). This dual origin justifies the presence of ammonia twice in table 17.1, that is, as relevant to the metabolism of amino acids and compounds of high phosphoryl-transfer potential (ATP → ADP → AMP → IMP). Ammonia is measured not in serum but in plasma, and the reference interval is 19 to 60 µg · dL^{-1}, or 11 to 35 µmol · L^{-1}.

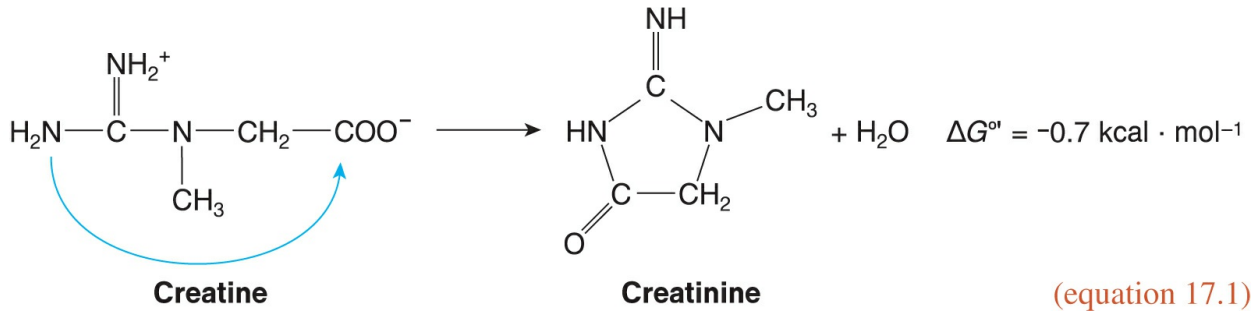
To convert µg · dL^{-1} into µmol · L^{-1}, divide by 1.7.

Plasma ammonium concentration rises during exercise and relates to lactate concentration. The two resemble each other in that they both exhibit an exponential increase with increasing exercise intensity and are higher in men than in women during exercise at the same relative intensity, as discussed by Henry Schulz and Hermann Heck. In men, plasma ammonium concentration may reach or exceed 200 µmol · L^{-1} after maximal exercise. The corresponding values in women are about 20% lower.

Measuring plasma ammonium helps to estimate the degree of AMP and amino acid deamination. If exercise is hard, then ammonium comes primarily from AMP; if exercise is of moderate intensity and prolonged, then ammonium derives primarily from amino acids.

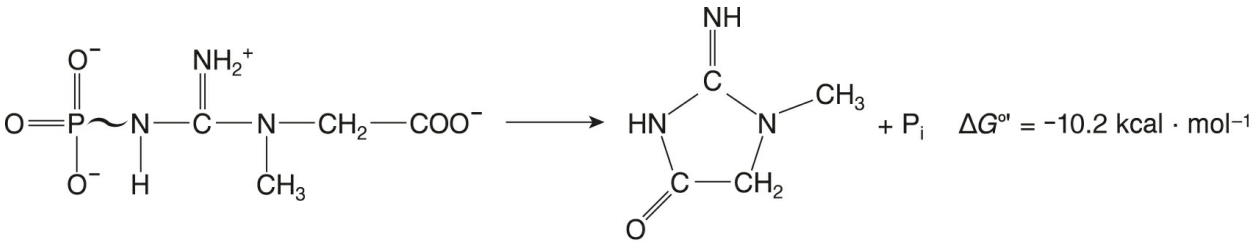
17.12 Creatinine

Creatinine is produced from **creatine** by **dehydration**.



(equation 17.1)

The curved, colored arrow indicates how the creatine molecule closes to form a ring. Creatinine is produced from **phosphocreatine** in a similar manner.



(equation 17.2)

Both reactions take place without the need for an enzyme in our tissues and especially in muscle, which has the highest creatine and phosphocreatine content. Creatinine then passes to the bloodstream, and the kidneys excrete it to the urine. Table 17.3 contains the reference intervals of serum creatinine concentration.

Table 17.3 Reference Intervals of Serum Creatinine Concentration

	Reference interval (mg · dL^{-1})		Reference interval (μmol · L^{-1})[a]	
	Male	**Female**	**Male**	**Female**
Children	0.3-0.7	0.3-0.7	27-62	27-62
Adolescents	0.5-1.0	0.5-1.0	44-88	44-88
18-60 years	0.9-1.3	0.6-1.1	80-115	53-97
61-90 years	0.8-1.3	0.6-1.2	71-115	53-106

[a]To convert mg · dL^{-1} into μmol · L^{-1}, multiply by 88.4.
Data from Tietz, Shuey, and Wekstein (1992); Wu (2006).

Like urea, creatinine serves as an index of renal function, because its plasma concentration rises if the kidneys do not remove it from blood. However, healthy athletes may have elevated creatinine for two other

reasons.

1. Because many athletes have a larger muscle mass than members of the general population, they have more creatine and phosphocreatine. As a result, they may form more creatinine.
2. Many athletes consume high amounts of meat or take creatine supplements, both of which may raise plasma creatinine.

Because of these particularities, the serum creatinine concentration of many athletes approaches the upper reference limit or may even exceed it without any other indication of a kidney problem.

17.13 Uric Acid

Uric acid appears in place of **urate** (figure 9.12, the predominant form at physiological pH) in biochemistry lab reports. It is the major product of purine degradation in our bodies, as described in section 9.6. Urate is carried by blood to the kidneys, which excrete it to urine. Table 17.4 contains the reference intervals of serum uric acid concentration.

Urate increases in serum if we consume foods that are rich in purines, such as organ meat (for example, liver and kidneys) and certain fish (for example, anchovies, herring, and sardines). Serum urate is also raised by impaired renal function. Excess urate forms crystals of sodium urate, which are deposited in joints and cause **gout**, a painful inflammatory disease.

Table 17.4 Reference Intervals of Serum Uric Acid Concentration

	Reference interval (mg · dL^{-1})		Reference interval (µmol · L^{-1})[a]	
	Male	**Female**	**Male**	**Female**
Children	2.0-5.5	2.0-5.5	119-327	119-327
18-60 years	4.4-7.6	2.3-6.6	262-452	137-393
61-90 years	4.2-8.0	3.5-7.3	250-476	208-434

[a]To convert mg · dL^{-1} into µmol · L^{-1}, multiply by 59.48.

Data from Tietz, Shuey, and Wekstein (1992); Wu (2006).

Because ATP and ADP are purine ribonucleotides, serum uric acid concentration is indicative of the metabolism of compounds with high phosphoryl-transfer potential. Exercise promotes the degradation of ATP to urate (sections 9.5 and 9.6), which results in increased urate in the circulation. The increase is usually moderate and transient, not posing a risk of gout. The increase is higher with higher exercise intensities. Measuring serum urate before and after exercise gives an indication of ATP degradation.

Finally, urate possesses antioxidant activity like that of ascorbate (vitamin C). In fact, thanks to its relatively high concentration, urate accounts for most of the total antioxidant capacity in human plasma. This fact justifies its presence twice in table 17.1, that is, as relevant to the metabolism of compounds with high phosphoryl-transfer potential and to the redox state.

17.14 Glutathione

Glutathione, in its reduced and oxidized forms (**GSH** and **GSSG**, respectively), is a major determinant of the redox state (section 14.24). It is present in a variety of cells, including erythrocytes. Given the growing appreciation for the importance of exercise- and training-induced changes in the redox state, I have included two major antioxidants (urate in the previous section and GSH in the current one) and some antioxidant enzymes (in the next chapter) in the biochemical assessment of exercisers.

Experts agree on the value of assessing the redox state of exercisers. As an example, Nathan Lewis and collaborators, in their review of alterations in redox homeostasis in elite endurance athletes, recommend monitoring biomarkers of the redox state in the basal state, before and after performance tests, before and after training cycles, and before and after altitude camps. Such monitoring, they suggest, may help identify individual tolerance for training loads, potentially allowing the prevention of *nonfunctional overreaching* (a term that we will explore in section 18.8) and optimization of training taper and the training program.

We can measure glutathione in whole blood in which we have prevented clotting by the addition of an anticoagulant or in erythrocytes isolated by centrifugation from such blood. In the former case, we usually express the

glutathione concentration in μmol · L^{-1}. In the latter case, we usually express the glutathione content of the erythrocytes relative to their hemoglobin content, that is, in μmol · g^{-1}.

Reference intervals have not been established for GSH. A tentative one, based on data from Tereza Moore and colleagues, is 630 to 1,170 μmol · L^{-1} of blood. Another tentative one, based on data from Wu, is 4.5 to 8.6 μmol · g^{-1} of hemoglobin. Reference intervals also have yet to be established for GSSG, which is hundreds of times less than GSH. A tentative reference interval of GSSG, on the basis of the study by Moore and colleagues, is 0.3 to 2.0 μmol · L^{-1} of blood.

Summary

Lactate is the metabolite displaying the most spectacular concentration changes in the muscles and blood with exercise. Measuring these changes enables estimation of the anaerobic and aerobic capacities of an athlete and helps coaches to program training. To draw safe conclusions from lactate testing, one must design an exercise protocol carefully and control a number of factors that affect its reproducibility. Serum glucose concentration shows the balance between supply of this important fuel to blood and uptake from it.

Triacylglycerols, total cholesterol, HDL cholesterol, and LDL cholesterol all relate to the risk of atherosclerosis. Monitoring these parameters warns against possible undesirable changes and helps assess the effectiveness of training programs in improving the lipidemic profile. Glycerol indicates the lipolytic rate.

Urea is an index of protein intake, amino acid breakdown, and renal function. Ammonia reflects the degradation of AMP and amino acids. Creatinine is a biomarker of creatine and phosphocreatine metabolism, as well as renal function. Uric acid shows purine intake, purine degradation, and antioxidant capacity. Finally, glutathione, in its reduced and oxidized forms,

is an index of the redox state.

Problems and Critical Thinking Questions

1. Draw a hypothetical lactate–intensity plot for a sprinter and one for an endurance athlete. How do the two plots differ, and why?
2. How would you explain a rise and a drop in the blood glucose concentration in two different exercises? How would you remedy the latter?
3. What is the serum LDL cholesterol concentration of an athlete whose total cholesterol is 220 mg · dL^{-1}, HDL cholesterol is 50 mg · dL^{-1}, and triacylglycerols are 100 mg · dL^{-1}?
4. Does the athlete in the previous problem have a healthy lipidemic profile?
5. Which of the two end lipolytic products is preferable to measure in plasma to estimate the lipolytic rate during exercise? Why?
6. A young male bodybuilder has a serum urea concentration of 104 mg · dL^{-1} and a serum creatinine concentration of 1.5 mg · dL^{-1} without any other sign of impaired renal function. What could be the reasons for these abnormal values, and what would you recommend as a remedy?
7. As mentioned in section 17.11, plasma ammonium concentration relates to lactate concentration during exercise. However, it is possible that, in a maximal graded exercise test performed by the same person for the second time within a few days, the lactate values are lower and the ammonium values are higher. Suggest an explanation.
8. In a lucid demonstration of the ability of training to upregulate the antioxidant capacity in the body, Ahmed Elodka and David Nielsen subjected four groups of healthy sedentary individuals to either of three training programs (endurance, resistance, or

combined endurance and resistance) or to no training (the control group) for six weeks. They found that all three training programs increased GSH and decreased GSSG in blood at rest. In addition, all three training programs mitigated the oxidation of GSH to GSSG during a maximal graded exercise test, a conversion indicative of oxidative stress. No such changes occurred in the control group. The following are the mean values (all in $\mu mol \cdot L^{-1}$) regarding the most successful of the three programs, the combined one.

	GSH		GSSG	
	Pre-exercise	**Postexercise**	**Pre-exercise**	**Postexercise**
Pre-training	1,030	860	2.2	3.2
Post-training	1,220	1,160	1.7	2.3

a. What were the training-induced percentage changes in the GSH–GSSG redox pair in the basal state? (Include GSH, GSSG, and their ratio in your calculations.)
b. What was the training-induced mitigation of exercise-induced GSH oxidation?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

L-lactate
maximal lactate steady state, MLSS
progression principle
glucose
diabetes mellitus
prediabetes
hypoglycemia
triacylglycerol, triglyceride
lipidemic profile

lipoprotein
VLDL
cholesterol
LDL
HDL
good cholesterol
bad cholesterol
anabolic androgenic steroid
atherogenic index
glycerol
urea
urea nitrogen
ammonia, ammonium
creatinine
creatine
dehydration
phosphocreatine
uric acid, urate
gout
glutathione
GSH
GSSG

References and Suggested Readings

Beneke R, Leithäuser RM, Ochentel O (2011). Blood lactate diagnostics in exercise testing and training. *International Journal of Sports Physiology and Performance* 6: 8-24.

Braun B, Mawson JT, Muza SR, Dominick SB, Brooks GA, Horning MA, Rock PB, Moore LG, Mazzeo RS, Ezeji-Okoye SC, Butterfield GE (2000). Women at altitude: Carbohydrate

utilization during exercise at 4,300 m. *Journal of Applied Physiology* 88: 246-256.

Elokda AS, Nielsen DH (2007). Effects of exercise training on the glutathione antioxidant system. *European Journal of Cardiovascular Prevention and Rehabilitation* 14: 630-637 (cited in problem 8).

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001). Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *Journal of the American Medical Association* 285: 2486-2497.

Faude O, Kindermann W, Meyer T (2009). Lactate threshold concepts: How valid are they? *Sports Medicine* 39: 469-490.

Lewis NA, Howatson G, Morton K, Hill J, Pedlar CR (2015). Alterations in redox homeostasis in the elite endurance athlete. *Sports Medicine* 45: 379-409.

Lundsgaard AM, Kiens B (2014). Gender differences in skeletal muscle substrate metabolism—molecular mechanisms and insulin sensitivity. *Frontiers in Endocrinology* 5: 195.

Moore T, Le A, Niemi AK, Kwan T, Cusmano-Ozog K, Enns GM, Cowan TM (2013). A new LC-MS/MS method for the clinical determination of reduced and oxidized glutathione from whole blood. *Journal of Chromatography B* 929: 51-55.

Nikolaidis S, Kosmidis I, Sougioultzis M, Kabasakalis A, Mougios V (2018). Diurnal variation and reliability of the urine lactate concentration after maximal exercise. *Chronobiology International* 35: 24-34.

Schulz H, Heck H (2003). Glycogen depletion as indication for ammonia determination in exercise testing. *European Journal of Sport Science* 3(3): 1-9.

Tietz NW, Shuey DF, Wekstein DR (1992). Laboratory values in

fit aging individuals–sexagenarians through centenarians. *Clinical Chemistry* 38: 1167-1185.

Wadley GD, Lee-Young RS, Canny BJ, Wasuntarawat C, Chen ZP, Hargreaves M, Kemp BE, McConell GK (2006). Effect of exercise intensity and hypoxia on skeletal muscle AMPK signaling and substrate metabolism in humans. *American Journal of Physiology Endocrinology and Metabolism* 290: E694-E702.

Wu A (2006). *Tietz Clinical Guide to Laboratory Tests* (Saunders, Philadelphia).

CHAPTER 18

Enzymes and Hormones

Learning Objectives

After reading this chapter, you should be able to do the following:

- Explain the special units used to express enzyme concentration in biological samples.
- Discuss the usefulness of measuring creatine kinase in serum and describe the values it exhibits in athletes.
- Describe what is shown by the serum γ-glutamyltransferase concentration.
- List antioxidant enzymes that may be useful in monitoring exercisers and explain why.
- Describe how the plasma concentrations of cortisol and testosterone are regulated.
- Describe the diurnal variation of cortisol, explain its biological actions, and discuss the utility of measuring it in exercisers.
- Explain how sex and biological age modulate serum testosterone concentration, list the biological actions of testosterone, and discuss how anabolic androgenic steroids interfere.
- Discuss how exercise modifies plasma testosterone concentration and explain the usefulness of measuring testosterone in

exercisers.

- Describe nonfunctional overreaching and overtraining syndrome and discuss whether measuring hormones can help diagnose them.

In this final chapter of part IV—and the book—we will examine how we can evaluate exercisers by measuring some enzymes (the catalysts of our chemical reactions) and hormones (our chemical messengers). Measurement of these biomarkers allows us to assess the integrity of organs, the redox state, training load, and recovery. At the end, I explain why I chose not to discuss certain substances in part IV and provide some perspective on the biochemical assessment of exercisers.

18.1 Enzymes

We will examine a few **enzymes** of interest to exercisers: creatine kinase, γ-glutamyltransferase, and four antioxidant enzymes. Creatine kinase and γ-glutamyltransferase abound in the muscles and liver, respectively, but do not play any known biological role in blood. Nevertheless, small amounts of them are detected in plasma because of leakage from the cells that contain them. By measuring these amounts, we can estimate the integrity of the organs from which the enzymes originate, in the sense that the more intact an organ is, the less of its characteristic enzyme will be in plasma. Conversely, if the organ is damaged, the enzyme will have a high plasma concentration. The antioxidant enzymes, on the other hand, abound in erythrocytes (among other cells), which makes their measurement in blood a practical means of assessing the redox state.

We measure the amount of an enzyme in a biological sample not by mass (say, in milligrams) or chemical quantity (say, in millimoles), as we measure other biochemical parameters, but by **enzyme activity** (section 3.15), which is easier to determine. The oldest and most frequently used unit of enzyme activity is called simply **unit** and symbolized as **U**. Because different

enzymes catalyze different reactions, the definition of U is not standard. Nevertheless, for many enzymes—including the ones that we will examine—1 U is the amount that catalyzes the conversion of 1 μmol of substrate into product within 1 min.

The unit of enzyme activity in the Système international is the **katal**. It is symbolized as **kat** and defined, for all enzymes, as the amount that catalyzes the conversion of 1 mol of substrate into product within 1 s. Expressing enzyme activity (whether in U or kat) relative to sample volume gives rise to a new form of concentration, termed **catalytic activity concentration**, the usual units of which are $U \cdot L^{-1}$ and $kat \cdot L^{-1}$.

A final introductory note is in order. Because the rate of enzyme reactions (and chemical reactions, in general) depends on temperature, a certain amount of an enzyme exhibits different activities at different temperatures. It is therefore necessary, when reporting values of enzyme activity, to also report the temperature at which the measurement was performed. This is usually 37 °C, the average temperature of the human body.

18.2 Creatine Kinase

Creatine kinase (CK) catalyzes the interconversion of phosphocreatine and ATP according to equations 9.4 and 14.10. The enzyme is present in almost all tissues but is highest in the three kinds of muscle (skeletal, heart, and smooth) and in the brain. Different CK isoforms predominate in these tissues (section 9.3), which lets us detect the origin of CK in plasma. The reference intervals of the catalytic activity concentration of CK in serum at 37 °C are as follows:

- 20 to 200 $U \cdot L^{-1}$, or 0.3 to 3.3 $\mu kat \cdot L^{-1}$, in men
- 20 to 180 $U \cdot L^{-1}$, or 0.3 to 3.0 $\mu kat \cdot L^{-1}$, in women

To convert $U \cdot L^{-1}$ into $\mu kat \cdot L^{-1}$, divide by 60.

More than 94% of serum CK is of the CK3 (CK-MM) isoform, which predominates in skeletal muscle.

Serum CK rises when an organ that contains the enzyme is damaged. Two typical cases are the following:

- Acute myocardial infarction, in which CK2 (CK-MB), the heart isoform, rises
- Myopathies, in which CK3 rises

Closer to our interests, serum CK increases in healthy persons after exercise, particularly after unaccustomed eccentric exercise, because of increased muscle fiber damage or muscle injury. Serum CK has been shown to be the best biochemical marker of muscle fiber damage. It rises slowly after exercise and usually peaks after two to five days. This rise is accompanied by decreased muscle strength and what is termed **delayed-onset muscle soreness**. Then CK declines even more slowly toward baseline (within about a week) as the musculature recovers (figure 18.1).

A frequent error is to associate the catalytic activity concentration of CK *in serum* with the power of the ATP-phosphocreatine system. However, the two parameters are not related in any way. To estimate the power of the ATP-phosphocreatine system one needs to measure the catalytic activity concentration of CK *in muscle*.

As a rule, athletes have higher serum CK than nonathletes do because of the regular strain imposed by training on their muscles. In fact, serum CK is profoundly affected by training, and values well above the upper reference limits listed earlier (which apply to physically inactive individuals) are very common among athletes. Based on this observation, I determined the following reference intervals for athletes in 2007:

- 82 to 1,083 $U \cdot L^{-1}$, or 1.4 to 18.1 $\mu kat \cdot L^{-1}$, in men
- 47 to 513 $U \cdot L^{-1}$, or 0.8 to 8.6 $\mu kat \cdot L^{-1}$, in women

Thus, values as high as about 1,000 $U \cdot L^{-1}$ in male athletes and 500 $U \cdot L^{-1}$ in female athletes should not be taken as pathologic without any other evidence for a health problem. Values twice as high (that is, up to 2,000 and 1,000 $U \cdot L^{-1}$, respectively) may indicate increased burden on the musculature, and values above those levels may signal excessive muscle fiber damage (figure 18.2).

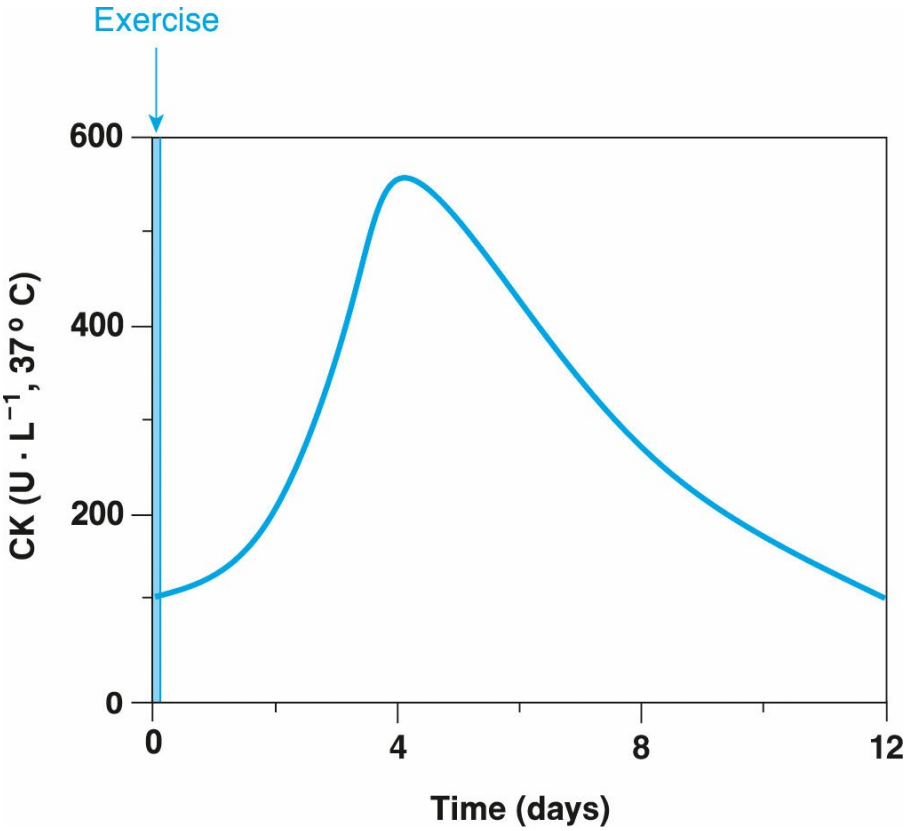


Figure 18.1 **Serum creatine kinase after eccentric exercise.** Eccentric exercise causes muscle fiber damage, which results in increased serum CK for several days afterward.

Even such high values, however, do not automatically mean that the athlete or coach should be discouraged from carrying on with a certain training routine. They just mean that the athlete and coach should take the values into consideration, weigh them against the specific contents of their training program, and judge whether they are expectedly or unexpectedly high. Based on the resulting conclusions, the athlete and coach should decide how to continue with the training routine.

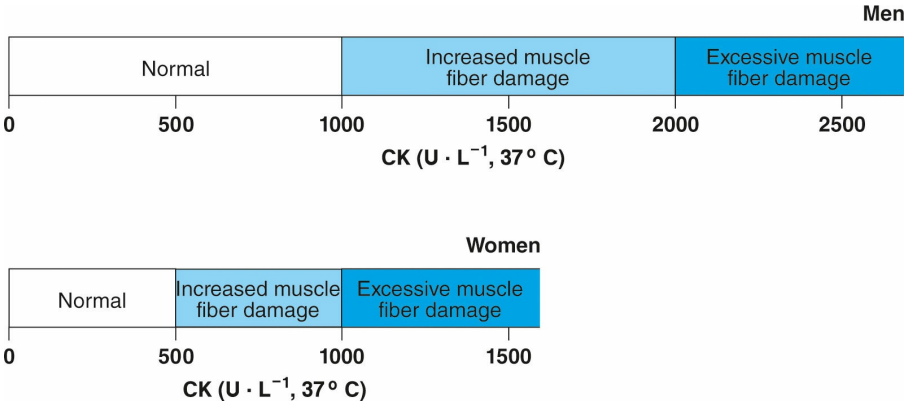


Figure 18.2 Suggested framework for the interpretation of serum CK values in athletes. Athletes have quite different serum CK values from nonathletes. Values up to about 1,000 U · L^{-1} in male athletes and 500 U · L^{-1} in female athletes should be considered normal. Values twice as high may show increased muscle fiber damage, and yet higher values may indicate excessive muscle fiber damage.

Although serum CK is generally higher in athletes than in nonathletes, its increase after a given exercise is lower in the former thanks to what has been termed the **repeated-bout effect**. That is, after several days or even weeks, repetition of an exercise, particularly eccentric exercise, causes less muscle fiber damage and a lower rise of CK than did the previous exercise. There is no agreement among investigators as to how this adaptation occurs. The following are the most probable mechanisms, as reviewed by Robert Hyldahl and collaborators:

- Neural adaptations. Following the initial bout, the central nervous system increases the number of motor units that it recruits during the subsequent bout. As a result, more muscle fibers share a certain load, and fewer are damaged.
- Adaptations of the extracellular matrix. Eccentric exercise results in remodeling of the connective tissue surrounding the muscle fibers and the connections between the two, thus strengthening them and protecting them against subsequent damage.

Regardless of the cause of the repeated-bout effect, its existence offers the opportunity to estimate muscle tolerance to successive training sessions through regular measurements of serum CK. To summarize, then, measuring CK in exercisers is useful in three intertwined ways:

- When measured over a few days after a single exercise session, it provides an estimate of how hard the exercise was on the musculature.
- When measured over a week following its peak, it gives an indication of the recovery process.
- When measured regularly during a training program, it becomes a biomarker of muscular adaptations.

18.3 γ-Glutamyltransferase

γ-Glutamyltransferase, or **GGT**, catalyzes the removal of the γ-glutamate residue from glutathione (figure 14.16), thus initiating glutathione degradation. The enzyme abounds in several organs, including the liver, and this organ is believed to be the main source of GGT in the circulation. The reference intervals of the catalytic activity concentration of GGT in serum at 37 °C are as follows:

- 8 to 61 U · L^{-1}, or 0.1 to 1.0 μkat · L^{-1}, in men
- 5 to 40 U · L^{-1}, or 0.1 to 0.7 μkat · L^{-1}, in women

> To convert U · L^{-1} into μkat · L^{-1}, divide by 60.

Serum GGT rises in cases of liver disease, such as cirrhosis and hepatitis. Thus, it serves as an index of the integrity of this vital organ and should be a component of the battery of tests used to assess the health of exercisers as well as the general population.

18.4 Antioxidant Enzymes

In section 14.24, we met four enzymes involved in the antioxidant defense of the body: **superoxide dismutase**, **catalase**, **glutathione peroxidase**, and **glutathione reductase**. Measurement of one or more of these enzymes—along with antioxidants such as uric acid and glutathione (sections 17.13 and 17.14)—in exercisers would be useful in assessing the redox state in the basal state and adaptations of the redox state to training.

Table 18.1 Reference Intervals for Antioxidant Enzyme Concentrations in Human Blood at 37 °C

	Male	Female
Superoxide dismutase (CuZnSOD)	558-1,350	515-1,420
Glutathione peroxidase	26-77	23-87

Values are in $U \cdot g^{-1}$ of hemoglobin.
Data from Habdous et al. (2003).

All four enzymes are present in erythrocytes. Thus, as in the case of glutathione, it is appropriate to measure them in whole blood or isolated erythrocytes. Reference intervals have not been firmly established for a number of reasons, including technical problems with the assays. Tentative reference intervals of two of the enzymes in adults are presented in table 18.1.

18.5 Hormones

In part III, we met **hormones** as signal-transducing biomolecules, through which the body achieves the concerted response of different organs to situations such as exercise. Measuring the serum concentration of a hormone may help to estimate the strength of the signal transduction pathway(s) in which the hormone participates. Hormone concentrations are much lower than those of the metabolites we considered in the previous chapter, as will become evident from the reference intervals that follow. This fact makes hormone action even more amazing.

We will examine two steroid hormones that are useful in the biochemical assessment of exercisers. These are **cortisol** and **testosterone**, both introduced in section 14.10 as the major **glucocorticoid** and major **androgen**, respectively. Their molecules (figures 18.3 and 14.7) resemble cholesterol, the parent compound.

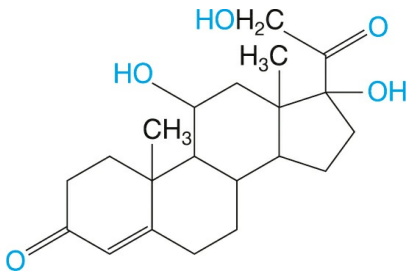


Figure 18.3 **Cortisol.** Its hydrophilic ends are shown in color, and the hydrophobic parts are shown in black.

The two hormones share several other features as well. Their production is controlled by the hypothalamus, which synthesizes two peptide hormones, **corticotropin-releasing hormone**, or **CRH**, and **gonadotropin-releasing hormone**, or **GnRH** (figure 18.4). These hormones are secreted to the anterior pituitary gland, which lies right underneath the hypothalamus in the brain. There, CRH stimulates the secretion of **corticotropin** (also known as **adrenocorticotropic hormone**, or **ACTH**), and GnRH stimulates the secretion of two **gonadotropins** to blood. All three **tropins** are proteins in nature.

Corticotropin then binds to receptors in the plasma membrane of target cells in the **adrenal cortex** (the outer layer of the adrenal glands) and stimulates cortisol synthesis. The gonadotropins, through their own receptors, control the development and function of the gonads (the testes and ovaries). Their individual names are **follicle-stimulating hormone**, or **FSH**, and **luteinizing hormone**, or **LH**. Testosterone synthesis and secretion are controlled by LH, which also controls testis development. FSH, on the other hand, augments sperm production. This effect completes two important relays of signaling events, the **hypothalamic-pituitary-adrenal axis** and the **hypothalamic-pituitary-gonadal axis**. (In females, the latter ends with estrogen and progestogen production in the ovaries.)

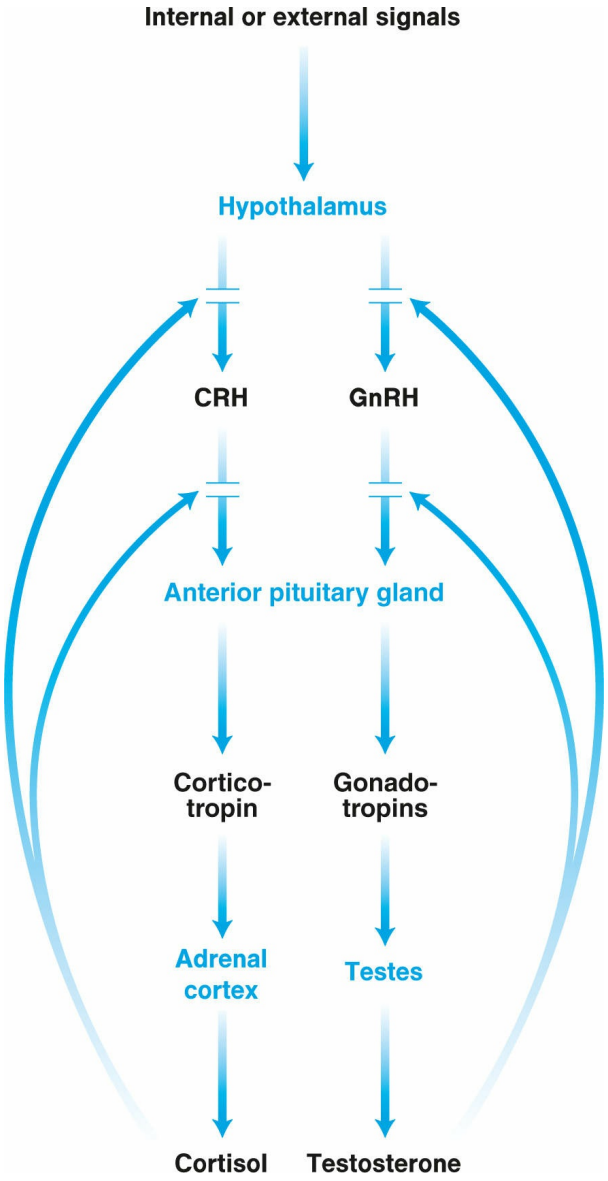


Figure 18.4 **Control of cortisol and testosterone production.** The hypothalamus controls the development and function of many organs through the hormones it secretes to the anterior pituitary gland in response to internal and external signals. Two of the hormones, CRH and GnRH, provoke the secretion of corticotropin and gonadotropins by the pituitary gland. These secretions, in turn, stimulate the synthesis of cortisol by the adrenal cortex and of testosterone by the testes. The plasma cortisol and testosterone concentrations are controlled by negative feedback (upward arrows): If either gets too high, it signals the hypothalamus and pituitary to lower the secretion of the hormones that stimulate its production.

Plasma cortisol and testosterone concentrations are controlled by reciprocal communication of the brain with the adrenal glands and testes. When cortisol concentration drops, the hypothalamus elevates CRH secretion

and the pituitary elevates corticotropin secretion, resulting in increased cortisol secretion. If blood cortisol rises excessively, the hypothalamus limits CRH secretion and the pituitary limits corticotropin secretion, resulting in decreased cortisol secretion.

Likewise, when blood testosterone concentration falls, the hypothalamus raises GnRH secretion and the pituitary raises gonadotropin secretion, causing increased testosterone secretion. If blood testosterone rises excessively, the hypothalamus curbs GnRH secretion and the pituitary curbs gonadotropin secretion, causing decreased testosterone secretion.

Cortisol and testosterone exert their actions in a way similar to that of estradiol (figure 14.8). That is, they cross the plasma membrane of target cells thanks to their amphipathic character. Once inside the cells, they bind to receptors, which then migrate to the nucleus. There, the receptors act as transcription factors, inducing genes that elicit effects characteristic of the two hormones.

Now let's examine the usefulness of measuring cortisol and testosterone in exercisers.

18.6 Cortisol

Serum cortisol concentration displays a distinctive *diurnal variation*: It is high in the morning and low in the evening. The reference intervals are as follows:

- 50 to 230 $\mu g \cdot L^{-1}$, or 138 to 635 $nmol \cdot L^{-1}$, at 8 a.m.
- 30 to 160 $\mu g \cdot L^{-1}$, or 83 to 442 $nmol \cdot L^{-1}$, at 4 p.m.
- less than 50% of the 8 a.m. value at 8 p.m.

To convert $\mu g \cdot L^{-1}$ into $nmol \cdot L^{-1}$, multiply by 2.76.

Cortisol secretion elicits a slow rise in plasma glucose concentration (slower than that elicited by glucagon), due partly to the stimulation of gluconeogenesis in the liver. In muscle, cortisol promotes proteolysis and inhibits protein synthesis. Cortisol also decreases bone formation.

At high plasma concentrations, cortisol and other glucocorticoids suppress the immune system. For this reason, glucocorticoids are used as anti-inflammatory and antiallergic drugs. Athletes may use these drugs to counter the acute inflammation caused by injury, thus enabling themselves to compete. WADA considers this practice unacceptable and prohibits the use of glucocorticoids, which form a separate category in the WADA's Prohibited List. WADA also lists corticotropin as a banned substance under the "peptide hormones, growth factors, related substances, and mimetics" category.

Cortisol secretion by the adrenal cortex increases in hypoglycemia, obesity, physical stress, and mental stress. Plasma cortisol concentration rises in endurance exercise exceeding 60% of $\dot{V}O_2max$ in intensity and 10 min in duration. Plasma cortisol also increases after resistance exercise of considerable volume. In all cases, plasma cortisol returns to baseline after maximally 2.5 hours of recovery from exercise.

Normal training does not seem to modify these acute responses to exercise or the resting cortisol concentration, as reviewed by Martine Duclos and Antoine Tabarin. However, this may not be the case in periods of intensified training and incomplete recovery, when one may see elevated resting cortisol in an athlete. I will return to this issue in section 18.8.

Thus, measuring cortisol before and after exercise may show how the body receives a physical load. High concentrations of cortisol are undesirable because it promotes catabolism in muscle and bone and suppresses the immune system. An additional utility of measuring cortisol will be discussed in section 18.8. In all cases, measurements that are going to be used in comparisons should be performed at the same hour of the day because of cortisol's diurnal variation.

18.7 Testosterone

Testosterone is synthesized and secreted primarily by the **Leydig cells** in the testes; small amounts are also produced by the adrenal cortex and the ovaries. Serum testosterone concentration is heavily influenced by sex and biological age, rising from childhood into adulthood and being more than tenfold higher

in men than in women. The reference intervals are shown in table 18.2.

Plasma testosterone concentration displays a diurnal variation like that of cortisol, though on a smaller scale: It peaks at around 7 a.m. and reaches a minimum that is 25% lower than the morning value at about 8 p.m.

Testosterone and other androgens exert a multitude of actions in the body. They are responsible for the development and maintenance of the organs of the male reproductive system, including the testes, prostate, epididymides, and penis. They cause sperm production. They are necessary for the development and maintenance of the secondary sex characteristics of the male, such as deep voice and hairiness. They determine aggressiveness and libido in the male. They have anabolic effects on the skeleton, skeletal muscles, and skin, as they promote protein synthesis and curb proteolysis. Finally, they increase erythropoietin synthesis in the kidneys and the responsiveness of immature bone marrow cells to erythropoietin, thus promoting erythropoiesis.

Table 18.2 Reference Intervals of Testosterone Concentration in Human Serum

	Reference interval (μg · L^{-1})		Reference interval (nmol · L^{-1})[a]	
	Male	**Female**	**Male**	**Female**
Children	0.03-0.3	0.02-0.2	0.10-1.04	0.07-0.69
Tanner stage 1[b]	0.02-0.23	0.02-0.1	0.07-0.80	0.07-0.35
Tanner stage 2	0.05-0.7	0.05-0.3	0.17-2.43	0.17-1.04
Tanner stage 3	0.15-2.8	0.1-0.3	0.52-9.72	0.35-1.04
Tanner stage 4	1.05-5.45	0.15-0.4	3.64-18.91	0.52-1.39
Tanner stage 5	2.65-8	0.1-0.4	9.20-27.76	0.35-1.39
Adults	2.8-11	0.15-0.7	9.72-38.17	0.52-2.43
After menopause	—	0.08-0.35	—	0.28-1.21

[a]To convert μg · L^{-1} into nmol · L^{-1}, multiply by 3.47.

[b]Tanner stages are numbers on a scale rating the sexual maturation of boys and girls during adolescence from 1 (initial) to 5 (final) on the basis of the development of secondary sex characteristics such as pubic hair, genital size, breast bud, and voice change.

Data from Wu (2006); Yu (2018).

Natural androgens and a wide variety of synthetic analogs termed **anabolic androgenic steroids**, or **AAS**, are apparently the most widely used

doping substances. This prevalence is apparently due to their powerful positive effects on muscle mass and strength, regardless of their serious detrimental effects on health. AAS are banned under the “anabolic agents” category in WADA’s Prohibited List.

The plasma testosterone concentration of a man using AAS decreases to values that may even be characteristic of women (unless he uses testosterone itself, in which case plasma concentration increases). The dramatic decrease with AAS use occurs because, being similar in structure to testosterone, these drugs trick the hypothalamus and pituitary into sensing an excessive plasma testosterone concentration and suppressing GnRH and gonadotropin secretion (figure 18.4).

This suppression results in decreased testosterone synthesis by the testes. Moreover, since the gonadotropins are needed for testis development and sperm production, their shortage results in testicular atrophy and suppression or even disruption of sperm production. Gonadotropins are also banned as doping substances by WADA under the “peptide hormones, growth factors, related substances, and mimetics” category.

Plasma testosterone concentration usually rises with endurance or resistance exercise in young men, as reviewed by Natalia Cano Sokoloff and colleagues. The magnitude of the rise depends on exercise intensity and total work. In general, it appears that considerable intensity and work are necessary to produce an increase, although findings in the literature differ so much that it is not possible to identify intensity and work thresholds.

In addition, a large muscle mass has to be involved in exercise for testosterone to increase. The rise in plasma testosterone with exercise is smaller in middle-aged and old men than in young men. Women, children, and adolescents exhibit small increases or no change in plasma testosterone in response to exercise, as reviewed by Jakob Vingren and associates.

If serum testosterone has increased at the end of exercise, it returns to baseline or below baseline within approximately one half hour. When endurance exercise lasts longer than 1 h, the elevated testosterone concentration begins to decrease and may drop below baseline after 3 h of exercise.

The chronic effect of exercise on serum testosterone concentration is less

clear than the acute effect, for which all possible outcomes (that is, increase, decrease, and no change) are reported in the literature. As with cortisol, the resting testosterone concentration may change with the training phase of an athlete, albeit in the opposite direction: Intensified training and inadequate recovery may suppress it, as Marcin Grandys and coworkers have found.

Because testosterone promotes protein synthesis and curbs proteolysis in muscle, high concentrations of it are considered desirable, especially in resistance training. For decades, it has been assumed that testosterone is the major promoter of muscle growth and strength increase in response to resistance training, at least in men. However, research from the laboratory of Stuart Phillips (for example, the study by Robert Morton and coworkers) shows that the rise in serum testosterone—or other anabolic hormones, such as growth hormone and IGF1, for that matter—after resistance exercise is not related to muscle protein synthesis, hypertrophy, or strength.

If confirmed by other laboratories, these findings certainly limit the utility of measuring the serum testosterone response to acute exercise. Nevertheless, baseline measurements may still be useful in assessing training load and recovery.

18.8 Overtraining Syndrome

Athletes or coaches may sometimes overestimate and exceed the athlete's natural capabilities in their desire to achieve rapid adaptations and boost performance. This overreaching, in combination with inadequate recovery, may lead to a series of undesirable signs and symptoms, including a drop in performance, chronic fatigue, disturbances in heart function, hormonal distress, susceptibility to infections (especially of the upper respiratory tract), slow healing of injuries, and sleep disorders.

To describe such cases, the European College of Sport Science and the American College of Sports Medicine, with lead author Romain Meeusen, issued a joint consensus statement adopting the terms **nonfunctional overreaching**, or **NFOR**, and **overtraining syndrome**, or **OTS**. The former is characterized by the appearance of the first signs and symptoms of prolonged training distress and will require weeks or months to reverse. The

latter is more serious and requires several months or even years for full recovery. Distinguishing the two entities is difficult, because the signs and symptoms are practically the same, possibly differing in severity. Meeusen and associates suggest that a distinction between NFOR and OTS can be achieved only retrospectively, when one can review the entire time course of the problem.

For decades, many investigators have directed their efforts toward, on the one hand, diagnosing NFOR and OTS and, on the other hand, detecting the risk of either one early enough to prevent it. These efforts have met with only limited success, since NFOR and OTS are complex conditions, involving a multitude of factors. In the context of these efforts, the biochemical assessment of athletes has also been recruited in the search for biochemical markers of NFOR and OTS.

One of the first markers to be proposed was the serum testosterone-to-cortisol ratio at rest. However, despite initial evidence, it is now rather clear that neither NFOR nor OTS is associated with abnormal basal concentrations of testosterone or cortisol (or any other hormone, for that matter), as Flavio Cadegiani and Claudio Kater found in a systematic review. Thus, it seems more likely that, rather than signaling NFOR or OTS, the ratio reflects how the body receives a training load (decreased ratio with increased load, according to the discussion in the previous two sections) and how it recovers.

As for detecting the two conditions, Meeusen and associates have suggested that studying hormonal responses to exercise may be more effective than measuring resting hormone concentrations. This suggestion is supported by Cadegiani and Kater's review, although their finding was based on a small number of studies and athletes. If appropriate exercise (or no-exercise) tests that challenge the endocrine system are validated through future research, they may become useful tools against NFOR and OTS.

18.9 Epilogue

In part IV, I have brought together two dozen blood parameters that I consider the most useful in assessing athletes and other persons who exercise regularly. I have left out a number of parameters that, although measured

frequently for research purposes, either are particularly demanding in technical terms or offer information of limited value for assessing the health or performance of an exerciser. Thus, I have omitted the following:

- Fatty acids, which could serve as indices of lipolysis. Glycerol is a better index of it.
- Proteins that abound in muscle, such as myoglobin, aldolase (the enzyme catalyzing the fourth reaction of glycolysis), and lactate dehydrogenase. Although serum concentrations of these proteins may serve as markers of muscle fiber damage, CK is a more sensitive marker.
- Two aminotransferases, that is, alanine aminotransferase and aspartate aminotransferase, which abound in the liver and are used as indices of liver integrity. Because these enzymes are also present in muscle, their catalytic activity concentrations in serum increase after hard exercise as a result of muscle fiber damage. Thus, they are not as useful in assessing exercisers as GGT, which is not affected by hard exercise.
- Catecholamines, which are useful in estimating the stimulation of the sympathoadrenergic system. Unfortunately, the stress caused by piercing the skin for blood sampling increases the plasma concentration of catecholamines; thus, blood sampling needs to be performed by inserting a catheter into a vein and waiting for one half hour. This need makes catecholamine measurements impractical outside of a research setting.
- Growth hormone. Its secretion is pulsatile; that is, it takes place in waves at intervals shorter than one hour. As a result, one needs to perform multiple blood samplings to obtain reliable results. Again, this need renders measuring growth hormone impractical outside of a research setting.
- Other hormones, such as aldosterone and angiotensin, listed at the end of section 14.23. Although they play pivotal roles in a variety of bodily functions, they do not seem to offer useful information to exercisers.
- Vitamins. Although they serve vital functions, there is no consensus that higher plasma vitamin concentrations are related to better health or

performance as long as they fall within the reference intervals. In addition, exercise does not seem to modify plasma vitamin concentrations. Finally, several vitamin tests are technically demanding and costly. For all of these reasons, measuring vitamin levels in well-nourished athletes with no signs of vitamin deficiency is not justified.

- Minerals, such as sodium, potassium, calcium, and magnesium, all of which are important for the neural and muscular processes of movement. Exercise does not affect their plasma concentrations considerably (except for a transient rise in $[K^+]$), because all are subject to powerful homeostatic mechanisms. Moreover, serum Ca^{2+} and Mg^{2+} concentrations are not trustworthy markers of their adequacy in the body unless their intake through the food is very low. Other minerals, such as zinc and chromium, do not seem to influence health or performance (again, as long as they fall within the reference intervals) or to be affected by exercise.

My intent in this presentation of the value of measuring many biomarkers throughout part IV has been to provide a clear and comprehensive picture of what the biochemical assessment of exercisers can offer. As compared with other ways of assessing exercisers—such as physiologically, nutritionally, biomechanically, and psychologically—biochemical assessment is more expensive due to the cost of chemical analyses. Moreover, to be as beneficial as possible, it must be regular and programmed.

Specifically, athletes subject to high training loads and frequent changes in training routines should be assessed biochemically several times during the year, preferably at milestones of their macrocycles that are of interest to their coaches. This approach allows the establishment of a long-term record for each athlete, which serves as a source of individualized reference values. This record, in turn, enables more accurate assessment of health and performance than resorting to general reference intervals.

It is, therefore, imperative to avoid meaningless expenses for analyses of limited utility. On the other hand, the cost of the biochemical assessment is a small fraction of the money usually spent in competitive sport. Therefore, sporting clubs and sport organizations should not hesitate to bear the cost of

biochemical assessment. They should realize that, by doing so, they invest in their own future.

Summary

The catalytic activity concentration of certain enzymes in serum shows the integrity of the organs in which they abound. Thus, CK serves as the most sensitive marker of muscle fiber damage, being the biochemical parameter that displays the largest differences in resting values between athletes and nonathletes. In addition, thanks to the repeated-bout effect, which lowers the increase of CK when a given exercise is repeated, CK serves as a biomarker of muscular adaptations. GGT serves as a marker of liver damage and, unlike the aminotransferases, is not affected by muscle fiber damage. Antioxidant enzymes—such as superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase—which abound in erythrocytes, are useful in assessing the redox state in the basal state and its adaptations to training when measured in whole blood or isolated erythrocytes.

The plasma concentrations of two steroid hormones, that is, cortisol and testosterone, are regulated by the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes, respectively. Both display diurnal variation, although this is more pronounced in cortisol, and both increase in response to exercise of considerable intensity and load. Their measurement at rest in athletes is useful in estimating the training load and the adequacy of recovery, although it is no longer considered useful in diagnosing NFOR or OTS.

Problems and Critical Thinking Questions

1. Which blood test could show the burden of a training session on the musculature of an exerciser?

2. What would you think of a serum CK value of 800 U · L^{-1} at 37 °C if it came from a male athlete? What if it came from a female athlete?
3. Suppose that serum CK increased by 500 U · L^{-1} in a novice exerciser a few days after performing resistance exercise. Which of the three—300, 500, or 700 U · L^{-1}—is the most probable increase when she performs the same exercise one week later?
4. Which blood test would you recommend to assess the integrity of the liver in an exerciser? Which would you not recommend, and why?
5. (Integrative problem) Which blood tests would you recommend to assess the redox state in an exerciser?
6. Which blood test could show the presence of physical or mental stress?
7. (Integrative problem) Which serum parameters could be affected by AAS use?

Key Terms

All key terms appear in boldface in the text. Terms in color are defined in the glossary.

enzyme
enzyme activity
unit, U
katal, kat
catalytic activity concentration
creatine kinase, CK
delayed-onset muscle soreness
repeated-bout effect
γ-glutamyltransferase, GGT
superoxide dismutase

catalase
glutathione peroxidase
glutathione reductase
hormone
cortisol
testosterone
glucocorticoid
androgen
corticotropin-releasing hormone, CRH
gonadotropin-releasing hormone, GnRH
corticotropin, adrenocorticotropic hormone, ACTH
gonadotropin
tropin
adrenal cortex
follicle-stimulating hormone, FSH
luteinizing hormone, LH
hypothalamic-pituitary-adrenal axis
hypothalamic-pituitary-gonadal axis
Leydig cell
anabolic androgenic steroid, AAS
nonfunctional overreaching, NFOR
overtraining syndrome, OTS

References and Suggested Readings

Cadegiani FA, Kater CE (2017). Hormonal aspects of overtraining syndrome: A systematic review. *BMC Sports Science, Medicine and Rehabilitation* 9: 14.

Duclos M, Tabarin A (2016). Exercise and the hypothalamo-pituitary-adrenal axis. *Frontiers of Hormone Research* 47: 12-26.

Grandys M, Majerczak J, Zapart-Bukowska J, Kulpa J, Zoladz JA (2011). Gonadal hormone status in highly trained sprinters and in untrained men. *Journal of Strength and Conditioning Research* 25: 1079-1084.

Habdous M, Herbeth B, Vincent-Viry M, Lamont JV, Fitzgerald PS, Visvikis S, Siest G (2003). Serum total antioxidant status, erythrocyte superoxide dismutase and whole-blood glutathione peroxidase activities in the Stanislas cohort: Influencing factors and reference intervals. *Clinical Chemistry and Laboratory Medicine* 41: 209-215.

Hyldahl RD, Chen TC, Nosaka K (2017). Mechanisms and mediators of the skeletal muscle repeated bout effect. *Exercise and Sport Science Reviews* 45: 24-33.

Meeusen R, Duclos M, Foster C, Fry A, Gleeson M, Nieman D, Raglin J, Rietjens G, Steinacker J, Urhausen A (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: Joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Medicine and Science in Sports and Exercise* 45: 186-205.

Morton RW, Oikawa SY, Wavell CG, Mazara N, McGlory C, Quadrilatero J, Baechler BL, Baker SK, Phillips SM (2016). Neither load nor systemic hormones determine resistance training-mediated hypertrophy or strength gains in resistance-trained young men. *Journal of Applied Physiology* 121: 129-138.

Mougios V (2007). Reference intervals for serum creatine kinase in athletes. *British Journal of Sports Medicine* 41: 674-678.

Sokoloff NC, Misra M, Ackerman KE (2016). Exercise, training, and the hypothalamic-pituitary-gonadal axis in men and women. *Frontiers of Hormone Research* 47: 27-43.

Vingren JL, Kraemer WJ, Ratamess NA, Anderson JM, Volek JS, Maresh CM (2010). Testosterone physiology in resistance

exercise and training: The up-stream regulatory elements. *Sports Medicine* 40: 1037-1053.

Wu A (2006). *Tietz Clinical Guide to Laboratory Tests* (Saunders, Philadelphia).

Yu S, Qiu L, Liu M, Li S, Tao Z, Zhang Q, Xia L, Li P, Hou L, Qin X, Yin Y, Ichihara K, Cheng X (2018). Establishing reference intervals for sex hormones and SHBG in apparently healthy Chinese adult men based on a multicenter study. *Clinical Chemistry and Laboratory Medicine* 56: 1152-1160.

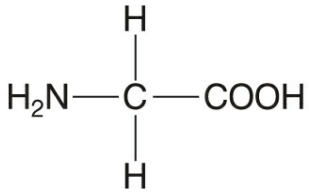
Answers to Problems and Critical Thinking Questions

Chapter 1

1. A molecular formula shows which atoms, and in what proportions, make up a molecule, whereas a structural formula shows how the atoms in a molecule are connected to one another. The former has the advantages of brevity and of allowing a fast calculation of molecular mass. The latter has the advantage of detail.

2. $C_3H_7NO_2$ and 89 Da

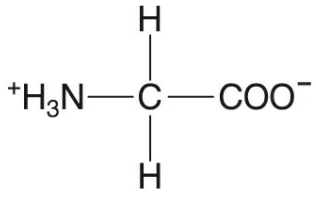
3. a. Structural formula:



Molecular formula: $C_2H_5NO_2$

b. 75 Da

c.



4. a. Nonpolar (because it contains only C and H); b. Polar (because it contains O connected to C and H); c. Polar (because it contains N connected to C and H); d. Polar (You know why!)

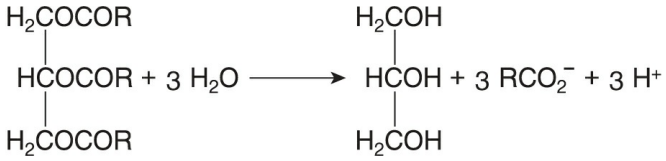
5. By chemical structure, detergents are both hydrophilic and hydrophobic. That way, they transfer the (usually hydrophobic) stains from fabrics, dishware, and other surfaces into water. Compounds that are hydrophilic and hydrophobic are called amphipathic. For natural amphipathic compounds, see sections 5.7, 5.9, and 5.10.

6. To go from mmol · L^{-1} to mg · dL^{-1}, first convert mmol into mg by multiplying by the molecular mass, which is 89 Da for alanine (recall problem 2). Thus 0.4 mmol of alanine is 35.6 mg. Then, because this amount is per liter, divide it by 10 in order to express it per deciliter. Thus, the concentration would be 3.56 mg · dL^{-1}.

Conversely, to go from mg · dL^{-1} to mmol · L^{-1}, first convert mg into mmol by dividing by the molecular mass, which is 75 Da for glycine (recall problem 3). Thus 1.5 mg of glycine is 0.02 mmol. Then, because this amount is per deciliter, multiply it by 10 in order to express it per liter. Thus, the concentration would be 0.2 mmol · L^{-1}.

7. The evaporation of a liquid is a physical process: The fluid merely goes from the liquid to the gaseous phase. The rusting of a metal is a chemical reaction: The metal reacts with oxygen to form oxides.

8. a.



b.

$$K_{eq} = \frac{[C_3H_8O_3]\,[RCO_2^-]^3\,[H^+]^3}{[C_6H_5O_6R_3]\,[H_2O]^3}$$

9. a. The cytosol goes from alkaline to acidic; b. On the basis of equation 1.3, the $[H^+]$ changes from $10^{-7.2}$ to $10^{-6.4}$ mol · L^{-1}. If you divide the latter by the former (by subtracting the exponents), you get $10^{-6.4-(-7.2)} = 10^{0.8} = 6.3$. Thus, the $[H^+]$ has increased 6.3 fold.

10. By applying the Henderson-Hasselbalch equation (equation 1.8), we get, $7 = 4 + \log [C_3H_5O_3^-]/[C_3H_6O_3]$. Hence, $3 = \log [C_3H_5O_3^-]/[C_3H_6O_3]$ and $[C_3H_5O_3^-]/[C_3H_6O_3] = 10^3$. Thus, the anionic form predominates over the uncharged one by a factor of 1,000.

11. a. Because pH equals –log [H^+], the pH would change by –log 15, that is, by –1.2; b. In reality, the pH of blood does not change because of the presence of effective buffer systems, which eliminate the additional acidity.

Chapter 2

10. Order the reactions in such a way that a product of one is a reactant in the next. Thus, a possible pathway is

(c) fructose 1,6-bisphosphate $\rightleftharpoons$ dihydroxyacetone phosphate + glyceraldehyde 3-phosphate $\Delta G°' = 4.7$ kcal · mol^{-1}

(reverse a) glyceraldehyde 3-phosphate + NAD^+ + P_i $\rightleftharpoons$ 1,3-bisphosphoglycerate + NADH + H^+ $\Delta G°' = 1.9$ kcal · mol^{-1}

(e) 1,3-bisphosphoglycerate + ADP $\rightleftharpoons$ 3-phosphoglycerate + ATP $\Delta G°' = -4.4$ kcal · mol^{-1}

(f) 3-phosphoglycerate $\rightleftharpoons$ 2-phosphoglycerate $\Delta G°' = 1$ kcal · mol^{-1}

(reverse d) 2-phosphoglycerate + H_2O $\rightleftharpoons$ phosphoenolpyruvate $\Delta G°' = -1$ kcal · mol^{-1}

An equally legitimate pathway would be the reverse, although then you would have to turn more reactions around. Reaction *b* does not fit into the pathway, because its product is not a reactant in any of the reactions; hence, it cannot serve as an intermediate compound. On the other hand, if we consider it as a possible end of the pathway, we can form a series of only two reactions—that is, *c* followed by *b*.

2. Reactions *e* and reverse *d* are exergonic, whereas reactions *c*, reverse *a*, and *f* are endergonic. The $\Delta G°'$ of the entire pathway is the algebraic sum of its component reactions, that is 2.2 kcal · mol^{-1}. Hence, the pathway is endergonic. (The reverse pathway would have a $\Delta G°'$ of –2.2 kcal · mol^{-1} and would be exergonic.)

3. To calculate the K_{eq}, we apply equation 2.4 and get 4.7 kcal · mol^{-1} = $-1.987 \cdot 10^{-3}$ kcal · mol^{-1} · K^{-1} · (273 + 37) K · ln K_{eq}, or $4.7 = -0.616 \cdot \ln K_{eq}$, or $\ln K_{eq} = -7.6$. Hence, $K_{eq} = 0.0005$.

To calculate the ΔG, we apply equation 2.3. The equation requires an

input of *molar* concentrations, whereas the ones I have given you are millimolar. Hence, the latter must be divided by 1,000 to convert them into the former. Then we get, $\Delta G = 4.7 \text{ kcal} \cdot \text{mol}^{-1} + 1.987 \cdot 10^{-3} \cdot 310 \cdot \ln (0.6 \cdot 10^{-3} \cdot 0.6 \cdot 10^{-3} / 0.8 \cdot 10^{-3}) \text{ kcal} \cdot \text{mol}^{-1}$, or $\Delta G = [4.7 + 0.616 \cdot \ln (0.45 \cdot 10^{-3})] \text{ kcal} \cdot \text{mol}^{-1}$, or $\Delta G = 0$.

4. Choice *d* is true (refer to equation 2.4).

5. See the following table.

	ATP	ADP	AMP
Phosphoryl groups	3	2	1
Phosphoanhydride linkages	2	1	0

AMP is not a high-energy compound because it lacks phosphoanhydride linkages.

6. See the following table.

Catabolism	Anabolism
Large metabolites → small metabolites	Small metabolites → large metabolites
ADP → ATP	ATP → ADP
Metabolites are usually oxidized.	Metabolites are usually reduced.

7. Since ATP hydrolysis releases 6.3 $\text{kcal} \cdot \text{mol}^{-1}$, gluconeogenesis must be coupled to the hydrolysis of at least 32/6.3 = 5 mol ATP. In reality, gluconeogenesis is powered by the hydrolysis of 6 ~P (see section 10.24).

8. When a substance burns, oxygen is added to it. Therefore, it is oxidized.

9. See the following table.

NAD^+	NADH
$NADP^+$	NADPH
FAD	FADH2
Oxidized forms	Reduced forms
They get reduced.	They get oxidized.
H acceptors	H donors
Oxidants	Reductants

10. See the following table.

Stage	Venue	Energy production
1	Digestive fluids, cytosol	None
2	Cytosol, mitochondria	Some

3	Mitochondria	A lot

Chapter 3

1. See the following table.

Nonpolar	Polar
Gly	Tyr
Ala	Ser
Val	Thr
Leu	Cys
Ile	Met
Pro	Asp
Phe	Glu
Trp	Lys
	Asn
	Gln
	Arg
	His

2. a. 999; b. 1; c. 1; d. Since there are 2 ends and 20 amino acids, the chances of any amino acid being at any end are 2 in 20, or 1 in 10; e. 999 (all but the *N*-terminal one); f. 999 (all but the *C*-terminal one); g. 50 (1,000/20); h. There would be 50 cysteine residues, which can form a maximum of 25 disulfide bonds.

3. Hemoglobin and mitochondrial superoxide dismutase.

4. At or above 100 °C, the proteins in the microbial cells are denatured, and the microbes die.

5. Cooking denatures the proteins in the fruits and vegetables, including the ones that cause allergy (the so-called *allergens*). The immune system does not recognize the denatured proteins and does not initiate an allergic reaction.

6. See the following table.

Protein	Category
Myoglobin	Transport and storage protein
Hemoglobin	Transport protein
Ribonuclease	Catalytic protein (enzyme)
Mitochondrial superoxide dismutase	Catalytic protein (enzyme)
Tropomyosin and troponin	Motile and regulatory proteins

Transferrin	Transport protein
Transferrin receptor	Receptor
Immunoglobulin A	Defensive protein (antibody)
Growth hormone	Messenger protein
Nuclear respiratory factors	Regulatory proteins

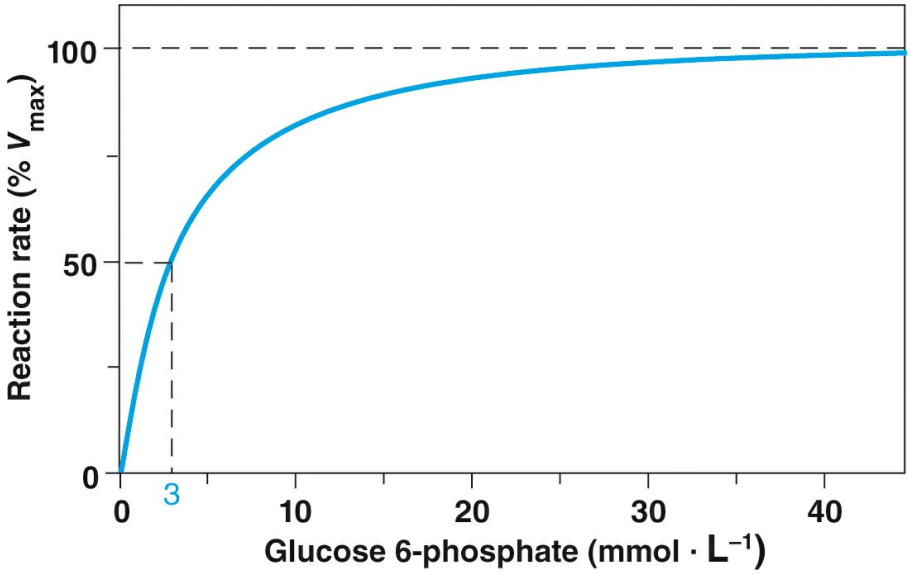
7. See the following table.

Similarities	Differences
Myoglobin and hemoglobin have similar tertiary structures.	Myoglobin consists of one polypeptide chain, whereas hemoglobin consists of four. (Alternatively, hemoglobin has a quaternary structure, whereas myoglobin does not.)
Both contain heme.	Myoglobin is found in muscle, whereas hemoglobin is found in erythrocytes.
Both bind O2.	Myoglobin has a higher affinity for O2 than hemoglobin does under physiological conditions.

8. Myoglobin has a higher affinity for O_2 than does hemoglobin. Muscle has a lower $[O_2]$ than does blood. Muscle has higher $[CO_2]$ and $[H^+]$ than does blood (the Bohr effect), especially during exercise.

9. a. Increase until the enzyme is saturated; b. Increase (see figure 3.23); c. Decrease; d. Decrease because of denaturation (see figure 3.22); e. Increase

10. a. Glucose 6-phosphate, H_2O, glucose, and P_i (all of the substrates and products); b. K_M = 3 mmol · L^{-1}. (See the following graph.)



c. To calculate the turnover number, we need to know how many molecules of glucose 6-phosphate are converted into the products by an

enzyme molecule in 1 s. If we divide the reaction rate by the enzyme concentration, we get $4 \cdot 10^4$ min^{-1}. Thus, an enzyme molecule hydrolyzes $4 \cdot 10^4$ molecules of glucose 6-phosphate per minute, or 667 molecules per second. Hence, the turnover number is $4 \cdot 10^4$ min^{-1}, or 667 s^{-1}.

Chapter 4

1. See the following table.

Feature	Protein	Nucleic acid
Building block (monomer)	Amino acid	Nucleotide
Number of different monomers	20	4
What links monomers	Peptide bond	Phosphodiester linkage
Conventional direction of chain	*N* → *C*	5' → 3'
Periodic secondary structure	α helix, β pleated sheet	Double helix
What stabilizes the secondary structure	Hydrogen bond	Hydrogen bond

2. If C is 23%, then G will also be 23%. That leaves 54% for A and T. Hence, they will be 27% each.

3. She will have to label a component of DNA that is missing from RNA. Such components are thymine and deoxyribose.

4. It belongs to RNA because it contains U. Its complementary sequence is 3' UGAUCGCGAU 5'.

5. 5' … UGCUGUACAA … 3'

6. One mRNA, four rRNA (in the ribosome), and 200 tRNA molecules

7. Look for the initiation codon, AUG, and write down Met. Continue with the next codons until you reach the termination codon, UGA. The peptide produced is Met–Arg–Ser–Tyr–Pro–Thr–Gln.

8. Since the first two amino acid residues in the new peptide are identical to the ones in the original peptide, the obvious thought is that the mutation has to be in the codon of the third residue, AGU. The codons encoding Cys are UGU and UGC. Thus, a substitution of U for A in AGU would yield Cys in place of Ser. However, this result would not change the remaining residues. We need to look for something more radical, something that will produce a start codon elsewhere. So, we start from the 5' end, looking for the possibility to create an initiation codon.

Indeed, if we insert a U after the second base, we create an AUG. Translation of the rest of the sequence up to the termination codon, UGA, yields the given peptide.

9. Whereas GAG encodes Glu, which is an amino acid bearing a negatively charged, hydrophilic side chain, the mutated codon GTG (in DNA), or GUG (in mRNA), encodes Val, which is an amino acid with a neutral, hydrophobic side chain. This change causes the development of abnormal hydrophobic interactions between hemoglobin molecules and makes them aggregate inside the erythrocytes, forming long fibers that change the shape of the erythrocytes from round to sickle-like. Sickle cells impair the circulation of blood and live less than normal erythrocytes. Both consequences of this genetic disease are life threatening.
10. Whereas TGG (or UGG in mRNA) encodes Trp, TAG (or UAG) is a termination codon. Thus, the mutation results in the production of a shorter erythropoietin receptor molecule. It appears that this form of the receptor is more active than the usual one in signaling erythrocyte formation.

Chapter 5

1. Cotton is almost pure carbohydrate (cellulose), whereas wool and silk are made of proteins. Wool and silk are so different to the touch because they are made up of different proteins: Wool contains α-keratin (in which α helices dominate), whereas silk contains fibroin (in which β pleated sheets dominate).
2. Corn, like all grains, contains a lot of starch. Being hydrophilic, starch retains water. When we heat the corn kernels, the water they contain goes from liquid to gas. Because a substance takes up much more volume as a gas than as a liquid, and because the kernel casing is hard and airtight, pressure builds up inside the kernels until finally they explode. As the water vapor expands violently, it pushes on the starch and leaves it fluffy and dry: popcorn.
3. They are polymers of glucose.
4. To find the omega notation of an unsaturated fatty acid, subtract the

number showing the position of the double bond that lies the farthest away from the carboxyl end (hence, closest to the methyl, or ω, end) from the number of carbon atoms of the fatty acid. In other words, subtract the highest number in the last column of table 5.2 from the number in the second column. By doing so, you get the following table:

Fatty acid	Omega notation
Palmitoleate	ω7
Oleate	ω9
Linoleate	ω6
α-Linolenate	ω3
Arachidonate	ω6
Eicosapentaenoate	ω3
Docosahexaenoate	ω3

5. Liquid oils become solid by hydrogenation, that is, addition of hydrogen atoms to the double bonds of their unsaturated fatty acids, which turns them into saturated fatty acids.
6. Cholesterol ester = sphingomyelin (one acyl group) < phosphatidyl inositol (two) < triacylglycerol (three).
7. Glycogen (hydrophilic) < phosphatidate (amphipathic with large hydrophilic part) < cholesterol (amphipathic with small hydrophilic part) < triacylglycerol (hydrophobic).
8. The egg yolk is rich in phosphatidyl choline, which, being amphipathic, lets oil and water mix just as detergents do (see problem 5 in chapter 1). Detergents and phospholipids are emulsifiers, as they give rise to emulsions (water-based liquids or creams in which lipid droplets remain suspended).

Chapter 6

1. Niacin can be synthesized from excess amounts of tryptophan, biotin is synthesized by bacteria in the gut, and vitamin D is synthesized from cholesterol in the skin with energy from the sun’s ultraviolet light.
2. A person who does not consume fruits and vegetables may be at risk for failing to obtain adequate amounts of vitamins C and K.
3. A strict vegetarian may be at risk for failing to obtain adequate amounts

of vitamin B_{12}.

4. g, b, k, j, a, h, f, d, i, m, e, c, l
5. See the following table.

Amino acid residue	Proteins	Vitamin
4-Hydroxyproline	Collagen	C
γ-Carboxyglutamate	Proteins involved in blood clotting and bone metabolism	K

6. e, j, n, d, h, f, b, m, l, i, g, c, k, a
7. Deficiency of vitamin B_6 or iron can cause microcytic hypochromic anemia. Deficiency of vitamin B_{12} or folate can cause megaloblastic anemia (also referred to as *pernicious* if due to vitamin B_{12} deficiency). Vitamin E deficiency can cause hemolytic anemia.
8. Vitamin B_2 is a precursor of FAD and FMN, and niacin is a precursor of NAD and NADP. Vitamin C, β-carotene (a form of vitamin A), and vitamin E act as antioxidants. Iron and copper alternate between their oxidized and reduced cationic forms in the active sites of many enzymes catalyzing redox reactions. Manganese is a cofactor of mitochondrial superoxide dismutase. Molybdenum is part of enzymes involved in the oxidation of amino acids and purines. Selenium is part of antioxidant enzymes.
9. Ascorbate, vitamin D, vitamin K, calcium, magnesium, phosphorus, and fluorine
10. i, f, a, h, b, c, g, e, d

Chapter 7

1. Na^+–K^+ pump, or Na^+–K^+ ATPase
2. In nerve cells, the Na^+ channel is responsible for the ascending phase, and the K^+ channel is responsible for the descending phase of the action potential. This is also true for muscle cells, although in that case the main responsibility for the ascending phase of the action potential lies with the acetylcholine receptor.
3. The resting potential is due to active transport, and the action potential is due to passive transport (an oxymoron).
4. Voltage-gated channels open when the membrane voltage changes,

whereas ligand-gated channels open when ligands bind to them. The Na^+, K^+, and Ca^{2+} channels are voltage-gated channels, whereas the acetylcholine receptor is a ligand-gated channel.

5. Multiple sclerosis affects the electrical transmission of signals in the nervous tissue through degradation of the myelin sheath. By contrast, myasthenia gravis affects the chemical transmission of nerve signals to the muscle tissue by blocking the acetylcholine receptor, which leads to muscle atrophy.
6. Because acetylcholinesterase catalyzes the hydrolysis of acetylcholine, and because acetylcholine signaling is deficient in myasthenia gravis, not multiple sclerosis, neostigmine is a drug used against the former. It fights myasthenia gravis by increasing the availability of acetylcholine in the synaptic cleft, thus offering more acetylcholine molecules to the few available acetylcholine receptor molecules.
7. By inhibiting acetylcholinesterase, organophosphates raise the concentration of acetylcholine in the synaptic cleft to abnormally high levels. In a healthy person (one with functional acetylcholine receptor), this condition results in uncontrolled muscle activity, leading to spasms and death by asphyxiation.

Chapter 8

1. One logical order (decreasing size) is muscle, muscle fiber, myofibril, sarcomere, thick and thin filaments. The reverse (increasing size) is also a logical order.
2. See the following table.

Thick filaments	Thin filaments	M line	Z line	Interfilament space
Myosin	Actin Tropomyosin Troponin	Myomesin M protein	α-Actinin	Nebulin Titin

3. See the following table.

	Rest	Concentric activity	Eccentric activity	Remarks
Sarcomere	2.5	2	3	Sarcomere length decreases or increases by 20%.
A band	1.5	1.5	1.5	Width of the A band does not change.
H zone	0.7	0.2	1.2	H zone shortens or lengthens by as much as the

				sarcomere shortens or lengthens, respectively (0.5 μm).
I band	1	0.5	0.5	I band shortens or lengthens by as much as the sarcomere shortens or lengthens, respectively.
Thick filaments	1.5	1.5	1.5	Width of the thick filaments does not change.
Thin filaments	0.9	0.9	0.9	Width of the thin filaments does not change.
Overlap	0.8	1.3	0.3	Overlap increases or decreases by as much as the sarcomere shortens or lengthens, respectively.

4. ATP is the direct energy source for both contraction and relaxation. Myosin uses it for contraction, and the Ca^{2+} pump uses it for relaxation.
5. Death stops almost all ATP production through catabolic processes. Thus, actin and myosin remain bound to each other (figure 8.10*c*) and make the muscles stiff.
6. It is. In fact, it is feasible regardless of the presence of Ca^{2+}.
7. Caffeine binds to the ryanodine receptor and facilitates its opening, thus releasing Ca^{2+} from the sarcoplasmic reticulum. This action may be part of the reason that coffee causes tremor in unaccustomed drinkers.
8. The factor is the sarcoplasmic reticulum.

Chapter 9

1. $$ADP + H_2O \rightleftharpoons AMP + P_i + H^+$$

   To calculate the $\Delta G°'$, notice that you can produce the equation by reversing equation 2.5 and adding equations 2.6 and 2.7 to it. If you do the same with the $\Delta G°'$ of the three reactions, you get 6.3 – 8.2 – 3.8 kcal · mol^{-1}, or –5.7 kcal · mol^{-1}.
2. Reverse equation 2.5 and double it. Then add equations 2.6 and 2.7 to it. Do the same with the $\Delta G°'$ of the three reactions.
3. Just add equations 2.5 and 9.4.
4. During 3 s of maximal exercise, 6 mmol of ATP will be hydrolyzed per kilogram of muscle. However, most of it will be replenished by phosphocreatine. Thus, the [ATP] in the end will be between 3 and 6 (for example, 4 or 5), and the [phosphocreatine] will be the balance up to 20

(16 or 15, respectively). The [creatine] will increase by as much as the [phosphocreatine] will decrease; thus, it will be 16 or 17, respectively. Finally, the [P_i] will increase by the amount of ATP hydrolyzed; thus, it will rise to 7.

5. Fluoro-2,4-dinitrobenzene inhibits creatine kinase.

6. First, creatine kinase replenishes ATP from phosphocreatine as ATP decreases and ADP increases. Second, adenylate kinase produces one ATP from two ADP as ADP increases. Third, adenylate deaminase is activated as ATP decreases and shifts the adenylate kinase reaction further toward ATP production by eliminating AMP.

7. ATP and phosphocreatine decrease; ADP, AMP, creatine, P_i, IMP, and ammonium increase.

8. By analogy to equation 9.4, we have

$$\text{Phosphoarginine} + \text{ADP} + H^+ \rightleftharpoons \text{ATP} + \text{arginine}$$

$$\text{Phospholombricine} + \text{ADP} + H^+ \rightleftharpoons \text{ATP} + \text{lombricine}$$

9. By analogy to equation 9.5, we have

$$\text{Phosphoarginine} + H_2O \rightleftharpoons \text{arginine} + P_i$$

$$\textit{Phospholombricine} + \textit{H2O} \rightleftharpoons \textit{lombricine} + \textit{Pi}$$

10. See the following table.

Compound	$\Delta G^{\circ\prime}$ of hydrolysis (kcal · mol^{-1})
Phosphocreatine	–9.5
Phosphoarginine	–7.8
ATP	–6.3
ADP	–5.7
Phospholombricine	–4.7

Don’t be surprised to find out that our direct energy source, ATP, did not make it to the top of the list of compounds with high phosphoryl-transfer potential. Being at the top would hamper its replenishment from other compounds.

Chapter 10

1. P_i, AMP, IMP, Ca^{2+}, and epinephrine

2. Although it yields ATP, glycolysis cannot start without a small amount of it, because ATP is needed in the first and third reactions.
3. Phosphoenolpyruvate and 1,3-bisphosphoglycerate climb to the top of the list.

Compound	$\Delta G^{\circ\prime}$ of hydrolysis (kcal · mol^{-1})
Phosphoenolpyruvate	–12.9
1,3-Bisphosphoglycerate	–10.7
Phosphocreatine	–9.5
Phosphoarginine	–7.8
ATP	–6.3
ADP	–5.7
Phospholombricine	–4.7

4. Glucose, glucose-6-phosphate, AMP, and ADP increase; ATP and phosphocreatine decrease.
5. The flow of protons from the mitochondrial matrix to the intermembrane space through the complexes of the electron-transport chain is a case of active transport (as defined in section 7.2), because they move against their concentration gradient, powered by the flow of electrons along the electron-transport chain. By contrast, the flow of H^+ from the intermembrane space to the mitochondrial matrix through ATP synthase is a case of passive transport (as defined in section 7.3), because they move down their concentration gradient.
6. *S. cerevisiae* breaks down the starch in the dough into glucose. Then it breaks down glucose into CO_2. As CO_2 accumulates, it forms bubbles that are trapped in the dough and make it rise. When the dough is heated during baking, the bubbles expand and make bread, cakes, cookies, and donuts fluffy.
7. Add reactions 10.6 (glucose to 2 pyruvate, $\Delta G^{\circ\prime} = -31.9$ kcal · mol^{-1}) and 10.18 (pyruvate to lactate, $\Delta G^{\circ\prime} = -6.6$ kcal · mol^{-1}) after doubling the latter. You should get $-31.9 + 2 \cdot (-6.6) = -45.1$ kcal · mol^{-1}.
8. See the following table.

		Aerobic	Anaerobic
ATP/glucose	30		2
ATP/glucose residue of glycogen	31		3

Maximal rate of ATP resynthesis (mmol · kg^{-1} · s^{-1})	0.5	1.5
Exercise tasks	Maximal lasting >1 min; hard or moderate-intensity of any duration	Maximal lasting about 7 s to 1 min

9. a. If 1 mol, or 1,000 mmol, of ATP is needed for the run and 50% (500 mmol) comes from the aerobic breakdown of glycogen, we will need to break down 500/31 = 16.1 mmol glucosyl units (since one glucosyl unit yields about 31 ATP aerobically). Similarly, 500 mmol ATP from the anaerobic breakdown of glycogen require 500/3 = 166.7 mmol glucosyl units (since one glucosyl unit yields 3 ATP anaerobically). The proportion is 16.1 to 166.7, or 1 to 10.3 (same as the proportion of 3 to 31); b. Again, if 1,000 mmol of ATP is needed for the run and 48% (480 mmol) comes from the aerobic breakdown of glycogen, we will need to break down 480/31 = 15.5 mmol glucosyl units. Similarly, 520 mmol ATP from the anaerobic breakdown of glycogen require 520/3 = 173.3 mmol glucosyl units. The proportion is 15.5 to 173.3, or 1 to 11.2; c. It is not! A 2% increase in anaerobic energy provision resulted in a 9% rise in the proportion of anaerobically catabolized glycogen (compare the 11.2 ratio with the 10.3 ratio). This result occurs because the anaerobic breakdown is uneconomical, thus requiring disproportionately higher glycogen breakdown.

10. The muscles and all of the cells in the body do not burn oxygen. Instead, oxygen burns fuel molecules. Moreover, if the muscles and all of the cells burn sugar, then CO_2, rather than lactic acid, is produced. An acceptable statement then would have been, “If the muscles and all of the cells in the body do not have enough oxygen to burn sugar, then lactic acid is produced.”

11. Gluconeogenesis and glycogenolysis are stimulated in the liver during exercise through increases in plasma glucagon and, possibly, epinephrine. In addition, glycogenolysis is stimulated by a drop in plasma glucose.

12. The increased uptake of glucose by the exercising muscles, due to augmented blood flow and movement of GLUT4 to the plasma membrane, tends to decrease the plasma glucose concentration. The increased release of glucose by the liver, due to enhanced secretion of

glucagon and, possibly, epinephrine, tends to increase the plasma glucose concentration.

13. a. Passive recovery is preferable because there was glycogen resynthesis, as opposed to active recovery, in which there was further glycogen degradation; b. Muscle glycogen was probably used to provide energy for the light exercise; c. Part of the lactate produced during exercise may have been used to resynthesize glycogen, as discussed in sections 10.24 and 10.32 (also presented in figure 10.37). In addition, blood glucose entering a resting muscle would preferentially be used to resynthesize glycogen, whereas glucose entering an exercising muscle would preferentially be degraded to provide energy; d. Because it takes two lactate molecules to synthesize one glucose residue of glycogen, 30 mmol of lactate were used per kilogram of muscle during the hour of passive recovery.

Chapter 11

1. Dietary triacylglycerols, phospholipids, and cholesterol esters are all digested in the small intestine by pancreatic enzymes. The products of digestion pass into the enterocytes, where triacylglycerols, phospholipids, and cholesterol esters are resynthesized. The newly synthesized lipids are all incorporated in chylomicrons, which distribute them to the body.
2. Woman's triacylglycerols > man's triacylglycerols > man's glycogen > woman's glycogen (For indicative figures, see sections 10.2 and 11.3.)
3. Pancreatic lipase hydrolyzes triacylglycerol to 2-monoacylglycerol and two fatty acids in the small intestine. Lipoprotein lipase hydrolyzes triacylglycerol to the same products at the capillaries. Adipose triacylglycerol lipase hydrolyzes triacylglycerol to 1,3-diacylglycerol or 2,3-diacylglycerol in adipocytes and muscle fibers.
4. ATGL catalyzes triacylglycerol hydrolysis to diacylglycerol and fatty acid. HSL catalyzes diacylglycerol hydrolysis to monoacylglycerol and fatty acid. MGL catalyzes monoacylglycerol hydrolysis to glycerol and fatty acid. CGI–58 controls ATGL activity. Perilipin controls access of HSL to the lipid droplets.
5. It is sped up by the rise in the plasma epinephrine concentration, the drop

in the plasma insulin concentration (not seen in all exercises), and the resulting rise in the adipocyte cAMP concentration.

6. C12 will yield 78 ATP, C14 will yield 92 ATP, and C18 will yield 120 ATP (see end of section 11.10).

7. Arachidonate has 20 C and four double bonds at positions 5, 8, 11, and 14. An elongase can add 2 C to the carboxylic end of linoleate (which has 18 C and two double bonds at positions 9 and 12), thus converting it into a fatty acid with 20 C, in which the double bonds have moved to positions 11 and 14. Then desaturases can introduce two additional double bonds at positions 5 and 8 to produce arachidonate. The same modifications could take place in a different order.

8. The triacylglycerol, total cholesterol, and LDL cholesterol concentrations decrease, and the HDL cholesterol concentration increases.

9. a. Because 400 kcal derived equally from carbohydrates and lipids, each source contributed 200 kcal. Because 1 g of carbohydrates yields 4 kcal, and 1 g of lipids yields 9 kcal (section 2.6), the man burned 50 (200/4) g of carbohydrates and 22.2 (200/9) g of lipids; b. According to the calculations presented in section 10.2, he has a total of 460 g of glycogen (85 g in the liver and 375 g in muscles). Hence, he consumed 10.9% of his glycogen stores. Likewise, according to the calculations presented in section 11.3, he has 11.25 kg, or 11,250 g, of body fat. Hence, he consumed just 0.2% of his fat stores. You can see the big difference.

10. First, we divide the carbohydrate and lipid masses burned by the corresponding molecular masses to convert them into moles. We get 0.309 (50/162) mol of carbohydrates and 0.087 (22.2/256) mol of lipids. Then we multiply these figures by the ATP yield of the aerobic breakdown of a glucosyl unit in glycogen (31; section 10.17) and palmitic acid (106; section 11.10). We get 9.6 mol of ATP from glycogen and 9.2 mol of ATP from palmitic acid. The two figures are comparable, as one would expect from the fact that the energy from carbohydrates and lipids was the same. (They are not equal, because of the approximations we used for the calculations in this and the previous problems.)

12. a. Because 300 kcal derived equally from carbohydrates and lipids, each

source contributed 150 kcal. The woman burned 37.5 (150/4) g of carbohydrates and 16.7 (150/9) g of lipids; b. According to the calculations presented in section 10.2, she has a total of 303 g of glycogen (70 g in the liver and 233 g in muscles). Hence, she consumed 12.4% of her glycogen stores. Likewise, according to the calculations presented in section 11.3, she has 14.26 kg, or 14,260 g, of body fat. Hence, she consumed just 0.12% of her fat stores. The difference is more pronounced than in problem 9 because, as compared with men, women have smaller carbohydrate stores but larger fat stores.

13. First, we divide the carbohydrate and lipid masses burned by the corresponding molecular masses to convert them into moles. We get 0.231 (37.5/162) mol of carbohydrates and 0.065 (16.7/256) mol of lipids. Then we multiply these figures by the ATP yield of the aerobic breakdown of a glucosyl unit in glycogen (31) and palmitic acid (106). We get 7.2 mol of ATP from glycogen and 6.9 mol of ATP from palmitic acid. As in problem 10, the two figures are similar.

Chapter 12

1. First, after either resistance or endurance exercise, the rates of muscle protein synthesis and breakdown both increase, regardless of dietary state. Second, protein or amino acid intake after either resistance or endurance exercise raises the rate of protein synthesis above the rate of proteolysis, resulting in positive protein balance.
2. If we consume no proteins or amino acids after resistance exercise, the negative baseline protein balance becomes less negative (that is, it increases). By contrast, if we consume no proteins or amino acids after endurance exercise, the protein balance becomes more negative (that is, it decreases).
3. All three are essential amino acids.
4. Valine is glucogenic, leucine is ketogenic, and isoleucine is partly glucogenic and partly ketogenic.
5. The major energy source that the liver provides is glucose. It comes from glycogenolysis (section 10.28) and gluconeogenesis (section 10.26). The raw material for gluconeogenesis can be pyruvate (section 10.24), lactate

(section 10.25), glycerol (section 11.8), and glucogenic amino acids (section 12.8). A minor energy source provided by the liver consists of ketone bodies through ketogenesis (section 11.23).

6. Carbamoyl phosphate makes it to third place.

Compound	$\Delta G^{\circ\prime}$ of hydrolysis (kcal · mol^{-1})
Phosphoenolpyruvate	–12.9
1,3-Bisphosphoglycerate	–10.7
Carbamoyl phosphate	–10.3
Phosphocreatine	–9.5
Phosphoarginine	–7.8
ATP	–6.3
ADP	–5.7
Phospholombricine	–4.7

7. a. Six percent of 400 kcal is 24 kcal. Since 1 g of proteins yields 4 kcal (section 2.6), the man burned 6 (24/4) g of proteins; b. According to the calculations presented in section 12.2, he has a total of 12 kg, or 12,000 g, of body proteins. Hence, he consumed just 0.05% of his protein stores.

8. a. Six percent of 300 is 18 kcal. Thus, the woman burned 4.5 (18/4) g of proteins; b. According to the calculations presented in section 12.2, she has a total of 8.7 kg, or 8,700 g, of body proteins. Hence, she consumed just 0.05% of her protein stores.

9. The muscle protein balance after resistance exercise and protein intake in figure 12.5 is 0.02% per hour, or 0.48% per day (multiply 0.02 by the 24 hours in a day). Average rates of muscle hypertrophy are 0.1% to 0.2% per day (section 12.12), thus considerably lower than the protein balance. A possible explanation for this difference is that the protein balance (which is usually measured a few hours after exercise) drops during the day or from one training session to another. Thus, the average protein balance throughout a training program is lower.

Chapter 13

1. The gradual rise in mRNA after the initial sessions may be due to a higher rate of transcription, RNA processing, or both as compared with RNA degradation. The subsequent drop in mRNA may be due to a reversal of the balance between these processes. The gradual rise in

protein after the initial sessions may be just the result of the rise in mRNA. The stabilization of the protein afterward, despite the drop in mRNA, may be due to a decrease in proteolytic rate.

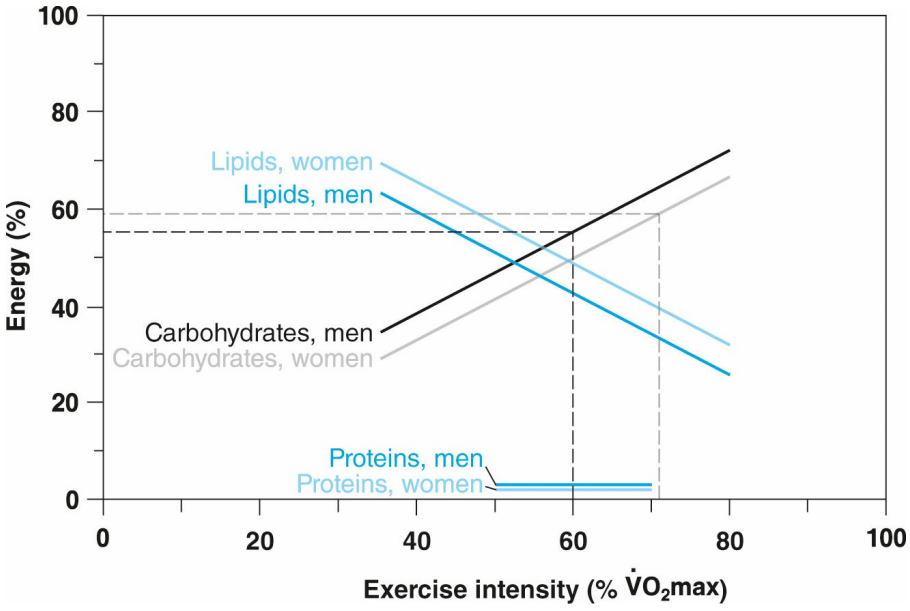
2. The lack of considerable change in mRNA may be due to equal rates of formation (including transcription and RNA processing) and degradation. The initial rise in the protein may indicate higher translation efficiency, which then subsides, resulting in stabilization of the amount of protein, in accordance with the stability in the amount of mRNA.
3. Messenger RNA may remain elevated throughout the training program thanks to higher rates of transcription and RNA processing as compared with RNA degradation. The absence of change in the protein may indicate that the translation rate increased but so did the proteolytic rate.
5. Increased protein synthesis and satellite cell proliferation do not coincide temporally. The former peaks a few hours after resistance exercise (section 12.4), whereas the latter takes about three days to peak (section 13.5).
6. Resistance training exerts its effects on gene expression primarily at the translational level (see figure 13.4). By contrast, endurance training exerts its effects mainly at the transcriptional level (see figure 13.8).

Chapter 14

1. Lactate system, 34%; oxygen system, 66%
2. ATP–phosphocreatine system, 15%; lactate system, 39%; oxygen system, 46%. The values differ from those in the previous problem because, although the contribution of the ATP–phosphocreatine system at the 50th second is null, the system offered a considerable amount of energy at the beginning of exercise, resulting in an overall contribution of 15%. On the other hand, although the contribution of the aerobic system at the 50th second is 66%, the system offered only a minor amount of energy at the beginning of exercise, resulting in an overall contribution of 46%.
3. Because the 200 m sprint lasts twice as long as the 100 m, it relies less on the ATP–phosphocreatine system and more on the oxygen system.
4. a. During prolonged exercise at constant intensity, the proportion of lipids

to carbohydrates goes up; therefore, RER goes down; b. As exercise intensity increases, the proportion of carbohydrates to lipids goes up; therefore, RER goes up as well; c. When we exercise after carbohydrate loading, the proportion of carbohydrates to lipids goes up; therefore, RER goes up as well; d. When we exercise after fat loading, the proportion of lipids to carbohydrates goes up; therefore, RER goes down; e. When we exercise after an aerobic training program, at the same absolute intensity as before training, the proportion of lipids to carbohydrates goes up; therefore, RER goes down.

5. a. The eumenorrheic woman will burn a higher proportion of lipids to carbohydrates and, hence, have a lower RER than the man; b. This is a tricky one. As mentioned in section 14.9, 60% of the man's $\dot{V}O_2max$ is 27 mL · kg $^{-1}$ · min^{-1}. This value corresponds to 71% (calculated as 27/38 · 100) of the woman's $\dot{V}O_2max$. In the graph of figure 14.6, you can draw a vertical line from the 60% $\dot{V}O_2max$ point along the x-axis to intersect the carbohydrate line for men and a vertical line from the 71% $\dot{V}O_2max$ point to intersect the carbohydrate line for women (see the following graph). Then draw horizontal lines from the points of intersection to meet the y-axis. You will see that women burn more carbohydrates than men do and therefore have a higher RER. (You would get the same result by working with the lipid lines.)



c. Because the surge of the sex hormones that signal puberty has not yet occurred, the boy and the girl will have similar RER; d. Oral contraceptives contain synthetic compounds that mimic estradiol and progesterone action. Thus, one would expect the woman taking oral contraceptives to have higher levels of sex hormones in her body, resulting in a higher proportion of lipids to carbohydrates and, hence, a lower RER than the woman not taking oral contraceptives. Nevertheless, most studies on the topic (reviewed by Devries) have shown no difference, possibly because the surplus hormones have no additional effect on exercise metabolism.

6. See the following table.

Cause of fatigue	Remedy
Hypoglycemia	Carbohydrate loading (in men), carbohydrate intake during exercise
Dehydration	Fluid intake during exercise
Hyperthermia	Fluid intake during exercise
Decreased muscle excitation	Caffeine supplementation
Glycogen depletion	Carbohydrate loading (in men), carbohydrate intake during exercise (in running events)
RONS accumulation	Endurance training

7. Creatine supplementation will raise the muscle phosphocreatine and creatine contents to 25 and 15 mmol · kg^{-1}, respectively. As in problem 4 in chapter 9, 6 mmol · kg^{-1} of ATP will be hydrolyzed during exercise. Again, most of it will be replenished by phosphocreatine. The [ATP] in the end will be between 3 and 6 (for example, 4 or 5), and the [phosphocreatine] will be the balance up to 25 (21 or 20, respectively). The [creatine] will increase by as much as the [phosphocreatine] will decrease; thus, it will be 19 or 20, respectively. Finally, the [P_i] will increase by the amount of ATP hydrolyzed; thus, it will rise to 7 as in problem 4 in chapter 9. The difference made by supplementation obtains in the phosphocreatine and creatine concentrations (both pre- and postexercise), not in ATP or P_i.

Chapter 15

1. Regular exercise elicits healthy cardiac hypertrophy; reduces the risk of

experiencing a heart attack; protects the heart against damage resulting from a heart attack; reduces the risk of dying during a heart attack; protects against ischemia-reperfusion injury; lowers the risk of atherosclerosis by decreasing plasma triacylglycerols, total cholesterol, and LDL cholesterol, while increasing HDL cholesterol; improves flow-mediated dilation; increases the diameter of the coronary arteries and the arteries nourishing the exercising limbs; increases the amount and diameter of the arterioles; and increases muscle capillarization.

2. Propranolol blunted the effect of training on both tumor growth and NK cell mobilization. That is, the sedentary and trained mice did not differ in these parameters any more.
3. Exercise increases the migration of GLUT4 to the plasma membrane of muscle fibers and, hence, glucose uptake in an insulin-independent way. Training increases the muscle GLUT4 content, thus further increasing glucose uptake. Training may enhance insulin's signal transduction pathway in muscle. Training improves blood flow, thus increasing the delivery of insulin, glucose, and fatty acids to tissues.
4. Regular exercise helps us lose weight primarily through the energy we spend during each exercise session. EPOC adds more energy expenditure, and resistance training may increase the energy expenditure at rest through muscle hypertrophy. Exercise does not seem to affect appetite. Exercise may fight the health consequences of obesity by inducing the secretion of anti-inflammatory myokines, such as IL6, IL1ra, and IL10, from muscle. These myokines may block the inflammatory and damaging actions of IL1β and TNFα, thus fighting T2D, CVD, and cancer.
5. Based on reaction 15.3 and on the atomic masses of the elements (table 1.1), you should be able to calculate that 856 g of triacylglycerol are burned by 2,464 g of O_2 to produce 2,420 g of CO_2 and 900 g of H_2O. Thus, most of the products of fat combustion (73%) are lost as CO_2 through the lungs.
6. Camels (like every animal) get not only energy but also water from burning fat. In fact, according to the answer to the previous problem,

they get more water than the fat they burn (900 g of water for every 856 g of fat). If this sounds odd, consider the huge amount of oxygen (2,464 g) that is needed to burn fat. In other words, camels get oxygen from the air for free and produce valuable water that weighs more than the fat they carry in their humps. Of course, this water is subsequently lost, mainly through respiration and perspiration, and camels end up severely dehydrated after a journey through the desert.

7. Regular exercise can prevent osteoporosis when applied throughout childhood and adolescence to maximize BMD. In addition, regular exercise can increase BMD in patients with osteoporosis. Endurance weight-bearing training, resistance training, and balance training are all advisable.
8. Exercise increases the concentrations of several biomolecules, including BDNF, in the brain and in the circulation. BDNF induces the formation of new neurons and new connections between existing neurons. As a result, exercise improves cognition and mood. It can also improve depression, anxiety, stress, and schizophrenia.
9. Physical inactivity increases morbidity and mortality from a variety of chronic diseases, such as CVD, T2D, breast cancer, colorectal cancer, dementia, depression, and anxiety. Inactivity reduces CRF, muscle mass, bone mass, and muscle strength. Inactivity speeds up biological aging.
10. Let's begin by converting 150 minutes into 2.5 hours per week to make calculations easier. If you multiply this number by 52 weeks in a year, you get 130 hours, or 5.4 days, per year. If you multiply this figure by the 66 years between the ages of 6 and 72, you get 356 days, or almost one year. Thus, you would need to exercise for a net time of 1 year to get 3 extra years of life. That's a 200% return on your investment.

Chapter 16

1. By inserting the given values into equation 16.1, we get

$$\text{(Plasma volume post)/(plasma volume pre)} = 0.96$$

Thus, the plasma volume decreased by 4%.

2. Again, by inserting the given values into equation 16.1, we get

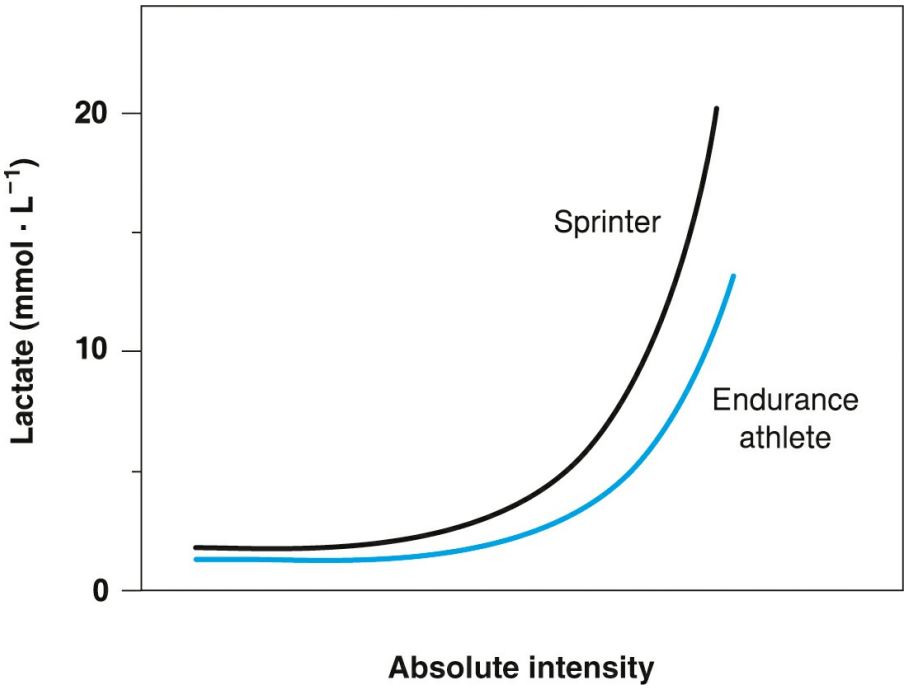
(Plasma volume post)/(plasma volume pre) = 1.02

Thus, the plasma volume increased by 2%.

3. Erythropoietin and blood doping
4. Iron deficiency increases TIBC and sTfR while decreasing transferrin saturation and ferritin.
5. The transferrin saturation was originally 30% (90/300 · 100). The new iron and TIBC values are 81 and 330 µg · dL^{-1}, respectively. Therefore, the new transferrin saturation is 24.5% (81/330 · 100). This decrease by 5.5 percentage units represents a relative decrease by 18% (5.5/30 · 100), which is larger than the individual relative changes of iron and TIBC. This difference exemplifies the fact that transferrin saturation is a more sensitive index of iron status than either iron or TIBC separately.
6. He or she probably has adequate iron stores but has reduced iron intake lately. We would advise this person to eat more iron-rich foods.
7. He or she probably has inadequate iron stores but has increased iron intake lately.

Chapter 17

1. A sprinter's plot will lie to the left of an endurance athlete's plot and will reach a higher lactate concentration because of genetic factors (the former usually has a higher percentage of type II muscle fibers and, hence, a higher lactate concentration at a given absolute intensity) and training factors (endurance training shifts the plot to the right). See the following graph.



2. A rise in blood glucose is explained by higher output from the liver as compared with uptake in the active muscles. Conversely, a drop is explained by higher uptake in the muscles as compared with output from the liver. We can remedy the latter by increasing carbohydrate intake before or during exercise, or both.

3. It will be 150 mg · dL^{-1} (220 – 50 – 100/5).

4. The triacylglycerol concentration is desirable. The total, HDL, and LDL cholesterol concentrations are borderline. The atherogenic indices (220/50 = 4.4 and 150/50 = 3) are desirable. Overall, the athlete has a rather healthy lipidemic profile.

5. Glycerol is preferable over fatty acids because it exhibits a rather consistent rise during exercise. By contrast, fatty acids go up and down depending on exercise duration and intensity.

6. The abnormal urea value could be the result of a high-protein diet or protein supplementation. The abnormal creatinine value could be the result of a high-meat diet or creatine supplementation. (This is a real case. The bodybuilder admitted that he had been taking a protein supplement in excess of 2 g per kg body weight daily and a creatine supplement of 20 g daily for some time.) The recommendation would be to not exceed a total daily protein intake of 2 g · kg^{-1} (from food and

supplements combined) and to limit long-term creatine supplementation to no more than 5 g per day.

7. The opposite changes in the lactate and ammonium values may be due to different glycogen stores in the two tests. If the exerciser has a low muscle and liver glycogen content (resulting from inadequate carbohydrate intake, or previous glycogen-depleting exercise, or both) in the second test, then he or she will not be able to produce as much lactate as in the first test, when glycogen stores were adequate. The exerciser may then have to resort more to amino acid degradation (in particular, to provide substrates for gluconeogenesis, given the lack of glycogen in the liver) and produce more ammonium.
8. a. GSH increased by 18% (from 1,030 to 1,220). GSSG decreased by 23% (from 2.2 to 1.7). The GSH/GSSG ratio went from 468 to 718, a 53% increase. These changes signify an improvement in antioxidant capacity ranging from 18% to 53%, depending on the index used.

   b. Pre-training, GSH dropped by 170 (1,030 – 860). Post-training, it dropped by 60 (1,220 – 1,160). This difference represents a mitigation of GSH oxidation by 65%.

Chapter 18

1. Serum CK
2. According to figure 18.2, the value would be normal for a male athlete and indicative of increased muscle fiber damage for a female athlete.
3. The increase will most probably be smaller, that is, 300 $U \cdot L^{-1}$, thanks to the repeated-bout effect.
4. We would recommend serum GGT. We would not recommend the aminotransferases, because their values are affected by muscle fiber damage.
5. We would recommend the metabolites discussed in chapter 17 (serum uric acid and blood or erythrocyte glutathione) and one or more of the antioxidant enzymes discussed in chapter 18 (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase).
6. Serum cortisol

7. Serum testosterone may be too high if testosterone itself has been used and too low if other AAS have been used. HDL cholesterol may be abnormally low in both cases. (Note, however, that none of these findings is acceptable as proof of AAS use in doping control. Rather, doping control is performed through the detection of AAS and their metabolites in urine.)

Glossary

α-tocopherol—Biologically active form of vitamin E.

β-D-2-deoxyribose—Five-carbon monosaccharide and component of DNA.

β-D-ribose—Five-carbon monosaccharide and component of RNA.

β oxidation—Breakdown of fatty acids into acetyl CoA.

Δ*G*—See free-energy change.

Δ*G*°—See standard free-energy change.

Δ*G*°'—Δ*G*° of a reaction at pH 7.

γ-glutamyltransferase (GGT)—Enzyme involved in glutathione degradation.

AAS—See anabolic androgenic steroid.

acetylation—Addition of an acetyl group to a compound.

acetylcholine—Neurotransmitter present at the neuromuscular junction and in the parasympathetic nervous system.

acetylcholine receptor—Protein that binds acetylcholine at the plasma membrane of skeletal muscle fibers and neurons of the parasympathetic nervous system.

acetyl coenzyme A—Activated carrier of the acetyl group.

acetyl group—Two-carbon group that participates in monosaccharide, fatty acid, and amino acid metabolism.

actin—Protein interacting with myosin in muscle cells during muscle activity.

action potential—Electrical potential difference across the plasma membrane of a neuron or muscle fiber when it is excited.

activator—Substance that increases the activity of an enzyme by binding to it noncovalently.

active site—Area on an enzyme where a reaction is catalyzed.

active transport—Passage of a substance from a compartment where its concentration is low to a compartment where its concentration is high at the expense of energy.

actomyosin—Complex of F-actin and myosin.

acyl group—Fatty acid devoid of its terminal OH or O^-.

adaptation to exercise—Change in a characteristic or function of the body (such as muscle hypertrophy or increased lipid combustion during exercise) due to exercise, usually chronic.

adaptation to training—See adaptation to exercise.

adenine—Nitrogenous base of DNA and RNA; a member of the purines.

adenosine diphosphate (ADP)—Compound consisting of adenine, β-D-ribose, and two phosphoryl groups.

adenosine monophosphate (AMP)—Compound consisting of adenine, β-D-ribose, and one phosphoryl group.

adenosine triphosphate (ATP)—Compound consisting of adenine, β-D-ribose, and three phosphoryl groups that serves as the major energy currency of cells.

adenylate—See adenosine monophosphate**.**

adenylate cyclase—Enzyme catalyzing the synthesis of cAMP from ATP.

adenylate deaminase—Enzyme catalyzing the deamination of AMP to IMP.

adenylate kinase—Enzyme catalyzing the reversible conversion of two ADP into AMP and ATP.

adipocyte—Fat cell.

adipocytokine—Compound (such as leptin and adiponectin) released from adipocytes and affecting the metabolism of other cells in the body.

adipokine—See adipocytokine.

adipose triacylglycerol lipase (ATGL)—Enzyme catalyzing the hydrolysis of triacylglycerol to diacylglycerol and fatty acid in adipose tissue and muscle.

ADP—See adenosine diphosphate.

adrenaline—See epinephrine.

adrenergic receptor—Protein that binds epinephrine and norepinephrine at the plasma membrane of cells.

aerobic—Oxygen dependent.

aerobic system—See oxygen system.

affinity—Binding strength of one chemical entity for another.

alanine aminotransferase—Enzyme catalyzing the reversible conversion of alanine and α-ketoglutarate into pyruvate and glutamate.

albumin—Protein carrying fatty acids and other substances in plasma.

allostery—Property of a protein that changes its affinity for a ligand when another ligand binds to a different site in the protein.

amino acid—One of multiple compounds containing an amino group and a carboxyl group and in some cases serving as the building blocks of proteins.

aminotransferase—Enzyme catalyzing a transamination.

AMP—See adenosine monophosphate.

AMP-activated protein kinase (AMPK)—Enzyme catalyzing the phosphorylation of proteins at the expense of ATP and activated by AMP.

amphipathic—Partly hydrophilic and partly hydrophobic.

amphiphilic—See amphipathic.

AMPK— See AMP-activated protein kinase.

anabolic androgenic steroid (AAS)—Member of a group of pharmaceutical agents that include natural androgens, such as testosterone, and synthetic analogs of these androgens.

anabolism—Phase of metabolism that includes biosynthetic processes.

anaerobic—Oxygen independent.

anemia—Disease characterized by low hematocrit and low blood hemoglobin concentration.

anticodon—Triplet of tRNA bases that base-pairs with a codon in mRNA.

ascorbate—See vitamin C.

aspartate aminotransferase—Enzyme catalyzing the reversible conversion of aspartate and α-ketoglutarate into oxaloacetate and glutamate.

atherogenic index—Serum concentration ratio of either total cholesterol or LDL cholesterol to HDL cholesterol, used to estimate risk for atherosclerosis.

atherosclerosis—Presence of fatty deposits inside the walls of blood vessels.

ATGL—See adipose triacylglycerol lipase.

ATP—See adenosine triphosphate.

ATP–ADP cycle—Interconversion of ATP and ADP accompanying cellular functions such as metabolism.

ATP–phosphocreatine system—Anaerobic energy system supplying a small amount of energy at high power during short maximal exercise.

ATP synthase—Enzyme synthesizing ATP from ADP and P_i in oxidative phosphorylation.

BCAA—See branched-chain amino acid.

BDNF—See brain-derived neurotrophic factor.

bicarbonate loading—See soda loading.

bioenergetics—Branch of the biological sciences dealing with energy transactions in living organisms.

bioinformatics—Interdisciplinary field of science applying computer technology to manage biological information.

biomolecule—Organic compound of biological origin.

biotin—A water-soluble vitamin.

blood doping—Transfusion of a large volume of blood to an athlete before an event, aimed at increasing his or her aerobic endurance performance.

branched-chain amino acid (BCAA)—One of three amino acids (leucine, isoleucine, and valine) that carry a branched side chain and are preferentially degraded in muscle during exercise.

brain-derived neurotrophic factor (BDNF)—Protein promoting the formation, growth, and survival of neurons.

buffer system—Pair, consisting of an acid and its conjugate base, that protects the pH of a solution against perturbations caused by the production of acids or bases.

Ca^{2+} ATPase—See Ca^{2+} pump.

Ca^{2+} channel—Membrane protein that lets Ca^{2+} through.

Ca^{2+} pump—Membrane protein that accumulates Ca^{2+} inside the sarcoplasmic reticulum in a muscle fiber at the expense of ATP.

calcium channel—See Ca^{2+} channel.

calmodulin—A Ca^{2+}-binding protein that mediates many of the ion's actions.

cAMP—See cyclic AMP.

cAMP cascade—Series of molecular interactions that begins with the binding of a hormone (such as epinephrine) or other ligand to a receptor at the plasma membrane, involves the synthesis of cAMP as an intermediate step, and ends in changing the rate of a metabolic process (such as glycogenolysis

or lipolysis).

cancer—Group of diseases characterized by abnormal and uncontrolled growth and proliferation of cells.

capillary density—Number of blood capillaries per unit of muscle cross-sectional area.

carbohydrate—Compound that contains at least two hydroxyl groups and either has the molecular formula $C_nH_{2n}O_n$ or derives from a compound of that formula.

carbohydrate loading—Nutrition and training intervention involving a high-carbohydrate diet and light training before an endurance event and aimed at maximizing muscle glycogen and increasing endurance performance.

carboxylation—Addition of a carboxyl group to a compound.

cardiac myocyte—Muscle cell of the heart.

cardiovascular disease (CVD)—Group of diseases affecting the heart and blood vessels.

catabolism—Phase of metabolism that includes degradation processes.

catalase—Enzyme catalyzing the breakdown of hydrogen peroxide into water and oxygen.

catecholamine—One of a group of compounds including epinephrine, norepinephrine, and dopamine.

cellulose—The structural polysaccharide of plants.

CGI–58—See comparative gene identification 58.

chemical kinetics—Branch of chemistry dealing with the rates and mechanisms of chemical reactions.

chemical thermodynamics—Branch of chemistry and physics dealing with energy changes in chemical systems.

chemiosmotic hypothesis—Hypothesis linking the electron-transport chain with oxidative phosphorylation through protons that are expelled from the mitochondrion as the electron-transport chain operates and then return through ATP synthase, thus powering ATP synthesis.

cholesterol—Lipid that forms part of cell membranes and serves as the precursor of steroids, bile acids, and vitamin D.

cholesterol ester—Ester of cholesterol with a fatty acid.

chromatography—Set of laboratory methods for separating components of a mixture based on their differing migration along a solid or liquid medium, through which they are forced to pass by pressure, gravity, or capillary action.

chromosome—Natural DNA molecule, with associated proteins, that contains part or all of an organism’s genome.

chylomicron—Lipoprotein carrying dietary triacylglycerols from the small intestine to extrahepatic tissues.

citrate—Compound of the citric acid cycle produced from acetyl CoA and oxaloacetate.

citric acid cycle—Cyclic metabolic pathway converting the acetyl group into two CO_2.

CK—See creatine kinase.

cobalamin—See vitamin B_{12}.

codon—Triplet of mRNA bases that encodes an amino acid.

coenzyme—Nonprotein organic compound present in the active site of an enzyme, where it participates in catalysis.

cofactor—Nonprotein chemical entity (either metal ion or organic compound) present in the active site

of an enzyme, where it participates in catalysis.

committed step—First irreversible reaction in a metabolic pathway.

comparative gene identification 58 (CGI–58)—Protein controlling the rate of lipolysis by interacting with ATGL.

complex carbohydrate—Carbohydrate consisting of more than two monosaccharide residues.

compound of high phosphoryl-transfer potential—Compound bearing a phosphoryl group and releasing a high amount of energy when hydrolyzed.

concentration—Amount of a substance dissolved in a certain amount of solution or solvent.

concentric—Moving toward the center; shortening.

Cori cycle—Transport to muscle of glucose synthesized in the liver from lactate that has been produced from glucose in muscle.

cortisol—Steroid hormone that is a member of the corticosteroids and is secreted by the adrenal cortex.

covalent modification—Addition of a chemical group to a molecule; term generally used with large molecules such as proteins.

creatine—Compound that is interconverted with phosphocreatine.

creatine kinase—Enzyme catalyzing the interconversion of ATP and phosphocreatine.

creatine phosphate—See phosphocreatine.

creatinine—Compound produced by dehydration of creatine or phosphocreatine.

cross-bridge—Myosin head in contact with F-actin in a myofibril.

CVD—See cardiovascular disease.

cyclic adenylate—See cyclic AMP.

cyclic AMP (cAMP)—Compound mediating the actions of several hormones through the cAMP cascade.

cytokine—Protein (such as TNFα and IL6) secreted from cells and affecting the metabolism of nearby cells, distant cells, or the very cells that secreted it.

cytoplasm—Interior of a cell except the nucleus in eukaryotic cells.

cytosine—Nitrogenous base of DNA and RNA; member of the pyrimidines.

cytosol—Fluid of a eukaryotic cell outside the intracellular organelles.

deacetylation—Removal of an acetyl group from a compound.

deamination—Removal of an amino group from a compound.

decarboxylation—Removal of a carboxyl group from a compound.

denaturation—Disruption of the tertiary and quaternary structure of a protein or nucleic acid.

deoxyribonucleic acid (DNA)—Large biomolecule serving as the repository of an organism's genetic material.

deoxyribonucleotide—Compound consisting of a nitrogenous base, a deoxyribose unit, and one to three phosphoryl groups and serving as a building block of DNA.

dephosphorylation—Removal of a phosphoryl group from a compound.

diabetes mellitus—Disease caused by inadequate insulin production or by insulin resistance.

dihydropyridine receptor—Voltage-sensing protein at the transverse tubule membrane in muscle fibers, mediating the release of Ca^{2+} from the sarcoplasmic reticulum in response to action potentials.

DNA—See deoxyribonucleic acid.

DNA polymerase—Protagonistic enzyme in DNA replication.

DNA replication—Duplication of DNA.

dyslipidemia—Abnormal concentrations of blood lipids.

eccentric—Moving away from the center; lengthening.

electron-transport chain—Transport of electrons from NADH and $FADH_2$ to O_2, accompanied by the release of large amounts of energy.

electrophoresis—Laboratory method of separating ions in a mixture along a gel to which an electric field is applied.

endergonic—Having $\Delta G > 0$ and being not favored.

endocytosis—Uptake of extracellular material by a cell through invagination of the plasma membrane and formation of intracellular vesicles.

endoplasmic reticulum—Extensive network of tubules and flattened sacs inside a eukaryotic cell.

endothelial nitrogen oxide synthase (eNOS)—Enzyme catalyzing the synthesis of NO from arginine, O_2, and NADPH.

endurance exercise—Exercise consisting of prolonged periods, either continuous or intermittent, of muscle activity against low resistance, such as a marathon run.

eNOS—See endothelial nitrogen oxide synthase.

enzyme—Protein catalyzing (that is, speeding up) a reaction.

enzyme activity—Increase in the rate of an enzyme reaction due to the enzyme.

epigenetics—Field of biological sciences examining the transmission of phenotypic characteristics from one generation to the next without their being encoded in the base sequence of genes.

epinephrine—Hormone that is a member of the catecholamines is produced primarily by the adrenal medulla.

EPOC—See excess postexercise oxygen consumption.

equilibrium constant (K_{eq})—Ratio of the mathematical product of the molar concentrations of a reaction's products to the product of the molar concentrations of the reactants at equilibrium.

ergogenic aid—Substance or method used to increase sport performance.

erythrocyte—Red blood cell.

erythrocyte count—Number of erythrocytes in 1 μL of blood.

erythropoietin—Hormone produced by the kidneys and causing erythrocyte formation in the bone marrow.

essential amino acid—Protein amino acid that needs be obtained from the diet, since we are unable to synthesize it.

essential fatty acid—Fatty acid that is necessary for cell function and needs be obtained from the diet, since we are unable to synthesize it.

ester linkage—Chemical linkage joining a carboxylic acid and an alcohol.

estradiol—Steroid hormone that is a member of the estrogens and is secreted primarily by the ovaries.

ethanol—Compound present in alcoholic beverages.

euglycemia—Normal blood glucose concentration.

eukaryotic cell—Cell having internal compartments such as nucleus and mitochondria.

excess postexercise oxygen consumption (EPOC)—Difference between the amount of oxygen consumed during recovery from exercise and that normally consumed at rest.

exercise—Planned and structured bodily movement, which, when repeated, results in learning and improving one or more physical skills, or in maintaining and improving one or more physical abilities.

exergonic—Having $\Delta G < 0$ and being favored.

exocytosis—Release of cellular material to the extracellular space by fusion of intracellular vesicles with the plasma membrane.

F_0F_1 ATPase—See ATP synthase.

FAD—Oxidized form of flavin adenine dinucleotide.

$FADH_2$—Reduced form of flavin adenine dinucleotide.

FAS—See fatty acid synthase.

fat—Adipose tissue; class of nutrients characterized by poor miscibility or immiscibility with water.

fatigue—See muscle fatigue.

fat loading—High fat intake for several days or weeks, aimed at maximizing fat utilization during prolonged exercise.

fat-soluble vitamin—Vitamin that does not dissolve readily in water but is contained in fat.

fatty acid—Lipid consisting of a long hydrocarbon chain with a carboxyl group attached to the end.

fatty acid synthase (FAS)—Enzyme catalyzing fatty acid synthesis from acetyl CoA, malonyl CoA, and NADPH.

feedback inhibition—Inhibition of an early step in a metabolic pathway by the pathway's product.

ferritin—Iron-storing protein found primarily in the liver, spleen, and bone marrow.

flavin adenine dinucleotide (FAD)—Compound participating in redox reactions.

fluorometry—Laboratory method of determining the concentration of a substance in solution by measuring how much light it emits after being excited by light of a higher energy.

folate—A water-soluble vitamin.

free-energy change (ΔG)—Thermodynamic term indicating whether a reaction is favored, which is the case if $\Delta G < 0$.

free radical—See radical.

fructose—Six-carbon monosaccharide similar in structure and function to glucose.

gene—Region of DNA that is transcribed into functional RNA.

gene doping—Transfer of nucleic acids or nucleic acid analogues and the use of normal or genetically modified cells in athletes, a practice prohibited by WADA.

gene expression—Synthesis of RNA or protein according to the information contained in a gene.

gene product—RNA or protein synthesized according to the information contained in a gene.

genetic code—Set of instructions for how the base sequence of any mRNA is translated into amino acid sequence during translation.

genetics—Field of biological sciences examining how phenotypic characteristics are inherited through the base sequence of genes.

genome—Genetic material of an organism.

genomics—The large-scale, comprehensive study of the genomes in terms of base sequence, gene

identification, and gene function.

genotype—Genetic makeup of an organism.

GGT—See γ-glutamyltransferase.

glucagon—Peptide hormone produced by the α cells in the pancreas.

glucagon receptor—Protein that binds glucagon at the plasma membrane of cells.

glucogenic amino acid—Amino acid that can serve as a precursor of glucose.

gluconeogenesis—Synthesis of glucose from compounds that are not carbohydrates.

glucose—Six-carbon monosaccharide serving primarily as an energy source.

glucose-alanine cycle—Transport to muscle of glucose synthesized in the liver from alanine that has been produced from glucose in muscle.

GLUT4—Protein importing glucose to muscle fibers, adipocytes, and other cells.

glutathione—Tripeptide involved in the antioxidant protection of cells.

glutathione peroxidase—Enzyme catalyzing the conversion of GSH and H_2O_2 into GSSG and H_2O.

glutathione reductase—Enzyme catalyzing the reversible conversion of GSSG and NAD(P)H into GSH and $NAD(P)^+$.

glycerol—Three-carbon compound; component of triacylglycerols and glycerophospholipids.

glycerophospholipid—Phospholipid containing a glycerol unit.

glycogen—Storage polysaccharide of animals.

glycogenesis—Synthesis of glycogen from UDP-glucose.

glycogenolysis—Breakdown of glycogen into glucose and glucose 1-phosphate.

glycogen phosphorylase—See phosphorylase.

glycogen synthase—The main enzyme catalyzing glycogenesis.

glycolysis—Breakdown of glucose into two pyruvates.

glycosidic linkage—Linkage joining two monosaccharide residues in an oligosaccharide or polysaccharide.

glycosylation—Addition of a carbohydrate unit to a compound (usually a protein).

guanine—Nitrogenous base of DNA and RNA; member of the purines.

HDL—See high-density lipoprotein.

health—State characterized not merely by the absence of disease or infirmity but by the presence of complete physical, mental, and social well-being.

hematocrit—Volume of the erythrocytes as a percentage of the total blood volume.

heme—Nonprotein part (prosthetic group) of hemoglobin, myoglobin, and other proteins that binds O_2.

hemoconcentration—Excessive loss of water from the blood vessels.

hemocondensation—See hemoconcentration.

hemodilution—Excessive entry of water into the blood vessels.

hemoglobin—The oxygen-carrying protein in the blood.

hepatocyte—Liver cell.

high-density lipoprotein (HDL)—Lipoprotein carrying cholesterol from extrahepatic tissues to the liver, chylomicrons, and VLDL.

histone—Protein around which DNA winds in the chromosomes.

homeostasis—Maintenance of a biological parameter at a relatively stable level within an organism despite temporary fluctuations.

hormesis—Biological principle whereby a low dose of an agent may result in a beneficial adaptation, whereas a high dose may have a toxic effect.

hormone—Compound secreted in the blood by an endocrine gland and serving as a messenger to other organs.

hormone-sensitive lipase (HSL)—Enzyme catalyzing the hydrolysis of diacylglycerol to monoacylglycerol and fatty acid in adipose tissue and muscle.

HSL—See hormone-sensitive lipase.

hydrogen bond—Attraction between a hydrogen atom with partial positive charge and an atom with partial negative charge (usually oxygen or nitrogen).

hydrolysis—Breakdown of a compound by water.

hydrophilic—Mixing readily with water.

hydrophobic—Not mixing readily with water.

hydroxylation—Addition of a hydroxyl group to a compound.

hyperglycemia—Abnormally high blood glucose concentration.

hypertension—Abnormally high arterial blood pressure.

hypoglycemia—Abnormally low blood glucose concentration.

hypoxia—Low oxygen concentration in a biological fluid such as the cytosol or blood.

IGF1—See insulin-like growth factor 1.

inflammation—Response of the body to a harmful stimulus that is aimed at eliminating the insult, removing damaged cells or tissue, and initiating tissue regeneration.

inhibitor—Substance that decreases the activity of an enzyme by binding to it noncovalently.

inorganic phosphate (P_i)—Phosphate ion, which, in biological fluids, is primarily of the HPO_4^{2-} form.

inorganic pyrophosphate (PP_i)—Pyrophosphate ion, which, in biological fluids, is primarily of the $HP_2O_7^{3-}$ form.

insulin—Peptide hormone produced by the β cells in the pancreas.

insulin-like growth factor 1 (IGF1)—Protein hormone involved in muscle hypertrophy.

insulin receptor—Protein that binds insulin at the plasma membrane of cells.

insulin resistance—Partial or total inability of insulin to exert its actions in the body (for example, to lower plasma glucose) due to malfunctioning of its signal transduction pathway.

insulin sensitivity—Ability of the body to respond (for example, with a drop in plasma glucose) to small increases in plasma insulin.

in vitro—In a test tube.

in vivo—In a living organism.

iron saturation—See transferrin saturation.

iron status—Concentration of iron in the various compartments of the body.

isoform—One of two or more similar forms of a protein.

isometric—Not changing in length.

K^+ channel—See potassium channel.

K_{eq}—See equilibrium constant.

ketogenesis—Synthesis of ketone bodies from acetyl CoA in the liver.

ketogenic amino acid—Amino acid that cannot serve as a precursor of glucose.

ketogenic diet—See low-carbohydrate high-fat diet.

ketolysis—Breakdown of ketone bodies into acetyl CoA.

ketone body—Compound resulting from the joining of two acetyl groups in the liver.

kinase—Enzyme catalyzing phosphorylation of a compound at the expense of ATP.

K_M—See Michaelis constant.

Krebs cycle—See citric acid cycle.

lactate dehydrogenase—Enzyme catalyzing the interconversion of pyruvate and lactate.

lactate system—Anaerobic energy system supplying a moderate amount of energy at moderate power during hard or maximal exercise through anaerobic carbohydrate catabolism.

L-carnitine—Compound carrying acyl groups from the cytosol to the mitochondrial matrix.

LDL—See low-density lipoprotein.

leukocyte count—Number of leukocytes per 1 μL of blood.

ligand-gated channel—transmembrane protein that lets substances through when a ligand binds to it.

lipid—Biological compound that is poorly soluble in water.

lipidemic profile—Serum concentrations of lipids, such as triacylglycerols, total cholesterol, LDL cholesterol, and HDL cholesterol, that are related to the risk of atherosclerosis.

lipolysis—Hydrolysis of triacylglycerol to three fatty acids and glycerol.

lipoprotein—Spherical aggregate of lipids and proteins serving the transport of lipids in the blood.

lipoprotein lipase—Enzyme hydrolyzing lipoprotein-borne triacylglycerols in the blood capillaries.

L-lactate—Product of anaerobic carbohydrate catabolism.

low-carbohydrate high-fat diet—Diet that provides most of the energy from fats and is poor in carbohydrates.

low-density lipoprotein (LDL)—Lipoprotein carrying cholesterol to extrahepatic tissues.

macronutrient—Nutrient, such as carbohydrate, fat, or protein, of which we regularly consume tens to hundreds of grams daily.

malonyl CoA—Compound produced from acetyl CoA and HCO_3^- at the expense of ATP and used in fatty acid synthesis.

mammalian target of rapamycin (mTOR)—Protein kinase involved in the muscle hypertrophic response to resistance training.

mass spectrometry—Laboratory method of identifying and measuring compounds on the basis of the mass-to-charge ratio of their fragments.

maximal oxygen uptake ($\dot{V}O_2max$)—Maximal amount of oxygen taken up by the tissues in the body per unit of time (usually a minute) during maximal exertion.

MCT—See monocarboxylate transporter.

mechanistic target of rapamycin—See mammalian target of rapamycin.

messenger RNA (mRNA)—Kind of RNA that contains information about the amino acid sequence of

proteins.

metabolic control—Coordinated change in the rates of reactions taking place in a living system in response to changing circumstances.

metabolic pathway—Series of reactions in which the product of one is a reactant in the next.

metabolic syndrome—Morbid state characterized by visceral obesity, hyperglycemia, dyslipidemia, and hypertension.

metabolism—Sum of the chemical reactions occurring in a living organism or part of it.

metabolite—Compound participating in metabolism.

metabolome—Sum of metabolites in an organism, tissue, cell type, or biological fluid.

metabolomics—Large-scale, comprehensive study of the metabolomes in terms of metabolite identity, quantity, and function.

MGL—See monoacylglycerol lipase.

Michaelis constant (K_M)—Substrate concentration causing half-maximal rate in an enzyme reaction.

micronutrient—Nutrient, such as vitamin or mineral, of which we regularly consume micrograms to grams daily.

microRNA (miRNA)—Kind of RNA involved in the regulation of gene expression by preventing translation.

mineral—Element considered to be an essential nutrient in the diet.

miRNA—See microRNA.

mitochondrial biogenesis—Generation of new mitochondria.

mitochondrial hypertrophy—Mitochondrial enlargement.

mitochondrion—Intracellular organelle in which most of the energy of a eukaryotic cell is produced through aerobic processes.

mitosis—Division of a somatic cell into two daughter cells.

monoacylglycerol lipase (MGL)—Enzyme catalyzing the hydrolysis of monoacylglycerol to glycerol and fatty acid.

monocarboxylate transporter (MCT)—Protein transporting an acid bearing one carboxyl group across the plasma membrane of cells.

monosaccharide—Carbohydrate containing three to seven carbons.

monounsaturated fatty acid—fatty acid bearing one double bond between two of its carbons.

motor unit—Motor neuron and the muscle fibers it innervates.

mRNA—See messenger RNA.

mTOR—See mammalian target of rapamycin.

muscle fatigue—Disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability and perceived fatigability.

muscle fiber—Muscle cell.

muscle hyperplasia—Increase in the number of muscle fibers.

muscle hypertrophy—Increase in the cross-sectional area of muscle fibers.

muscle remodeling—Reorganization of muscle structure and function.

mutation—Change in the DNA base sequence.

myelin—Relatively inert substance surrounding the axons of many vertebrate neurons.

myofibril—Rod of contractile proteins inside a muscle fiber.

myoglobin—Protein storing O_2 in vertebrate skeletal muscles.

myokinase—See adenylate kinase.

myokine—Compound (such as IL6) released from muscle fibers and affecting the metabolism of other cells in the body.

myosin—The major muscle protein, which moves the muscle at the expense of ATP.

Na^+ channel—See sodium channel.

NAD^+—Oxidized form of nicotinamide adenine dinucleotide.

NADH—Reduced form of nicotinamide adenine dinucleotide.

$NADP^+$—Oxidized form of nicotinamide adenine dinucleotide phosphate.

NADPH—Reduced form of nicotinamide adenine dinucleotide phosphate.

Na^+–K^+ ATPase—See sodium–potassium pump.

Na^+–K^+ pump— See sodium–potassium pump.

neuromuscular junction—Interface between a motor neuron and a muscle fiber.

neuron—Cell of the nerve tissue.

neurotransmitter—Compound transmitting a signal from a neuron to another neuron or other cell type.

niacin—Water-soluble vitamin.

nicotinamide adenine dinucleotide (NAD)—Compound participating in redox reactions.

nicotinamide adenine dinucleotide phosphate (NADP)—Compound participating in redox reactions.

nicotinate—See niacin.

NMR spectroscopy—See nuclear magnetic resonance spectroscopy.

nonessential amino acid—Protein amino acid that we can synthesize.

nonpolar—Having even distribution of charges.

noradrenaline—See norepinephrine.

norepinephrine—Neurotransmitter produced mainly in the sympathetic nervous system.

normoxia—Normal oxygen concentration in a biological fluid such as the cytosol or blood.

nuclear magnetic resonance spectroscopy—Laboratory method of identifying and measuring compounds on the basis of the magnetic properties of certain atomic nuclei.

nucleic acid—Biomolecule consisting of nucleotide residues joined in a row or circle through phosphodiester linkages.

nucleolus—Spherical body inside the nucleus of a eukaryotic cell where rRNA is synthesized.

nucleotide—Compound consisting of a nitrogenous base, a monosaccharide, and one to three phosphoryl groups and serving as a building block of nucleic acids.

nucleus—The organelle of a eukaryotic cell where most of its DNA resides.

nutrient—A food component that serves the provision of energy, provision of raw materials to build tissues, or regulation of bodily functions.

obesity—Excessive accumulation of body fat that may impair health.

oligosaccharide—A carbohydrate consisting of 2 to 10 monosaccharide residues.

osteoporosis—Abnormally low bone mass.

oxaloacetate—A compound of the citric acid cycle serving as a precursor of glucose.

oxidation—The removal of electrons from a compound.

oxidative phosphorylation—The synthesis of ATP from ADP and P_i, compliments of the electron-transport chain.

oxidative stress—A perturbation of the balance between antioxidants and oxidants in a biological system in favor of the oxidants.

oxygen debt—See excess postexercise oxygen consumption.

oxygen saturation (SO_2)—The percentage of the total oxygen-binding sites in the molecules of an oxygen carrier, such as hemoglobin and myoglobin, that are occupied by O_2.

oxygen system—The energy system supplying a large amount of energy at low power during exercise through carbohydrate, lipid, and protein oxidation.

packed cell volume—See hematocrit.

pancreatic lipase—The enzyme catalyzing the hydrolysis of triacylglycerol to monoacylglycerol and two fatty acids in the small intestine.

pantothenate—A water-soluble vitamin.

partial pressure—The pressure that a gas in a mixture would exert if it alone occupied the volume of the mixture.

passive transport—The spontaneous passage of a substance from a compartment where its concentration is high to where its concentration is low.

peptide—A compound made up of amino acid residues linked in a row through peptide bonds. Typically, the term is used for small numbers of amino acid residues (up to about 50).

peptide bond—The bond linking amino residues in a peptide or protein.

perilipin—A protein at the surface of lipid droplets in adipocytes and other cells.

PGC1α—See PPARγ coactivator 1α.

phenotype—The visible or measurable characteristics of an organism.

phosphagen—See compound of high phosphoryl-transfer potential.

phosphatase—An enzyme catalyzing dephosphorylation of a compound.

phosphoanhydride linkage—The linkage joining two phosphoryl groups.

phosphocreatine—A compound of high phosphoryl-transfer potential, which replenishes ATP during maximal exercise.

phosphodiester linkage—The linkage joining two nucleotides in a nucleic acid.

phosphoglyceride—See glycerophospholipid.

phospholipid—A lipid having an amphipathic character, a major component of cell membranes.

phosphorylase—The main enzyme catalyzing glycogenolysis.

phosphorylation—Addition of a phosphoryl group to a compound.

photosynthesis—The synthesis of glucose from CO_2 and H_2O, compliments of solar energy.

phylloquinone—See vitamin K.

physical activity—Any bodily movement produced by muscle activity that results in energy expenditure above the resting level.

P_i—See inorganic phosphate.

PI3K cascade—A series of molecular interactions that begins with the binding of a hormone (such as insulin) or other ligand to a receptor at the plasma membrane, involves the activation of phosphatidyl inositol 3 kinase as an intermediate step, and ends in changing the rate of a metabolic process (such as glycogenesis).

plasma—The fluid of blood outside blood cells. Plasma is obtained as supernatant after centrifugation of a blood sample in which coagulation has been inhibited.

plasma membrane—The membrane surrounding a cell.

platelet count—The number of platelets per usually 1 μL of blood.

polar—Having uneven distribution of charges.

polynucleotide chain—A chain of nucleotide residues (deoxyribonucleotide in the case of DNA, ribonucleotide in the case of RNA) linked in a row through phosphodiester linkages. Typically, the term is used for chains of over 20 residues and is synonymous with DNA or RNA.

polypeptide chain—A chain of amino acid residues linked in a row through peptide bonds. Typically, the term is used for chains of over 20 residues and is synonymous with protein.

polysaccharide—A carbohydrate consisting of over 10 monosaccharide residues.

polyunsaturated fatty acid—A fatty acid bearing more than one double bond between its carbons.

potassium channel (K^+ channel)— A protein at the plasma membrane that lets K^+ out of a cell.

PPARγ coactivator 1α (PGC1α)—A transcriptional coactivator considered the master regulator of the increase in muscle mitochondrial content and, generally, of the changes in muscle phenotype induced by endurance training.

PP_i—See inorganic pyrophosphate.

progesterone—A steroid hormone, member of the progestogens, secreted primarily by the ovaries.

prokaryotic cell—A primitive cell having no internal compartments.

propionyl CoA—A compound produced from the breakdown of odd-number fatty acids.

protease—An enzyme catalyzing the hydrolysis of a protein.

proteasome—A complex of proteins hydrolyzing other proteins to small peptides.

protein—A biomolecule consisting of amino acid residues linked in a row through peptide bonds.

protein turnover—The cyclic process of protein synthesis and degradation.

proteolysis—The hydrolysis of a protein to amino acids or peptides.

proteome—The sum of proteins in an organism, tissue, cell type, or biological fluid.

proteomics—The large-scale, comprehensive study of the proteomes in terms of protein identity, quantity, and function.

purine—A nitrogenous base of DNA and RNA consisting of a six-membered and a five-membered ring.

pyridoxine—See vitamin B_6.

pyrimidine—A nitrogenous base of DNA and RNA consisting of a six-membered ring.

pyruvate—The product of glycolysis.

pyruvate dehydrogenase complex—An assembly of enzymes catalyzing the conversion of pyruvate into acetyl CoA.

radical—A chemical entity possessing an unpaired electron.

reactive oxygen and nitrogen species (RONS)—Chemical entities exhibiting high reactivity in redox reactions and being extremely labile.

receptor—A protein that possesses high affinity for another biomolecule and, upon binding it, changes structure and function.

redox reaction—A reaction in which a reactant is reduced and another is oxidized.

redox state—The ratio of the concentrations of oxidized to reduced substances in a chemical or biological system.

reduction—The addition of electrons to a compound.

reference interval—The range of values of a biochemical or hematologic parameter in the vast majority (usually, the central 95%) of a population.

reference limit—The lower or upper end of the reference interval.

repeated-bout effect—The occurrence of less muscle fiber damage when an exercise, particularly eccentric exercise, is repeated several days or weeks after the initial bout.

RER—See respiratory exchange ratio.

resistance exercise—Exercise consisting of short periods of muscle activity against high resistance, for example, weightlifting.

respiratory chain—See electron-transport chain.

respiratory exchange ratio (RER)—The ratio of the volume of expired CO_2 to the volume of O_2 consumed by the entire body.

respiratory quotient (RQ)—The ratio of the volume of CO_2 released to the volume of O_2 taken up at the cellular level.

resting potential—The electric potential difference across the plasma membrane of a neuron or muscle fiber when it is not excited.

rhodopsin—A light-receiving protein in the retina.

riboflavin—See vitamin B_2.

ribonucleic acid (RNA)—A large biomolecule serving the transmission of genetic information from DNA to proteins and the control of gene expression.

ribonucleotide—A compound consisting of a nitrogenous base, a ribose unit, and one to three phosphoryl groups. Ribonucleotides are the building blocks of RNA.

ribosomal biogenesis—Formation of new ribosomes in a cell.

ribosomal RNA (rRNA)—Kind of RNA that is part of the ribosomes.

ribosome—Complex of rRNA and proteins that performs protein synthesis.

ribozyme—Kind of RNA that exhibits catalytic activity.

RNA—See ribonucleic acid.

RNA polymerase—The main enzyme catalyzing transcription.

RONS—See reactive oxygen and nitrogen species.

RQ—See respiratory quotient.

rRNA—See ribosomal RNA.

ryanodine receptor—Protein at the sarcoplasmic reticulum membrane that acts as the Ca^{2+} channel through which Ca^{2+} is released in response to action potentials.

sarcolemma—Plasma membrane of a muscle fiber.

sarcomere—Segment of a myofibril between two Z lines that constitutes the minimal complete functional unit in a muscle fiber.

sarcoplasm—Cytoplasm of a muscle fiber.

sarcoplasmic reticulum—Membranous system of sacs surrounding the myofibrils in a muscle fiber and containing Ca^{2+} at high concentration.

satellite cell—Cell lying between the basal lamina and the sarcolemma of a muscle fiber, containing little more than a nucleus, and capable of proliferation and fusion with the muscle fiber, thus offering its genetic material for repair or growth.

saturated fatty acid—Fatty acid bearing only single bonds between its carbons.

serum—The fluid obtained as supernatant after centrifugation of a blood sample that has been left to coagulate.

siderophilin—See transferrin.

signal transduction pathway—Series of molecular interactions through which a signal (for example, the secretion of a hormone) causes a response (for example, the acceleration of a reaction).

simple carbohydrate—Monosaccharide or disaccharide.

siRNA—See small interfering RNA.

sliding filament model—Model positing that muscle contraction is due to the active sliding of thick and thin filaments past each other.

small interfering RNA (siRNA)—Kind of exogenous RNA involved in gene silencing within a cell.

small nuclear RNA (snRNA)—Kind of RNA that participates in RNA splicing in the nucleus.

small nucleolar RNA (snoRNA)—Kind of RNA that participates in rRNA processing in the nucleolus.

snoRNA—See small nucleolar RNA.

snRNA—See small nuclear RNA.

SO_2—See oxygen saturation.

SOD—See superoxide dismutase.

soda loading—Intake of $NaHCO_3$ before maximal exercise, aimed at increasing performance by mitigating the drop in blood and muscle pH; possible cause of fatigue.

sodium bicarbonate loading—See soda loading.

sodium channel (Na^+ channel)—Protein of the plasma membrane that lets Na^+ into the cell.

sodium–potassium pump—Protein of the plasma membrane that pumps Na^+ out of the cell and K^+ into the cell at the expense of ATP.

soluble transferrin receptor (sTfR)—Plasma-borne, water-soluble form of the transferrin receptor, the protein that binds transferrin at the plasma membrane of cells.

spectrophotometry—Laboratory method of determining the concentration of a substance in solution by measuring how much light it absorbs.

sprint exercise—Exercise consisting of short periods of maximal muscle activity against low resistance, such as a competitive 100 m run.

standard free-energy change ($\Delta G°$)—ΔG of a reaction when the concentration of every participating substance in solution is 1 mol · L^{-1}.

starch—Storage polysaccharide of plants.

steroid—Lipid containing three six-membered and one five-membered carbon rings, as in cholesterol.

steroid hormone—Member of a family of hormones that derive from cholesterol.

sTfR—See soluble transferrin receptor.

substrate—Reactant in an enzyme reaction.

substrate-level phosphorylation—Synthesis of ATP or another ribonucleoside triphosphate in a reaction in which a substrate is a compound of high phosphoryl-transfer potential.

subunit—Polypeptide chain that is part of a protein consisting of more than one polypeptide chain; one of the two parts of a ribosome.

superoxide dismutase (SOD)—Enzyme catalyzing the conversion of $O_2{\cdot}^-$ into H_2O_2 and O_2.

synapse—Area of contact between neurons or between a neuron and another cell type.

synaptic cleft—Space between the presynaptic and postsynaptic membranes in a synapse.

synaptic vesicle—Vesicle near a synapse that gathers neurotransmitter molecules.

systems biology—Study of complex biological systems, such as cells, tissues, and organisms, through the integrated and combined examination of all of their components in terms of both structure and function.

testosterone—Steroid hormone that is a member of the androgens and is secreted primarily by the testes.

thermogenin—See uncoupling protein 1.

thiamine—See vitamin B_1.

thick filament—Filament containing myosin inside a muscle fiber.

thin filament—Filament containing actin, tropomyosin, and troponin inside a muscle fiber.

thymine—Nitrogenous base of DNA, a member of the pyrimidines.

TIBC—See total iron-binding capacity**.**

total iron-binding capacity (TIBC)—Serum iron concentration in the hypothetical case that the iron-binding proteins in the plasma are fully saturated.

transaminase—See aminotransferase.

transamination—Transfer of an amino group from an α-amino acid to an α-keto acid.

transcription—Synthesis of RNA on the basis of the genetic information contained in DNA.

transcription coactivator—Protein binding to transcription factors in the process of initiating transcription.

transcription factor—Protein binding to specific DNA base sequences, thus serving as docking site for RNA polymerase to initiate transcription.

transcriptome—Complete set of transcripts in an organism, tissue, cell type, or biological fluid.

transcriptomics—Large-scale, comprehensive study of the transcriptomes in terms of RNA identity, quantity, and function.

transferrin—The major iron-carrying protein in the plasma.

transferrin saturation—Serum iron concentration as a percentage of TIBC.

transfer RNA (tRNA)—Kind of RNA that transfers amino acids for protein synthesis.

translation—Protein synthesis based on the information contained in mRNA.

transverse tubules—System of tubules conducting action potentials to the interior of a muscle fiber.

triacylglycerol—Lipid consisting of three acyl groups and a glycerol unit; the major energy depot in the body.

tricarboxylic acid cycle—See citric acid cycle.

triglyceride—See triacylglycerol.

tRNA—See transfer RNA.

tropomyosin—Muscle protein controlling the interaction of F-actin and myosin.

troponin—Muscle protein mediating control of muscle activity by Ca^{2+}.

T system—See transverse tubules.

turnover number—Number of substrate molecules converted into product by an enzyme molecule in a specified time when the enzyme is saturated with substrate.

ubiquitin—Polypeptide that, when attached to a protein, earmarks it for degradation in the proteasome.

UCP1—See uncoupling protein 1.

uncoupling protein 1 (UCP1)—Protein at the inner mitochondrial membrane of brown adipocytes, which uncouples the electron-transport chain from oxidative phosphorylation, resulting in heat dissipation.

unsaturated fatty acid—Fatty acid bearing one or more double bonds between its carbons.

uracil—Nitrogenous base of RNA; member of the pyrimidines.

urate—The main product of purine degradation in the human body.

urea—Compound through which most of the nitrogen is removed from the human body.

urea cycle—Cyclic metabolic pathway producing urea in the liver.

uric acid—See urate.

very low-density lipoprotein (VLDL)—Lipoprotein carrying hepatic triacylglycerols to extrahepatic tissues.

vitamin—Organic compound present in food in minuscule amounts and participating in metabolic regulation.

vitamin A—Fat-soluble vitamin.

vitamin B_1—Water-soluble vitamin.

vitamin B_2—Water-soluble vitamin.

vitamin B_6—Water-soluble vitamin.

vitamin B_{12}—Water-soluble vitamin.

vitamin C—Water-soluble vitamin.

vitamin D—Fat-soluble vitamin.

vitamin E—Fat-soluble vitamin.

vitamin K—Fat-soluble vitamin.

VLDL—See very low-density lipoprotein.

$\dot{V}$**Omax**—See maximal oxygen uptake.

voltage-gated channel—Transmembrane protein that lets substances through when the membrane voltage changes.

water-soluble vitamin—Vitamin that dissolves readily in water.

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